Wave length of the cardiac impulse and reentrant arrhythmias

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Joseph Léon Robert Marie Smeets

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Joseph Léon Robert Marie Smeets
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Promotor: Prof. Dr. F.I.M. Bonke, Rijksuniversiteit Limburg, Maastricht.

Co-promotor: Dr. M.A. Allessie, Rijksuniversiteit Limburg, Maastricht

Referenten: Dr. M.J. Janse, Universiteit van Amsterdam, Amsterdam.

Prof. Dr. H.J.J. Wellens, Rijksuniversiteit Limburg, Maastricht.

Prof. A.L. Wit, PhD, Columbia University, New York.

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1. INTRODUCTION.

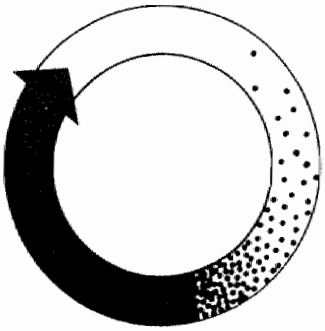
Historical background.

Tachyarrhythmias can be divided in those based on abnormal impulse formation and those based on abnormal impulse conduction, leading to circulating excitation in the heart (Wit and Cranefield 1978, Hoffman and Rosen 1981). The existence of circulating excitation was already discovered in the beginning of this century. Mayer (1906) demonstrated that in a ring, cut from the paralyzed jelly-fish, rhythmic activity could be initiated if only one stimulus was given. This regular activity was based on the occurrence of one contraction wave, which circulated in this ring. The first to describe circulating excitation in cardiac tissue was Mines (1913). In a ring structure, made from atrial and ventricular muscle of the tortoise heart, a rhythm was originated in which the activation went from atrium to ventricle and back to atrium and so on. This was described as a "reciprocating rhythm". It is essential for the initiation of such a reciprocating rhythm or circus movement that the stimulated impulse is blocked in one direction of the ring structure (unidirectional block). Furthermore the impulse, going in the opposite direction, must travel along such a route that the area of block has enough time to restore its excitability to be activated in a retrograde fashion by the circulating impulse.

Lewis et al. (1920) demonstrated that atrial flutter initiated in a canine heart was based on a circulating excitation around the superior and inferior caval veins. Further evidence that this arrhythmia was based upon circus movement around the orifices in the atrium was obtained by Rosenblueth and Garcia Ramos (1947). They showed that induction of atrial flutter was markedly facilitated if the muscular area between superior and inferior caval vein was crushed. The importance of the size of the anatomical obstacle around which the impulse is circulating was further elucidated by the fact that the cycle length prolonged if the anatomical obstacle was enlarged (Kimura et al. 1954). An additional argument for circus movement as the underlying mechanism, was the sudden termination of the arrhythmia if the anatomical obstacle was enlarged in the direction of the atrio-ventricular groove, resulting in an interruption of the pathway.

CIRCUS MOVEMENT

Anatomically determined
(Mines, 1913)



1) Fixed length of circuit

2) Circuit length equal to anatomical pathway

3) Excitable gap between head and tail of impulse

4) Rate proportional to conduction velocity and length of pathway

Functionally determined
(Allessie et al., 1977)

1) Variable size of circuit

2) Circuit length equal to length of the excitation wave

3) No gap of full excitability

4) Rate proportional to refractory period

FIGURE 1.1: Illustration of the differences in properties of an anatomically determined (left) and a functionally determined (right) circus movement.

An example of a clinical arrhythmia which is based on a circus movement in an anatomically determined pathway is the Wolff-Parkinson-White-syndrome. In this arrhythmia the anatomically determined pathway consists of atrium, atrio-ventricular node, bundle of His, bundle branch, ventricle and accessory pathway (Durrer and Wellens 1974).

Two types of circus movement.

In addition to circulating excitation in an anatomically determined pathway, circus movement also can exist without involvement of an anatomical obstacle. It was first shown by Allessie et al. (1973, 1976, 1977a) that a rapid circus movement could be initiated in an isolated atrium of the rabbit which did not contain any anatomical obstacle. This kind of circus movement without an anatomically preformed pathway is fully determined by the electrophysiological properties of the myocardium. The circulating wave will take the shortest possible route in which the impulse just can activate the tissue ahead, which is recovering from the previous activation. In this situation a tight fit exists between the head and tail of the wave front. The central area is continuously invaded by centripetal wavelets, which collide on each other and thus prevent a short-cut of the circuit. In other words this central area acts as a functional block, around which the impulse revolves. If the electrophysiological properties of the tissue change, the pathway of the impulse changes too. As a consequence the localization and size of this functionally determined circuit is not fixed and can change from moment to moment. For instance if the refractory period is shortened the tissue in the center will recover its excitability earlier and thus allows an impulse to take a shorter route, eventually resulting in a short-cut of the circuit. On the other hand if the conduction velocity is increased the impulse is forced to take a longer way since the tissue is not yet restored from the previous activation. However the time needed to complete one revolution will not be affected. From this it follows that the rate of this circus movement is related to the refractory period of the myocardium. In figure 1.1 the properties of an anatomically determined and a functionally determined circuit are compared.

Arrhythmias based on intra-myocardial reentry.

Arrhythmias which might be based on such functionally determined circuit are atrial flutter and fibrillation. It was proposed by Moe and Abildskov (1959) that fibrillation could be based on the simultaneous wandering of multiple wavelets, changing continuously in width, position and number. Recently Allessie et al. (1982) provided experimental evidence for this hypothesis. Using an extensive mapping technique they demonstrated that during atrial fibrillation in an isolated blood perfused canine heart several functionally determined circuits were present simultaneously. These reentrant circuits continuously changed in size and position. The number of simultaneously present circuits depends on the size of the circuits relative to the size of the part of the heart in which they are circulating. If the size of the circuit is small in relation to the heart, there might be room for many circuits. In this situation the statistical chance that all wavelets die out at the same time is small and spontaneous termination of fibrillation is unlikely. On the other hand if the size of the circuit is large, only a limited number of wave fronts can be present simultaneously and termination of fibrillation becomes more likely.

The role of inhomogeneity in conduction and refractoriness for the initiation and continuation of fibrillation.

Measurement of the refractory period at several sites in atrial and ventricular myocardium have revealed considerable differences in the rate of recovery of excitability (Alessi et al. 1958, Han and Moe 1964, Janse 1971, Allessie et al. 1976). In general if the heart rate is low these differences play no role because the myocardium has sufficient time to restore its excitability completely before the next impulse arrives. As a consequence the activation front is conducted uniformally in all directions. If however an extrasystole arises in the relative refractory period the conduction is less uniform since not all fibers have completely regained their excitability. The activation front is conducted in fibers, which have recovered from the previous activation, whereas in neighboring fibers the conduction is blocked because they did not yet restore their excitability. As a consequence the activation front will have an irregular contour and becomes fractionated. From this it follows that if the dispersion in recovery of excitability is large, the likelihood of occurrence of areas of block is high. As a result

fractionation of the wave fronts will occur frequently, making degeneration into arrhythmias likely. Interventions which increase the inhomogeneity in conduction and refractoriness, are associated with an increased risk of fibrillation. It was demonstrated that vagal stimulation enhances the non-uniform recovery of excitability in the atrium (Alessi et al. 1958), and increased sympathetic activity increases the dispersion of recovery of the excitability of ventricular myocardium (Han and Moe 1964). However not only local inhomogeneities in refractoriness and conduction but also the dimension of an intra-myocardial circuit determines whether or not an impulse can be trapped in a circuitous route.

The role of the length of the excitation wave for the initiation and continuation of fibrillation.

In considering the occurrence of a circus movement three factors have to be taken into account, as was already described by Lewis (1925): the length of the pathway, the conduction velocity of the impulse and the duration of the refractory period. In an anatomically determined circuit the length of the pathway is larger than the length of the excitation wave (product of conduction velocity and refractory period). Under these circumstances a gap of full excitability exists between head and tail of the wave front. However, in a functionally determined circuit there is a tight fit between head and tail of the wave front and no gap of full excitability is present. Then the length of the pathway is equal to the wave length of the circulating impulse.

In figure 1.2 the prerequisite conditions for the initiation of a functionally determined circuit are depicted. In general the activation front will propagate uniformally from the point of origin to all directions, as illustrated in panel A. If due to inhomogeneity in excitability the impulse is blocked, the activation front circumvents this area. By doing so the myocardium at the other side of the block is activated (panel B and C). If the impulse travelled such a route that the myocardium which was previously blocked had enough time to restore its excitability, the block area can be activated in a retrograde fashion (panel D) and the impulse can accomplish the first reentrant loop.

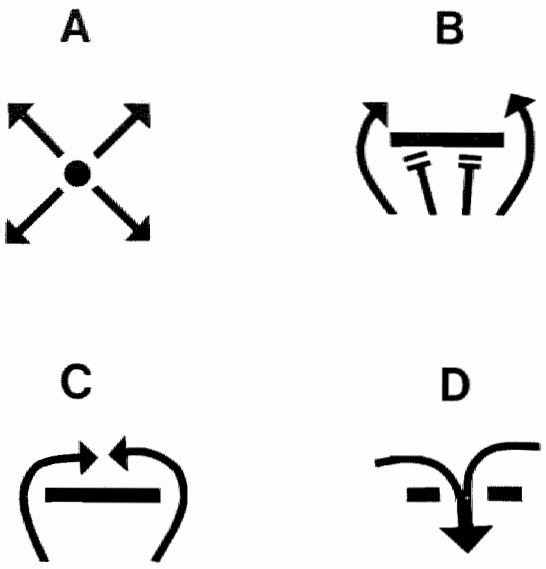


FIGURE 1.2: Prerequisite conditions for initiation of reentry. In general an activation front is conducted uniformally in all directions (panel A). Sometimes however the impulse is blocked (panel B) and the activation front circumvents this area of block (panel B and C). If the impulse arrives at the backside of the block area at the moment the myocardium has restored its excitability, retrograde activation of this area occurs (panel D).

Whether such a reentrant circuit occurs depends on a delicate interplay between the size of the block and the length of the excitation wave. If the route the impulse must travel to reach the backside of the block area is shorter than the length of the excitation wave, no circuit can be established because the myocardium had not yet restored its excitability. Only if the pathway is equal to the length of the excitation wave, the block area can be invaded and a circuit can be completed since the myocardium has just regained its excitability. If the pathway which the impulse travels around the block area becomes larger than the length of the excitation wave the activation front will activate the block area as soon as the myocardium becomes

excitable again. From this it follows that if the wave length is relatively short, a small area of block will be sufficient to set the stage for a reentrant circuit. Since small areas of block can occur rather frequently in the myocardium initiation of these circuits becomes more likely. On the other hand if the wave length is relatively long, reentry only can occur if a large arc of conduction block will be present. However occurrence of a large area of block is unlikely, making the chance on initiation of a reentrant circuit low.

Measurement of the length of the excitation wave.

In figure 1.3 a functionally determined circuit is schematically represented. The black area indicates the myocardium which is in the absolute refractory phase, whereas the dotted area indicates the relative refractory tissue. Since a tight fit exists between the head and tail of the wave fronts, the size of the reentrant circuit is equal to the length of the circulating wave. Measurement of the wave length can be done in two ways. The wave length of the circulating impulse can be measured directly by mapping the activation pathway during an intra-myocardial reentry. Allessie et al (1977b) showed that under the influence of acetylcholine the minimal dimension of this functionally determined circuit diminished considerably due to a change in refractoriness. However measurement of changes in the minimal dimension by mapping the activation pathway is a rather time consuming investigation.

The wave length can also be measured in a narrow bundle of myocardium, as if the circuit is opened and unrolled. If one knows the conduction velocity and the refractory period under conditions comparable with that of the tachycardia the length of the excitation wave can be calculated from the product of these two parameters. The advantage of this method is that the measurement of the wave length can be done quickly and no elaborate equipment is needed.

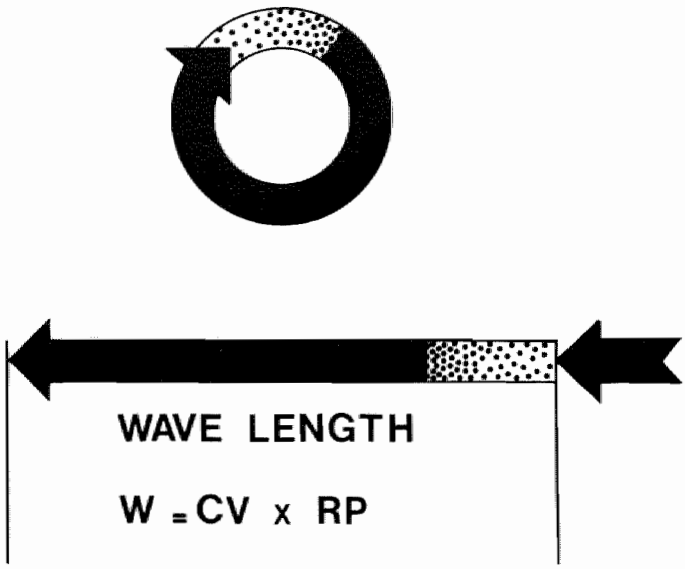


FIGURE 1.3: Schematic representation of a functionally determined circuit. The black area indicates the tissue which is in its absolute refractory phase, whereas the dotted area represents the relative refractory tissue. Since a tight fit exists between the head and tail of the circulating activation wave, the size of the circuit is equal to the wave length of the impulse. The wave length can be measured by mapping the activation sequence of a reentrant arrhythmia, or by calculating the product of conduction velocity and refractory period, measured under conditions comparable with that of the reentrant arrhythmia.

Possible mode of action of some arrhythmogenic and antiarrhythmic interventions in relation to reentrant arrhythmias.

If our hypothesis is correct that the size of the circuit cq. the wave length is one of the factors determining the chance of initiation and continuation of fibrillation we should expect that interventions which influence the occurrence of fibrillation will also change the length of the excitation wave. To test this hypothesis we measured the wave length in the isolated atrium of the rabbit during arrhythmogenic and antiarrhythmic interventions.

It is a well known fact that atrial fibrillation can easily be induced when the vagal nerve is stimulated or when the atrium is exposed to acetylcholine. According to our hypothesis we expect that acetylcholine shortens the length of the excitation wave. In this case the induction of fibrillation would be facilitated (only a small area of block is needed), and persistence of fibrillation becomes more likely (many circuits can be present simultaneously).

On the other hand drugs which can terminate atrial fibrillation (quinidine, digitalis, amiodarone) are expected to prolong the length of the excitation wave. When the excitation wave is long the induction of fibrillation would be more difficult (a large block area is needed) and the termination of fibrillation more likely (only a limited number of wavelets can be present).

In addition we tested this hypothesis by measuring the effects of antiarrhythmic drugs, which do not influence the occurrence of atrial fibrillation (verapamil, lidocaine). If no change in the length of the excitation wave was observed during administration of these drugs, this would support our hypothesis.

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2. METHODS

2.1 Preparation

Young New Zealand rabbits of both sexes weighing between 1.5 and 2.0 kg were stunned with a blow in the neck and the thorax was opened by a mid-sternal incision. The heart was rapidly excised and transferred to a tissue bath where further dissection was performed. A left oblique lateral view of the rabbit heart is shown in figure 2.1 panel A. The hatched area of the left atrium indicates the part of the heart which was used for these studies. The atria and ventricles were separated by a cut along the atrial side of the atrio-ventricular groove. Another cut was made along the border of the atrial appendage, removing the floor of the atrium. After this procedure the oxygenated Tyrode solution could reach freely the endocardial surface of the left atrium. This part of the operation - from the cervical dislocation till the opening of the left atrium - was done as quickly as possible to prevent hypoxic damage of the endocardial cells and was accomplished within 2 minutes. Figure 2.1 panel B is a view of the atria as seen from below. The right atrium is still intact, showing the atrio-ventricular orifice. In the left atrium the floor has been removed showing the complete endocardial surface of the roof. The last part of the operation was to isolate a long and narrow strip of atrial myocardium. In figure 2.1 panel C the actual form of the left atrial preparation is shown which was used for our experiments as seen from the endocardial side. The L-shaped strip is about 20 mm long, 2-3 mm in width and not more than 0.5 mm thick. After isolation from the rest of the atria, the preparation becomes quiescent. The short "leg" was used for stimulation, whereas in the long part the conduction characteristics were measured.

2.2. Superfusing- and experimental equipment.

The strip of left atrial myocardium was placed in a 50 ml tissue bath which is perfused at a rate of 100 ml per minute. The Tyrode solution had the following composition (mM): NaCl 130, KCl 4.5, $CaCl_2$ 2.2, $MgCl_2$ 0.6, $NaHCO_3$ 24.2, NaH_2PO_4 1.2, glucose 11 and sucrose 13. The perfusion fluid was saturated by a mixture of 95% O_2 and 5% CO_2. The pH of the Tyrode was

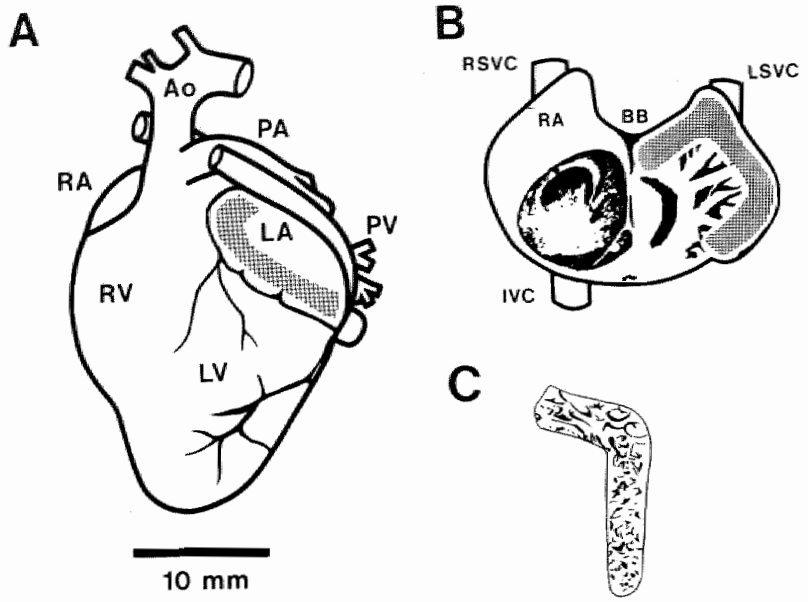

FIGURE 2.1 Schematic drawings of the different stages of dissection of the preparation. Panel A shows the rabbit heart from a left oblique lateral view. The hatched area in the left atrium indicates the part that will be isolated. In panel B the left and right atrium are seen from below. The right atrium is still intact, whereas the floor of the left atrium has been removed. In panel C the preparation is shown as it appears after the hatched area in panel B has been excised. The strip is L-shaped, about 20 mm long, 2-3 mm in width and not more than 0.5 mm thick. The short part of the L-shaped strip is used to stimulate the preparation, whereas in the long part the conduction velocity is measured. Ao = aorta, BB = Bundle of Bachmann, IVC = inferior vena cava, LA = left atrium, LSVC = left superior vena cava, LV = left ventricle, PA = pulmonary artery, PV = pulmonary veins, RA = right atrium, RSVC = right superior vena cava, RV = right ventricle.

maintained at 7.35 $\pm$ 0.05. After being oxygenated in a 10 liter reservoir the Tyrode solution was transported by a roller pump to an airchamber, where the pulsatile flow was changed to a more continuous flow. Before entering the tissue bath the fluid was warmed in a heating coil to a temperature of 37°C $\pm$ 0.1. After coming out of the tissue bath the Tyrode is led back again to the 10 liter reservoir.

Figure 2.2 gives a schematic drawing of the experimental set-up.

A programmable stimulator delivers constant current pulses (duration 1-2 msec, strength 2-5 times diastolic threshold) to the strip of left atrium through 2 platinum plates (2 x 4 mm) embracing the short leg of the bundle. The stimulating electrode is positioned in such a way that the preparation can move freely between the 2 plates. From the point of stimulation the impulse is propagated along the bundle. The fact that the strip is so narrow guarantees that the pathway of activation can show only minor variations. The activation sequence was recorded with a multiple unipolar recording electrode, consisting of a row of 8 teflon coated silver wires 0.3 mm in diameter. The distance between the recording electrodes is fixed at 2 mm.
A minimum distance of 3 mm was kept between the point of stimulation and the

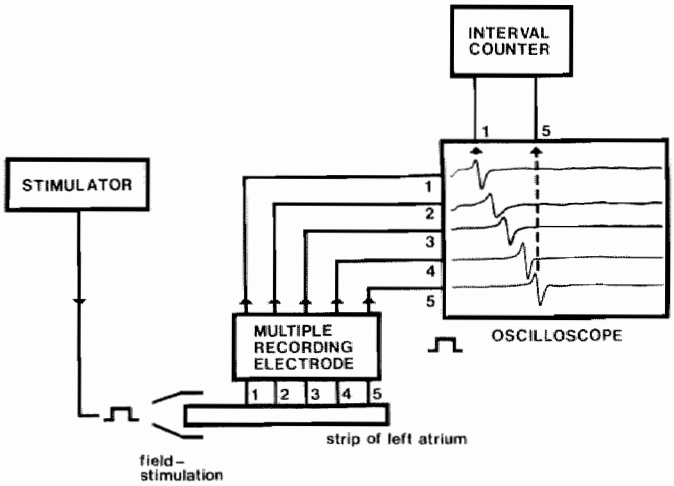

FIGURE 2.2 Schematic representation of the experimental set- up. A strip of left atrial myocardium is mounted in a tissue bath. A programmable stimulator delivers square wave pulses, 1 or 2 msec in duration, through a set of platinum plates placed just above and beneath one end of the left atrial strip. A row of 8 unipolar recording electrodes is gently positioned on the remaining part of the bundle. The electrograms are individually amplified and displayed on an oscilloscope. An example of the recorded activation sequence is shown at the right. The beam of the oscilloscope is started on the stimulus artefact. The time between activation of the first and last electrode on the atrial strip is displayed on an interval counter. In this case five electrodes fitted on the preparation, the distance between the first and last recording electrodes being 8 mm.

first recording electrode. A large silver plate in the tissue bath was used as indifferent electrode. The unipolar electrograms recorded with this multiple recording electrode were individually amplified (bandwith 5 - 400 Hz) and displayed on an oscilloscope (Tektronix 1503N). In this way the activation sequence all along the strip of left atrium can be easily monitored. The fast part of the intrinsic deflection of the unipolar electrogram, representing the moment of activation of the tissue beneath the recording electrode, was detected by a trigger unit designed by our electronic workshop. The time difference between arrival of the activation front at the first and last recording electrode was continuously measured with an interval counter (Hewlett Packard 5300B)

2.3. Electrical stimulation

Point- versus field-stimulation.

Stimulation of the myocardium with extracellular electrodes can be done either locally, using a point electrode, or more regionally, using a large electrode (field stimulation). To evaluate which way of stimulation should be used in our studies, we compared both ways of stimulation in 12 preparations. Table 2.I gives the individual values of these experiments. For both ways of stimulation the shortest possible pacing interval and the conduction velocity at that rate are plotted. Note that, compared to point stimulation, during field stimulation the average minimal pacing interval is shorter (90.4 versus 110.4 msec) and the conduction is slower (32.8 versus 36.3 cm/sec). These differences can be explained by the different amount of tissue, which is stimulated. In case of a point electrode the electrophysiological properties of a small amount of tissue determines whether or not a propagated response will be initiated. If at the spot of stimulation the refractory period is long, the maximum pacing rate will be found to be relatively low. On the other hand if the local refractory period is short a higher maximum pacing rate will be measured. In case of field stimulation the area of myocardium, which is exposed to the electrical stimulus, is larger. Therefore the localisation of the stimulating electrodes is less critical. The part of the tissue between the plates with the shortest refractory period will determine the maximum pacing rate. As a consequence the minimal pacing cycle length is

TABLE 2.I

Prepa-ration	POINT-STIMULATION Interval Fmax (ms)	POINT-STIMULATION Conduction Velocity (cm/sec)	FIELD-STIMULATION Interval Fmax (ms)	FIELD-STIMULATION Conduction Velocity (cm/sec)
1	110	29	100	34
2	125	24	90	35
3	80	24	80	40
4	125	27	85	34
5	110	55	100	33
6	110	37	80	33
7	105	40	80	28
8	95	43	100	33
9	120	57	90	33
10	110	21	95	32
11	130	43	85	31
12	105	35	100	28
MEAN	110.4	36.3	90.4	32.8
SD	±13.9	±11.9	±8.4	±3.2

shorter, and also the conduction at this higher pacing rate is slower. Furthermore the variability between the different preparations, as indicated by the standard deviation is smaller for field stimulation. Because of these reasons we decided to use field stimulation in these studies.

Release of neurotransmitters by field stimulation

It has been described that electrical stimulation of the heart can liberate neurotransmitters like acetylcholine and/or (nor)epinephrine from the cardiac nerve terminals (Vincenzi and West 1963, Spear et al. 1979). To check whether the field stimulation we wanted to use could release acetylcholine and (nor)epinephrine, we applied this kind of stimulation on the isolated rabbit sinus node. It is well known that in the sinus node area a large number of both sympathetic and parasympathetic nerve terminals are present. If the stimulation procedure releases one or both neurotransmitters this should become apparent by a change in sinus rhythm. In figure 2.3 an example is shown of the effects of electrical stimulation on the rate of spontaneous discharge of the isolated sinus node. Two platinum plates were

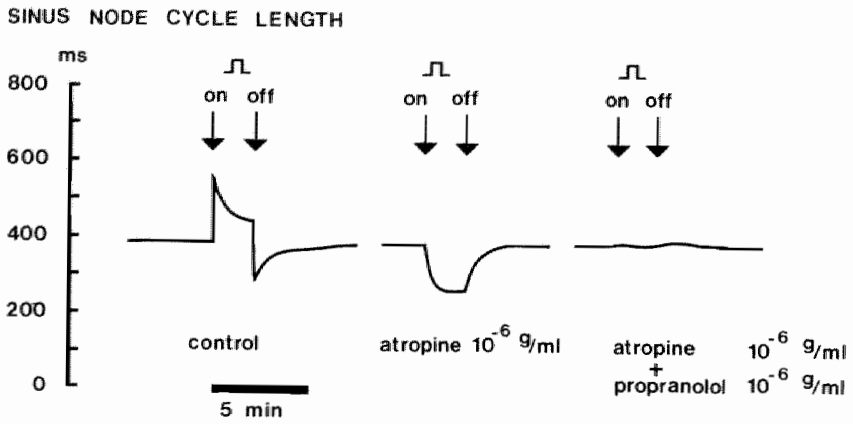

FIGURE 2.3 The effects of electrical stimulation of the sinus node. The spontaneous cycle length of the sinus node is plotted on the ordinate. The effects of electrical stimulation are shown during control, during administration of atropine (10^{-6} gr/ml) and during administration of atropine together with propranolol (10^{-6} gr/ml). During the period delineated by the arrows, a train of 7 stimuli (duration 1 msec, interval 2 msec, strength 10 mA) is applied to the sinus node region after every spontaneous discharge. During control, stimulation resulted in a prolongation of the sinus node cycle length. In contrast after administration of atropine a marked shortening of cycle length was observed. When in addition propranolol was added no effect of the electrical stimuli was observed at all.

positioned at each side of the opened superior Vena Cava, at the area where the sinus node is located. After every spontaneous discharge of the sinus node a train of 7-10 stimuli (duration 1 msec, interval 2 msec, strength 10 mA) are given through the two plates. Because the atrium has been depolarized by the sinus node just before this train of stimuli is given, the myocardium is still in its absolute refractory phase and can not be excited. However the nerve fibers, having a very short refractory period, will be stimulated. In figure 2.3 the effects during control, after administration of atropine and after atropine together with propranolol are illustrated. During control electrical stimulation clearly prolonged the spontaneous sinus node cycle length. This phase of slowing was preceded by a decelaration directly after initiation of stimulation, and was followed by an acceleration after termination. After administration of atropine no increase in cycle length was

observed anymore. Instead the cycle length decreased from about 400 to 300 msec. If atropine and propranolol were administered the sinus node cycle length was not changed at all during the stimulation procedure. These results indicate that field stimulation indeed can release both (nor)epinephrine and acetylcholine from the nerve terminals.

To check whether also in the isolated left atrium acetylcholine and (nor)epinephrine are released, we applied the same stimulation protocol to the atrium. However instead of using the rate of spontaneous discharge of the sinus node, we used the refractory period as an indicator for the liberation of neurotransmitters. Acetylcholine shortens the refractory period at 500 msec pacing interval (2 Hz) in the rabbit atrium whereas epinephrine prolongs refractoriness (see chapter 5). As can be seen in table 2.II no differences in refractory period were detected with or without electrical stimulation. Also addition of atropine in a concentration of 10^{-6} g/ml did not change the refractory period. These results indicate that even strong stimulation of the

TABLE 2.II

Preparation	REFRACTORY PERIOD (ms) Control	Stimulation*	Atropine**	INTERVAL Fmax (ms) Control	Atropine**
1	70	70	85	100	100
2	60	60	65	90	90
3	45	45	45	95	90
4	60	60	60	85	95
5	70	65	70	-	-
MEAN	61.0	60	65	92.5	93.8
SD	±10.3	±9.4	±14.6	±6.5	±4.8

* train of 7 stimuli, duration 1 msec, interval 2 msec, strength 10 mA

** 10^{-6} g/ml atropine

isolated left atrium does not cause a major release of neurotransmitters and will not influence the values of refractoriness and conduction velocity.

The stimulus strength

Since the measured value of the refractory period may strongly depend on the stimulus intensity used, an appropriate stimulus strength has to be chosen. The relation between stimulus strength and shortest possible coupling interval (strength-interval curve) is shown in figure 2.4. At lower stimulus

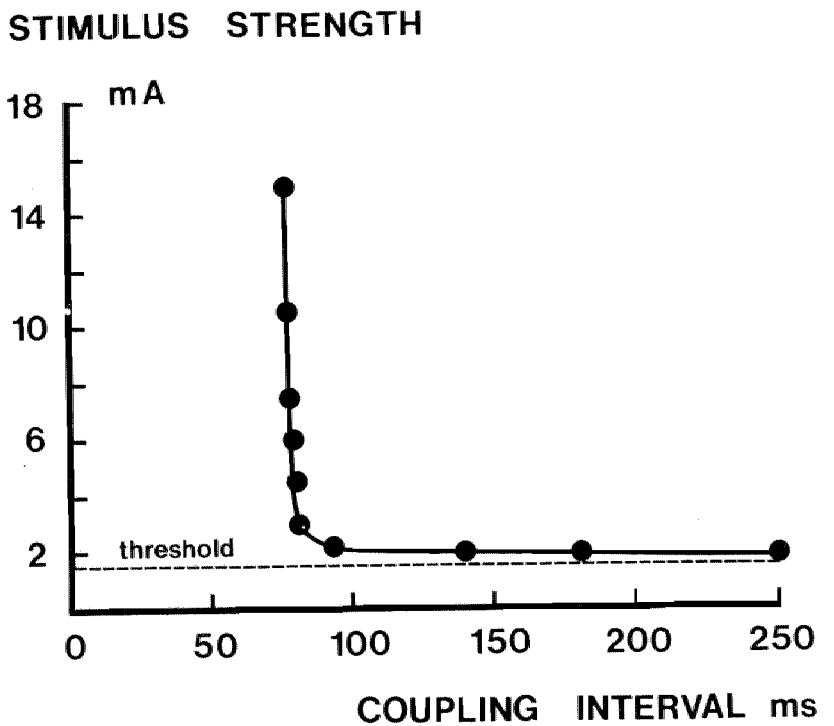

FIGURE 2.4 The strength-interval curve of isolated left atrial myocardium using field stimulation. The refractory period (the shortest possible A1-A2 interval) is shortened markedly when the stimulus strength is increased from threshold to about 2 times threshold (3 mA). Further increase to 10 times diastolic threshold (15 mA) hardly affects the A1-A2 interval. For the determination of the refractory period we used a stimulus strength of 4-5 times diastolic threshold (pacing rate 2Hz).

intensities minor changes in stimulus strength result in large differences in the measured refractoriness. However at a higher stimulus strength, starting from 3 times diastolic threshold (4.5 mA) the refractory period is hardly affected by the stimulus strength. For this reason a stimulus of 4 to 5 times diastolic threshold was used for the measurement of the refractory period.

2.4. Measurement of the conduction velocity.

The conduction velocity of the electrical impulse in the heart can be calculated if one knows the conduction time of the activation wave over a certain distance. There are two different ways which are used to measure the conduction time. The simplest and most widely used method is to measure the time between delivery of a stimulus through a pair of stimulating electrodes and the arrival of the activation front at a remote recording electrode. Another method uses an additional recording electrode between the point of stimulation and the remote recording electrode. The time lapse between the arrival of the activation front at the first and second recording electrode is taken as the conduction time required to cover the distance between the electrodes. To evaluate which of the methods is to be prefered, we compared both ways of conduction time measurement in a isolated piece of atrial myocardium. Figure 2.5 gives an example. The conduction time was measured pacing the preparation at a rate of 10 Hz at different stimulus strengths. The conduction time measured between the stimulus artefact and the remote recording electrode is plotted as solid circles, whereas the squares indicate the conduction time measured between the proximal and distal recording electrode. The inset of the figure shows the experimental set-up. The conduction time shortened with increasing stimulus strength, if measured between stimulus artefact and the remote recording electrode. However, if the conduction time is measured between two recording electrodes it was found to be almost independent of the stimulus strength. Because a reproducible and accurate measurement of the conduction time can only be done if it is independent of the stimulus intensities used, we decided to measure the conduction time using two recording electrodes.

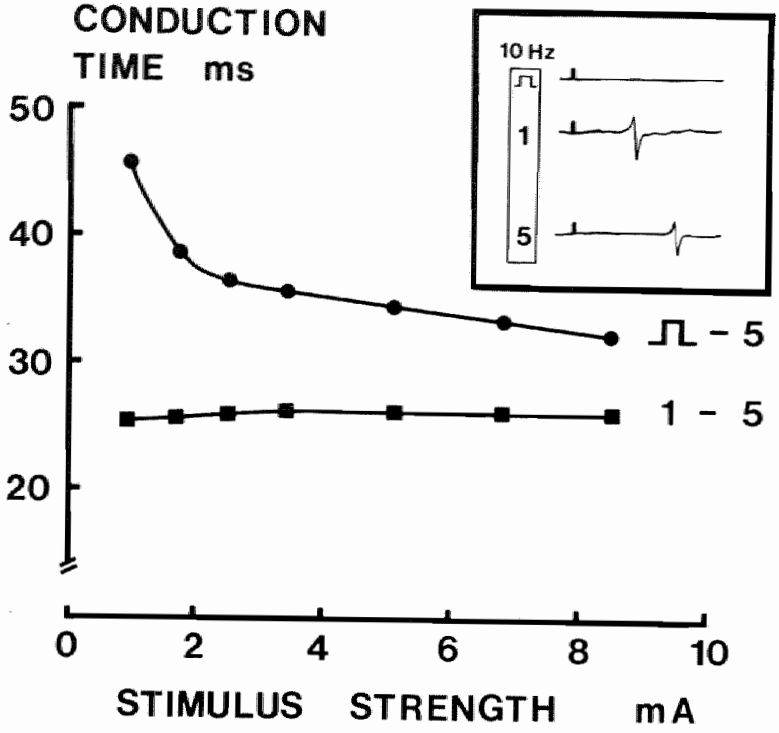

FIGURE 2.5 Conduction time along a bundle of atrial myocardium as a function of stimulus strength. Two methods to measure the conduction time are compared. If the conduction time is expressed as the time difference between the stimulus artefact and the moment of arrival at a remote recording electrode, the values indicated by the filled circles are found. If the conduction time is measured between two recording electrodes, in this example between the first and fifth lead of a multiple recording electrode, the values indicated by the filled squares are found. The inset at the top gives a schematic representation of the experiment. In this case the preparation was paced at a frequency of 10 Hz. It is clear that using the stimulus artefact to determine the conduction velocity, the measured value of the conduction time is not independent of the stimulus strength used.

2.5. Homogeneity in conduction.

Figure 2.6 gives an example of the activation sequence in a strip of atrial myocardium at a pacing rate of 2 Hz (500 msec pacing interval). Recording electrode 1 was about 3 mm from the site of stimulation and the distance between the recording electrodes was 2 mm. In the left panel the

simultaneously recorded unipolar electrograms of electrodes 1 to 5 are displayed, whereas in the right panel the conduction time is plotted as a function of distance. During this slow rhythm the impulse propagates at a constant speed, the conduction time showing a linear relationship with distance. Also at higher pacing rates the conduction remained homogeneous. Figure 2.7 shows the conduction in another strip of atrial myocardium measured at 4 different pacing rates. At the top of each panel the conduction time over a distance of 8 mm between the first and fifth recording electrode is indicated. An increase of the pacing rate from 2 to 4 Hz causes a minor increase in conduction time from 12 to 13 msec. Increasing the pacing rate to 8 Hz causes a further prolongation of the conduction time to 14 msec, whereas a lengthening to 16 msec is observed when the pacing rate is increased to 12 Hz. Even at this high rate there is still a uniform conduction.

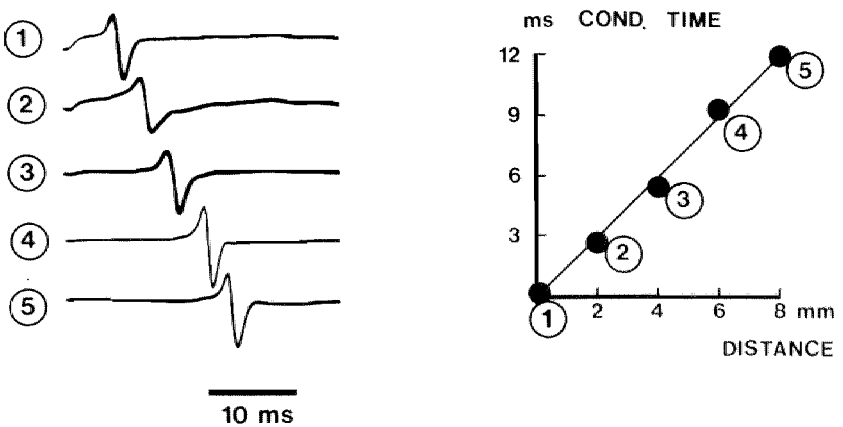

FIGURE 2.6 Conduction characteristics in a bundle of isolated atrial myocardium. In the left panel the activation sequence at a constant pacing interval of 500 msec is shown. The electrodes are indicated by the encircled numbers. It is clear that the conduction between the different electrodes is very uniform. At the right, the conduction time is plotted as a function of the recording distance. Here again it can be noted that the conduction in this strip of atrial myocardium at a pacing interval of 500 msec is highly uniform. The conduction time between two succesive recording electrodes (inter-electrode distance 2 mm) was 3 msec.

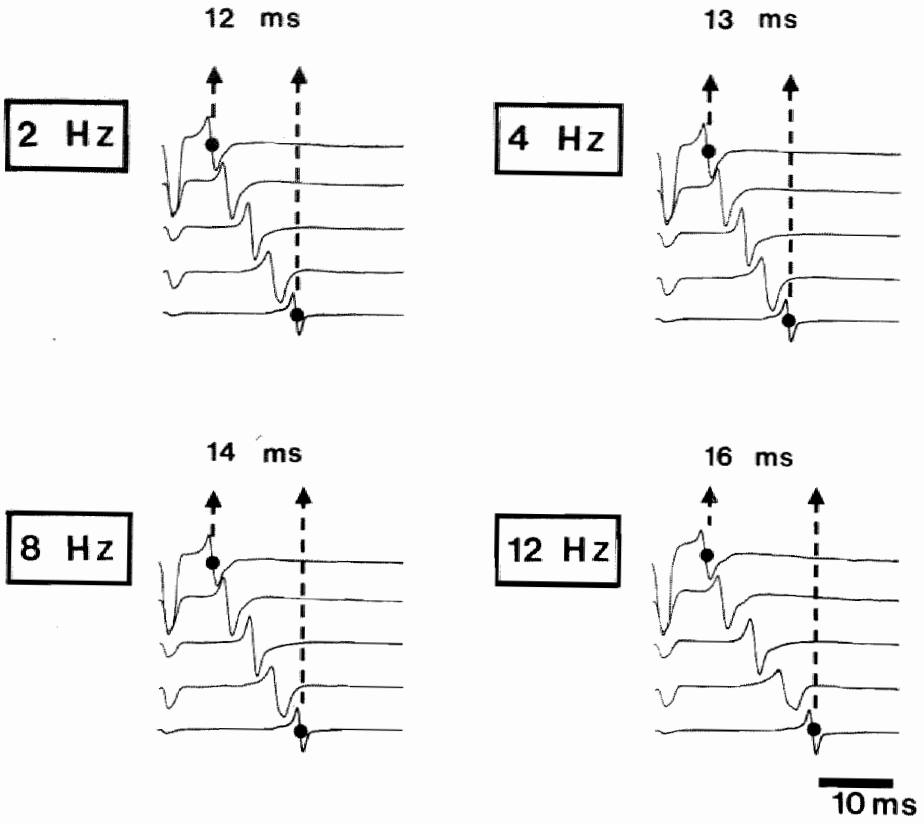

FIGURE 2.7 Influence of rate on conduction in the atrium. In the 4 panels the conduction is shown over a distance of 8 mm at the following pacing rates: 2, 4, 8 and 12 Hz. The conduction time was 12 msec at a pacing rate of 2 Hz (left top panel). Increasing the pacing rate to 4,8 and 12 Hz caused a prolongation of the conduction time to 13, 14 and 16 msec respectively. It may be noted that at this high rate the conduction is still highly homogeneous.

2.6. Characteristics of conduction block.

Figure 2.8 is taken from the same experiment as figure 2.7. The pacing rate has been increased to 13 Hz and every impulse is still conducted along the bundle. If the pacing rate is further increased to 14 Hz conduction block occurred at the end of the strip between the fourth and fifth recording electrode. At this high rate not every impulse could be conducted all along

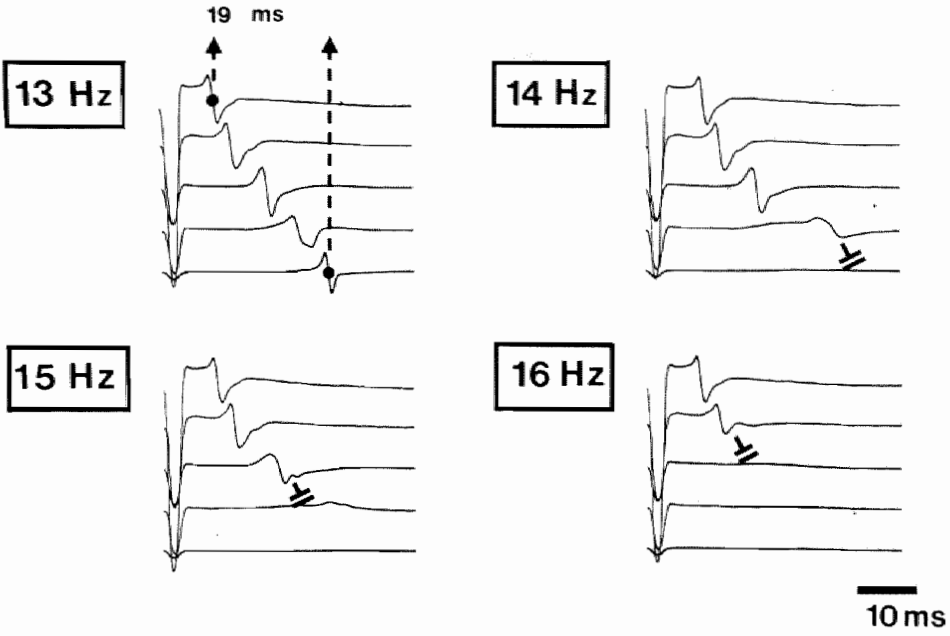

FIGURE 2.8 Intra-atrial conduction block at high pacing rates. Impulse propagation along the bundle of atrial myocardium during constant pacing with 13, 14, 15 and 16 Hz is shown. The data are taken from the same preparation as figure 2.7. At a pacing rate of 13 Hz every impulse is conducted throughout the bundle as shown in the top left panel. If however the pacing rate is increased to 14 Hz conduction block is observed between the fourth and fifth electrode. At pacing rates of 15 and 16 Hz the impulse already dies out closer to the site of stimulation , between the third and fourth electrode and between the second and third electrode respectively.

the bundle. When the pacing rate is increased to as high as 15 and 16 Hz, the conduction was blocked at progressively shorter distances from the site of stimulation. During pacing with 15 Hz the impulse died out between the third and fourth electrode and at 16 Hz conduction block occurred between the second and third recording electrode. The site where conduction is blocked differed from preparation to preparation and was closer to the site of stimulation at increasing pacing rates. This indicates that the localisation of block is determined by functional properties of the myocardium, making an anatomically determined "weak link" within the bundle unlikely.

Intra-atrial conduction block as found during rapid pacing is not observed during propagation of a premature impulse. An early premature beat

will either be conducted all along the bundle or not be conducted at all, not even to the first recording electrode. A possible explanation for the fact that rapid pacing may lead to conduction block whereas a single premature beat does not, is offered in figure 2.9. The A_1-A_2 intervals as measured with the row of recording electrodes are given for the shortest possible premature beat (S_1-S_2 is 62 msec). At this coupling interval the time between the basic and premature response at the different recording electrodes was 64, 66, 69, 72 and 74 msec respectively. It is clear that the A_1-A_2 interval is getting longer as the distance from the site of stimulation increases. In other

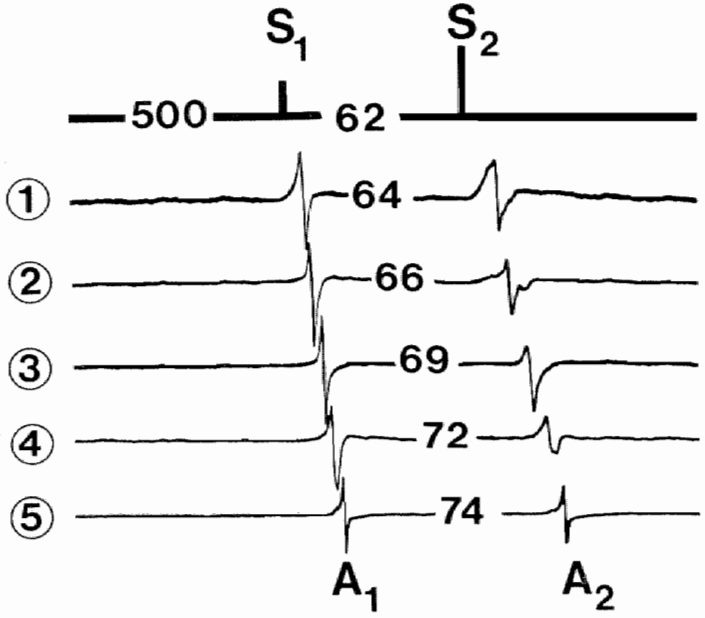

FIGURE 2.9 The prematurity of an extrasystole at increasing distance from the site of stimulation. Conduction of the last basic beat and the earliest possible premature beat in atrial myocardium are shown as recorded with the brush electrode. The top tracing indicates the stimulus protocol, the subsequent tracings represent the electrograms 1 to 5. The shortest possible S1-S2 which provoked a propagated response was 62 msec. At the first recording electrode, near the site of stimulation, the A1-A2 interval is 64 msec. At the second, third, fourth and fifth electrode the A1-A2 interval increased to 66, 69, 72 and 74 msec. It is clear that the prematurity decreases with increasing distance from the site of origin of the premature beat. This increase in the A1-A2 interval is caused by the fact that the premature impulse is conducted slower than the basic impulse.

words, at greater distance the degree of prematurity is less than in the direct vicinity of the site of origin of a premature beat. This decrease in prematurity, caused by the fact that the premature impulse conducts more slowly than the basic impulse, makes it more unlikely that the impulse is being blocked.

On the other hand, during rapid pacing there is no sudden change in conduction velocity. Thus all along the bundle the cycle length is equal to the pacing interval and not dependent on the distance from the site of stimulation. Consequently during maximum pacing, minor differences in local excitability may cause intra-atrial conduction block.

2.7. Measurement of the refractory period.

During programmed electrical stimulation of the heart, refractoriness of the myocardium can be measured with the extra stimulus technique. The actual value of the refractory period at well defined stimulus qualities depends on the criteria used to determine the refractory period. Some widely used definitions of refractoriness of atrial or ventricular myocardium are:

Absolute refractory period (ARP): the longest coupling interval between a basic stimulus and a premature stimulus at which the atrial or ventricular myocardium is not captured.

Effective refractory period (ERP): the shortest coupling interval (S_1-S_2) eliciting a propagated response in the myocardium.

Functional refractory period (FRP): the shortest interval between two induced and propagated responses.

In figure 2.10 the A_1-A_2 interval is plotted as a function of the S_1-S_2 interval as measured with field stimulation in a bundle of left atrium. It is clear that the A_1-A_2 interval is equal to the S_1-S_2 interval at long coupling intervals. However if the coupling interval is progressively shortened the A_1-A_2 interval is shortened to a lesser extent, and at the shortest S_1-S_2 intervals the A_1-A_2 interval even prolongs. This prolongation of the interval between successive responses is caused by an increased latency between

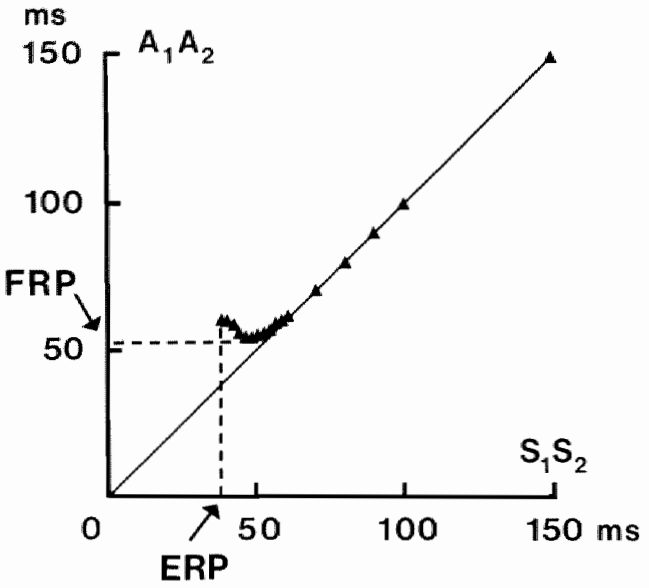

FIGURE 2.10 The relationship between A1-A2 and S1-S2 interval. The A1-A2 interval, measured on the recording electrode near the site of stimulation, is plotted as a function of the coupling interval S1-S2. At coupling intervals longer than 70 msec the S1-S2 and A1-A2 interval are equal. If the S1-S2 is shortened the A1-A2 interval shortens also but to a lesser extent and becomes longer than the coupling interval. The shortest possible S1-S2 interval is the effective refractory period, the shortest attainable A1-A2 interval is the functional refractory period.

stimulation and initiation of a response. This explains why in this case the ERP is shorter than the FRP. To avoid this kind of measuring faults we decided to use the shortest possible interval between two successive responses as criterium for refractoriness (FRP).

Since the distance from the site of stimulation influences the prematurity (see 2.6) we measured the refractory period close to the site of stimulation at the first recording electrode (distance 3 mm). Thus the functional refractory period is defined as the shortest possible A_1-A_2 interval, measured at the first recording electrode at a stimulus strength of 4 times diastolic threshold, which is conducted throughout the bundle of atrial myocardium. The actual measurement was done by selecting a premature stimulus S_2 which did not capture the atrium, followed by increasing the

S_1-S_2 interval in steps of 2 msec until the shortest possible A_1-A_2 interval was measured.

2.8. Measurement of the maximum pacing rate.

Figure 2.11 (top panel) gives the protocol of incremental pacing used to determine the highest possible pacing rate. The top tracing indicates the conduction time between the first and last recording electrode on the atrial strip, the bottom tracing gives the pacing interval. The pacing interval is initially decreased with big steps from 500 to 400 to 300 to 200 to 170 and 150 msec. These changes in pacing interval only caused a minor prolongation in conduction time. At pacing rates above 400 beats per minute (interval less than 150 msec) the cycle length is shortened in steps of 10 msec and above 600 beats per minute (interval less than 100 msec) in steps of 5 msec. At these high pacing rates small changes in pacing interval caused a considerable prolongation in conduction time. The shortest possible pacing interval at which every impulse was still conducted all along the atrial myocardium during at least 400 to 500 consecutive beats is indicated by an arrow. If the pacing interval was further decreased with 5 msec intra-atrial conduction block occurred.

In figure 2.11 (bottom panel) the conduction time is plotted as a function of the pacing interval. Here again it is clear that at pacing *intervals longer than about 150 msec variations in rate hardly affect* conduction velocity. However at pacing intervals shorter than 150 msec a marked prolongation of the conduction time is seen. In this example the conduction time during the highest pacing frequency (Fmax) was more than 50 % longer than during the slow rate of 2Hz.

2.9. Calculation of the length of the excitation wave.

The term wave length in cardiac electrophysiology indicates the distance travelled by the impulse during the time the myocardium needs to restore its excitability. This distance is given by the product of conduction velocity and refractory period. Whether an impulse can be trapped in a circuitous route depends on the length of the reentrant pathway in relation to the wave

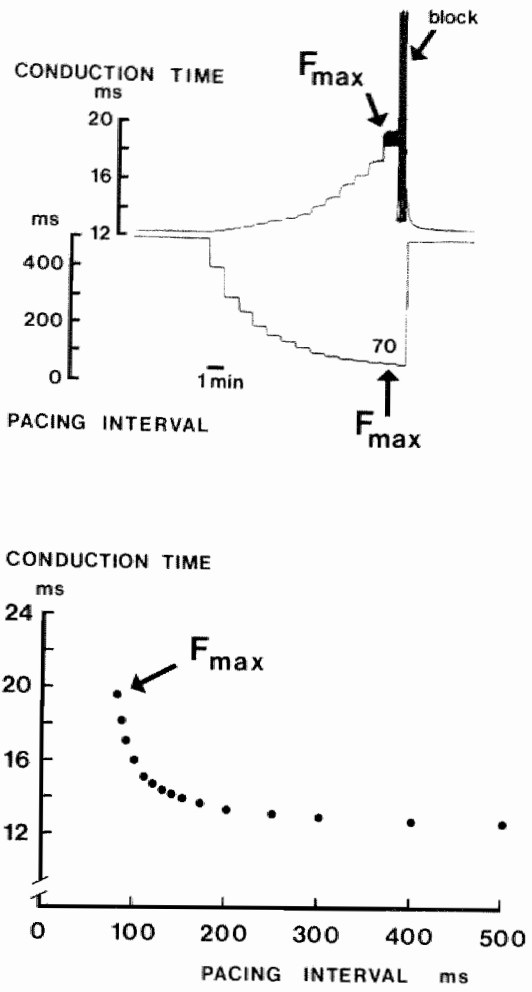

FIGURE 2.11 Measurement of the maximum pacing rate. In the top panel the conduction time (top tracing) is shown at different pacing intervals (bottom tracing). Shortening of the pacing interval from 500 to 150 msec in big steps prolongs the conduction time only slightly. However, at intervals shorter than 150 msec a small decrease of the pacing interval (5 or 10 msec) causes a marked prolongation in the conduction time. The shortest possible pacing interval (Fmax) at which every impulse was conducted all along the atrial strip was in this preparation 70 msec. The conduction time at this pacing interval was about 19 msec. If the pacing interval is shortened further from 70 to 65 msec the conduction is blocked in the bundle. In the bottom panel the conduction time is plotted as a function of the pacing interval. Here again it is shown that the conduction time only increases slightly at pacing interval longer than 150 msec. At pacing intervals shorter than 150 msec a marked increase in conduction time can be observed. The shortest possible pacing interval and conduction time at this rate is indicated by the arrow.

length. A continuous circus movement can only be established if the pathway of the reentrant loop is equal to or larger than the wave length. Thus the wave length is defined as the distance the impulse travels during the time the myocardium restores its excitability to such a level that a second wave can just be propagated. It can be calculated as follows :

WAVE LENGTH (mm)= CONDUCTION VELOCITY (mm/msec) x REFRACTORY PERIOD (msec)

The wave length during a regular rhythm.

An example of the simultaneous determination of conduction velocity and refractory period during regular pacing is shown in figure 2.12. The electrograms numbered 1 and 5 are taken from the first and last electrode on the bundle. The distance between these recording electrodes is 8 mm. The bottom tracing gives the stimulation protocol. The left panel shows the 14th and 15 th basic stimulus and the premature stimulus, while in the right panel the last basic impulse and the premature response are given at a higher sweep speed. In this example the basic pacing interval (S_1-S_1) is 500 msec. The

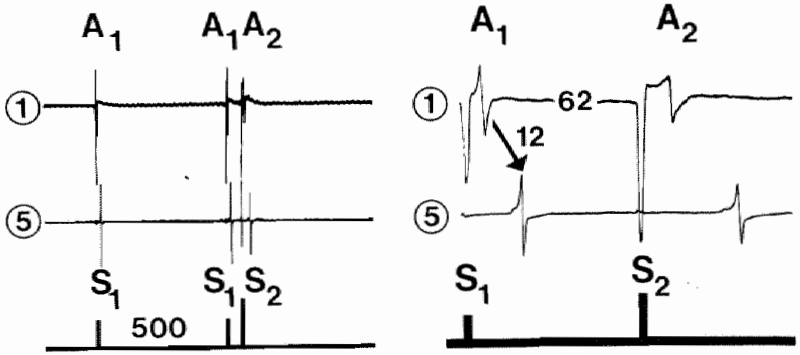

FIGURE 2.12 The calculation of the wave length of a regular impulse. In the left panel the 14th and 15th basic beat and the premature beat are shown at a slow sweep speed. At the right the last basic beat and the premature beat are depicted at a fast sweep speed. The tracings numbered 1 and 5 are from the recording electrodes 1 and 5 of the brush electrode and the bottom tracing gives the stimulation protocol. The conduction time of the basic beat (S1-S1 is 500 msec) is 12 msec over a distance of 8 mm (conduction velocity is 0.67 mm/msec). The shortest possible A1-A2 interval is 62 msec. The wave length, being the product of the conduction velocity and the refractory period is calculated to be 42 mm.

conduction time of this regular impulse as measured between the intrinsic deflection of electrograms 1 and 5 was 12 msec. Since the distance between electrode 1 and 5 was 8 mm the conduction velocity during this slow rhythm was 67 cm/sec (0.67mm/msec). The shortest possible A_1-A_2 interval at this rate was 62 msec. Thus the wave length could be calculated to be 42 mm (0.67 mm/msec x 62 msec).

The wave length of a premature impulse.

The length of the excitation wave of a premature impulse is calculated in an analogous way as the wave length of a regular impulse. An example is shown in figure 2.13. It is taken from the same experiment used for figure 2.12. Again the left panel shows the electrograms 1 and 5 and the stimulation protocol at a slow time base, the right panel at a fast time base. The conduction time of the premature impulse (A_2) was 22 msec over a distance of 8 mm, the conduction velocity being 36 cm/sec (0.36 mm/msec). The refractory period of this premature impulse was measured by giving a third stimulus (S_3). The shortest possible A_2-A_3 interval, indicating the functional refractory period of the A_2 impulse, turned out to be 58 msec. Thus the

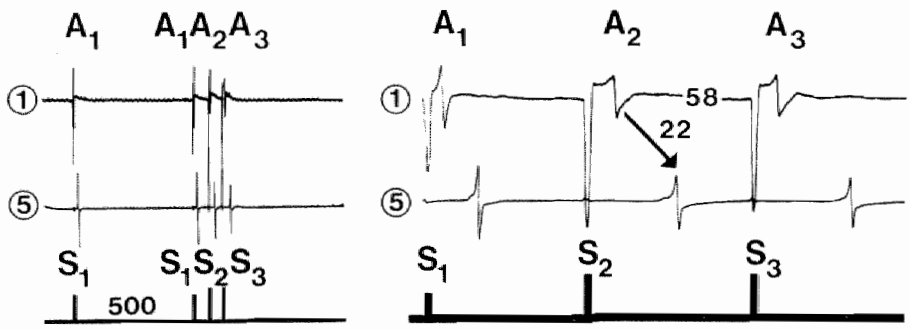

FIGURE 2.13 The calculation of the wave length of a premature beat. At the left side the electrograms 1 and 5 and the stimulation protocol are given at a slow sweep speed. In the right panel the last basic beat and the premature beat are shown at a fast sweep speed. The conduction time of the premature response A2 over 8 mm is in this example 22 msec (conduction velocity is 0.36 mm/msec). To measure the refractory period of the A2 impulse a third impulse has to be evoked as short as possible after A2. In this example the shortest attainable A2-A3 interval was 58 msec. The wave length of this premature impulse is 21 mm.

length of the excitation wave of this premature beat was 21 mm (0.36 mm/msec x 58 msec).

The wave length during maximum pacing.

As illustrated in figure 2.11 the conduction of the excitation wave slows progressively during incremental pacing. Also the time needed for restoration of excitability shortens. Figure 2.14 gives an example of the conduction during the highest pacing rate (cycle length 75 msec) at two different time scales. As shown in the left panel every impulse is conducted all along the bundle. The conduction time between electrodes 1 and 5 (right panel) was 20 msec. Therefore the conduction velocity can be calculated to be 40 cm/sec (0.40 mm/msec). Since the shortest possible interval between two impulses was 75 msec the wave length was calculated to be 30 mm (0.40 mm/msec x 75).

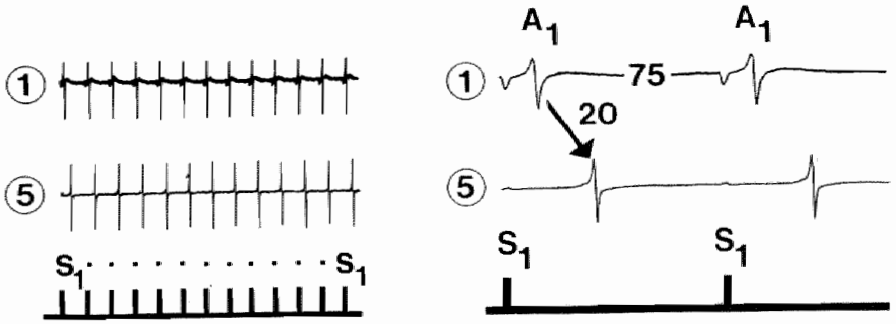

FIGURE 2.14 The calculation of the wave length at the maximum pacing rate. The left panel gives the recordings of electrode 1 and 5 during the shortest possible pacing interval and the stimulation protocol at a slow sweep speed, the right panel shows 2 beats at a fast sweep speed. The conduction time over 8 mm is 20 msec (conduction velocity is 0.4 mm/msec). The shortest possible pacing interval is 75 msec. The wave length, being the product of the conduction velocity and this pacing interval is 30 mm.

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3. INFLUENCE OF RATE AND RHYTHM ON THE LENGTH OF THE EXCITATION WAVE.

It is now widely recognized that reentrant arrhythmias can be initiated and terminated by programmed electrical stimulation. One, two or more premature impulses, if properly timed, can initiate reentrant arrhythmias in the heart. Only premature stimuli within a narrow zone of short coupling intervals will initiate a reentrant rhythm. Besides the timing of a premature beat its site of origin is also a critical factor for the initiation of reentrant arrhythmias (Wellens 1978). Furthermore the success rate for induction of arrhythmias increases if more premature stimuli (2, 3 or 4) are given. Finally also heart rate itself influences the chances for reentry.

If our hypothesis is correct that the wave length is an indicator for the chance on initiation and continuation of reentrant arrhythmias, we must expect that programmed electrical stimulation, leading to the induction of reentrant arrhythmias, should be associated with a shortening of the wave length. To test whether this hypothesis is correct we determined the wave length during changes in rate and rhythm. We measured the wave length of a basic beat at different pacing rates, and the wave length of premature beats at different prematurities and at different pacing rates.

3.1 Experimental protocol.

The effects of changes in rate and rhythm were investigated in 30 left atrial preparations. In all preparations the wave length of the basic impulse and the wave length of the earliest premature impulse was measured during incremental pacing. Additionally in 7 experiments we measured the wave length of premature beats of different prematurities as induced during a fixed pacing rate of 2 Hz. In two studies an extensive stimulation protocol was performed in which both the effect of pacing rate and the degree of prematurity of a single premature beat on the wave length was studied.

Figure 3.1 gives an example of the effects of 4 different pacing rates on refractoriness and conduction of a regularly driven impulse (A_1). The top left panel gives the control situation at a basic pacing interval of 500 msec. Only electrogram 1 and 5 of the multiple recording electrode are displayed. The conduction time of the basic impulse (A_1) at this rate of 2 Hz was 12 msec. After every 15th A_1 impulse a premature impulse A_2 was

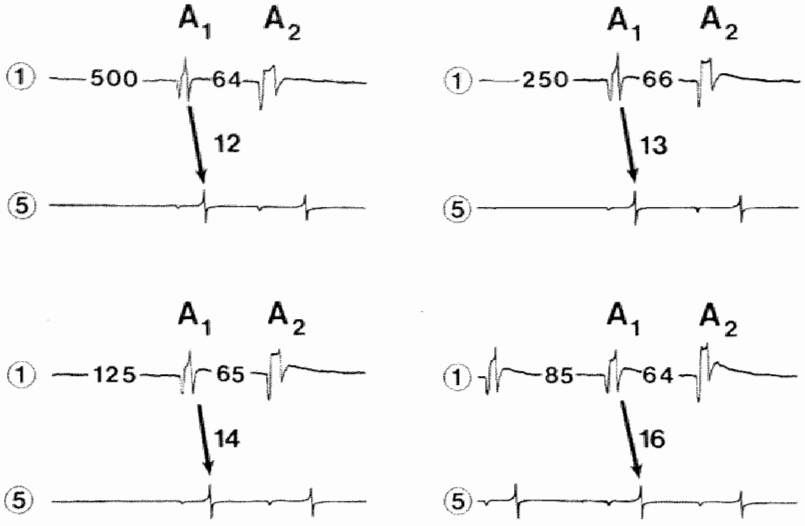

FIGURE 3.1 The effects of incremental pacing on the wave length of a basic impulse. The 4 panels show the effects of shortening of the pacing interval from 500 to 250, 125 and 85 msec on refractoriness and conduction. The distance between the recording electrodes 1 and 5 is 8 mm. The conduction time of the A1 impulse prolongs from 12 to 13, 14 and 16 msec if the pacing interval is shortened. Simultaneously the functional refractory period is almost unchanged (64, 66, 65 and 64 respectively). Calculation of the wave length of the basic impulse revealed a progressive shortening from 43 to 41, 37 and 32 mm.

introduced. The shortest A_1-A_2 interval at this rate was 64 msec and the wave length could be calculated to be 43 mm. A progressive, gradual shortening of the pacing interval to 250, 125 and 85 msec, as depicted in the next three panels, caused a prolongation of the conduction time to 13, 14 and 16 msec. The refractory period changed only slightly. Calculation of the wave length revealed a progressive shortening from 43 to 41, 37 and 32 mm respectively.

Figure 3.2 shows the effects of 4 degrees of prematurity on refractoriness and conduction of a premature beat. The shortest A_1-A_2 impulse was 63 msec (top left panel). The other panels show premature beats with an A_1-A_2 interval of 73, 98, and 120 msec. The conduction time of the earliest premature impulse was 22 msec. Its refractory period was 59 msec (shortest A_2-A_3 interval). Thus the wave length of the earliest possible premature beat was 22 mm. A premature beat induced 10 msec after the functional refractory period (top right panel) showed a conduction time of 17 msec and a refractory

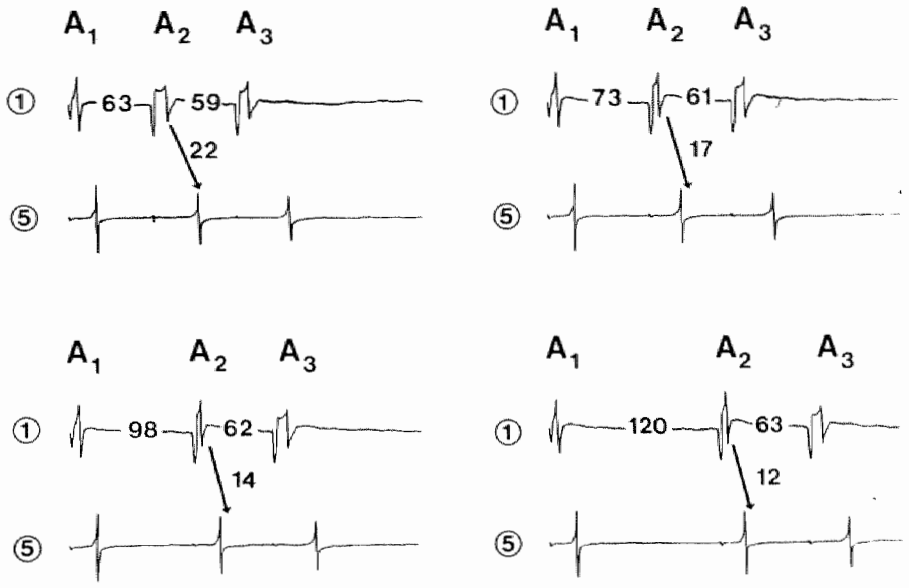

FIGURE 3.2 The effects of the degree of prematurity on the wave length of a premature beat. The effects of 4 different timings of the A2 impulse (63 msec (FRP), 73 msec (FRP + 10 msec), 98 (FRP + 35 msec) and 120 msec (FRP + 57 msec)) on conduction and refractoriness are shown. The distance between the recording electrodes 1 and 5 is 8 mm. The basic interval A1-A1 is fixed at 500 msec. Decreasing the prematurity by prolonging the A1-A2 interval from 63 to 73, 98, 120 msec caused a shortening of the conduction time from 22 to 17, 14, 12 msec respectively. The refractory period (the shortest A2-A3 interval) is prolonged slightly from 59 to 61, 62 and 63 msec. As a consequence the wave length of these premature beats is 23, 29, 35 and 43 mm respectively. These records are taken from the same experiment as was used for figure 3.1.

period of 61 msec (wave length = 29 mm). Further prolongation of the wave length to 35 mm and 42 mm was observed when the A_1-A_2 interval was prolonged to 98 and 120 msec.

3.2 RESULTS.

3.2.1 The influence of rate on the length of the excitation wave.

The effects of incremental pacing on refractoriness, conduction velocity and wave length of a regularly driven impulse are shown in figure 3.3. The mean values of 30 atrial preparations are plotted. The length of the excitation wave is clearly affected at higher pacing rates. Shortening of the

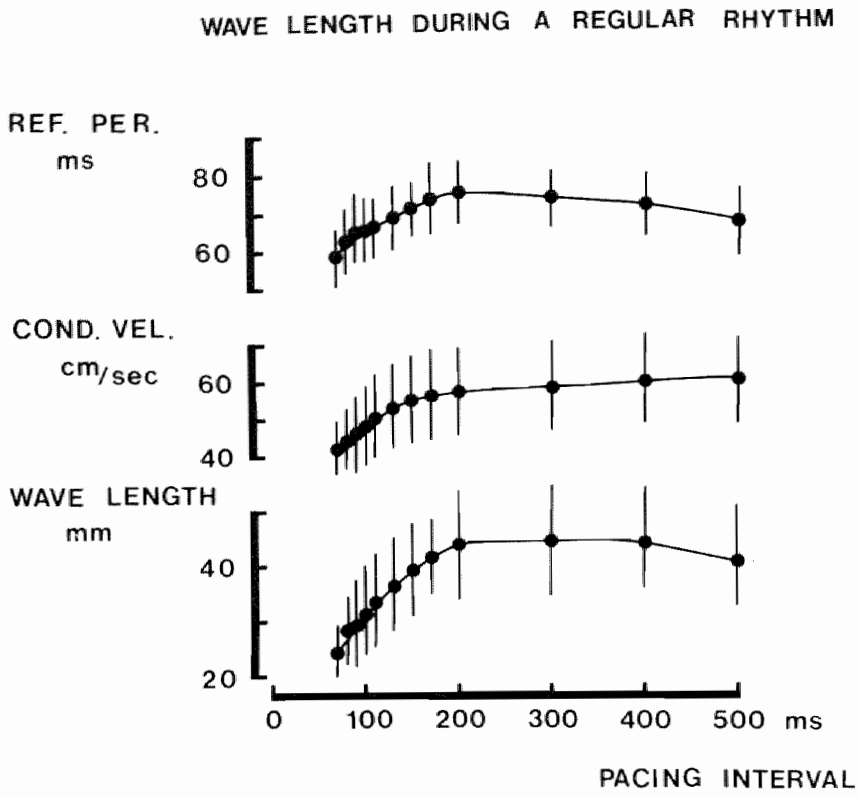

FIGURE 3.3 The effects of incremental pacing on the wave length of a regularly driven impulse. The mean value and standard deviation (n=30) of refractory period, conduction velocity and wave length are plotted as a function of the pacing interval. The wave length of a basic beat is progressively shortened if the pacing interval is shorter than 200 msec. The length of the excitation wave is constant between 200 and 400 msec. The shortening of the wave length is caused by both a shortening of the refractory period and a depression in conduction. The refractory period of the regular impulse shortens at pacing intervals shorter than 200 msec. The conduction velocity is depressed at pacing intervals shorter than 130 msec.

pacing interval from 200 msec to the shortest possible pacing interval (70-90 msec) causes a gradual, progressive shortening of the length of the excitation wave of the basic impulse from 44 to an average of 28 mm. If the pacing interval is varied between 400 and 200 msec the wave length is not changed, whereas at a pacing interval of 500 msec the mean wave length is slightly shorter. The shortening of the wave length at the highest rate with

40 % is caused both by a shortening of the refractory period and by a slowing of the conduction velocity at these high rates. The refractory period of the basic impulse (the shortest possible A_1-A_2 interval) is shortened from 76 msec at 200 msec pacing interval to 63 msec at 80 msec pacing interval. The conduction velocity at 200 msec pacing interval was 54 cm/sec. It was depressed to 42 cm/sec at the highest possible pacing rate.

At relatively slow rates (slower than 5 Hz) the length of the excitation wave was constant. This was true although the values for the refractory period and conduction velocity were not. The refractory period prolonged slightly at pacing intervals from 500 to 200 msec, whereas the speed of propagation was somewhat slowed. Because refractoriness and conduction velocity changed in a different direction their effects on the length of the excitation wave were opposite, resulting in a complete cancellation of their effects on the wave length.

3.2.2 The wave length of a premature impulse.

The effect of the degree of prematurity of an impulse on refractoriness, conduction velocity and wave length during a stable rhythm of 2 Hz (500 msec interval) are shown in figure 3.4. The abscissa gives the timing of the premature impulse relative to the functional refractory period of the basic beat (A_1), starting with the shortest possible A_1-A_2 interval (FRP) at the left, followed by FRP + 5 msec, FRP + 10 msec and so on. Premature beats occurring more than 40 msec after the refractory period were not different from a basic impulse with respect to both conduction velocity and refractoriness. Consequently also the wave length was unchanged.

However if premature beats are given in a small range of 40 msec after the refractory period, marked changes in refractoriness and conduction velocity occurred. The most outspoken changes in electrophysiological properties of these early premature beats are a pronounced slowing of the speed of propagation together with a slight shortening of the refractory period. Compared to a premature beat elicited late in the atrial cycle, the conduction of the earliest premature beat was depressed from 55 to 33 cm/sec, whereas the refractory period was shortened from 68 to 61 msec. Because both a shortening of the refractory period as well as a slowing in conduction exerts their effects on the wave length in the same direction, a marked shortening of the length of the excitation wave was observed. The wave length

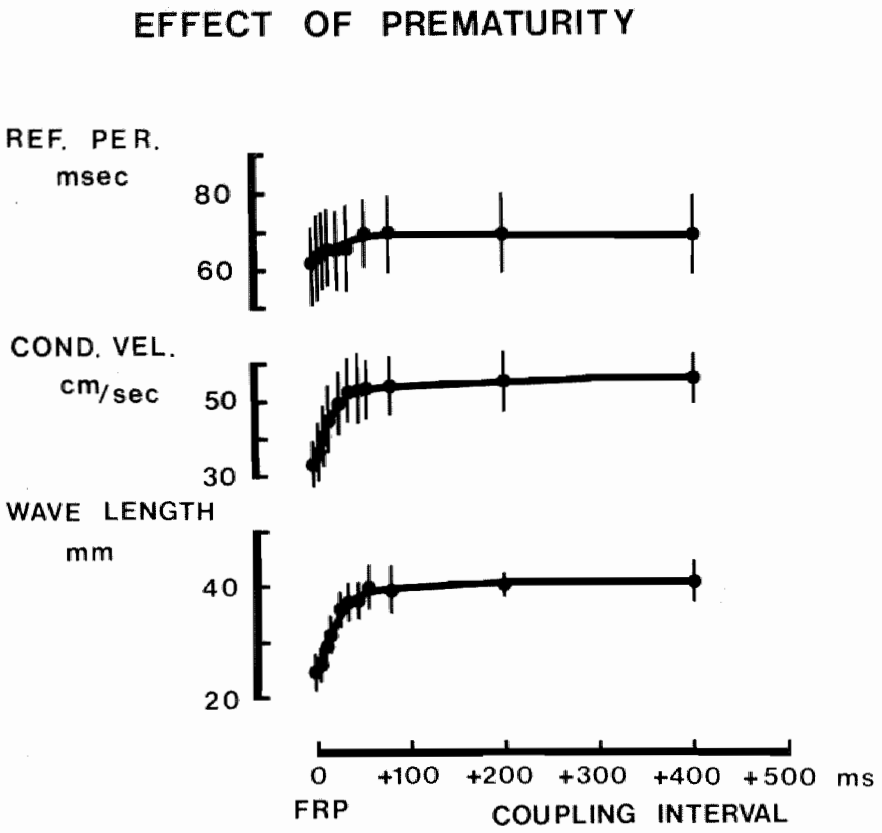

FIGURE 3.4 The effects of the degree of prematurity on the wave length of a premature beat. The mean value and standard deviation (n=7) of refractory period, conduction velocity and wave length are plotted as a function of the prematuriy of the A2 impulse relative to the FRP of the basic beat. The abscissa starts at the shortest possible A1-A2 interval (FRP) followed by FRP +5 msec, FRP+10 msec and so on. The wave length of a premature beat is constant over a wide range of A1-A2 intervals. A marked shortening is observed if a premature beat arises within 30 msec after the shortest possible A1-A2 interval. The wave length is shortest at the shortest A1-A2 interval. This shortening is caused by a marked depression of the conduction and a slight shortening of the refractory period at these early premature beats.

of an extrasystole arising 40 msec after the shortest possible A_1- A_2 interval was 37 mm, whereas the earliest possible premature beat had a wave length of only 23 mm. Thus an abrupt shortening of the length of the excitation wave of a premature beat with 38 % is achieved in a narrow zone of 40 msec of A_1-A_2 intervals. Note that during a regular rhythm a comparable shortening of the wave length was observed if the pacing interval was lowered from 200 to 80 msec, thus over a range of 120 msec.

3.2.3 The influence of rate on the wave length of the earliest premature impulse.

After having studied the effects of rate and the degree of prematurity on the length of the electrical impulse we investigated the effects of incremental pacing on the wave length of the earliest possible premature beat. In figure 3.5 the mean values of refractory period, conduction velocity and wave length (n=30) of the earliest premature impulse are given at a wide range of pacing intervals. The refractory period (the shortest A_2-A_3 interval) is shown in the top panel. Shortening of the pacing interval slightly shortened the refractory period of the earliest premature impulse, reaching a minimum value at a pacing interval of 100 msec. A further shortening of the pacing interval, up to the maximum rate, caused a slight prolongation.

The conduction velocity of the earliest premature impulse (A_2, middle panel) is constant at almost all pacing intervals. Only at intervals shorter than 130 msec the conduction of the earliest premature impulse is somewhat slowed.

As a consequence the wave length of the earliest premature impulse is hardly affected by changes in pacing rate. Only at pacing intervals shorter than 170 msec it is slightly shortened. At the highest pacing rate the length of the shortest impulse was 18-19 mm, compared to 23 mm at a slow pacing rate.

In figure 3.6 the effects of changes in rate and rhythm on the wave length are illustrated. In this experiment an extensive stimulation protocol was done. Not only the wave length during regular pacing (top thick curve) and the wave length of the earliest possible premature beat (bottom thick curve) were measured at different pacing rates, but in addition also the degree of prematurity was varied (thin curves). It is clear that an increase

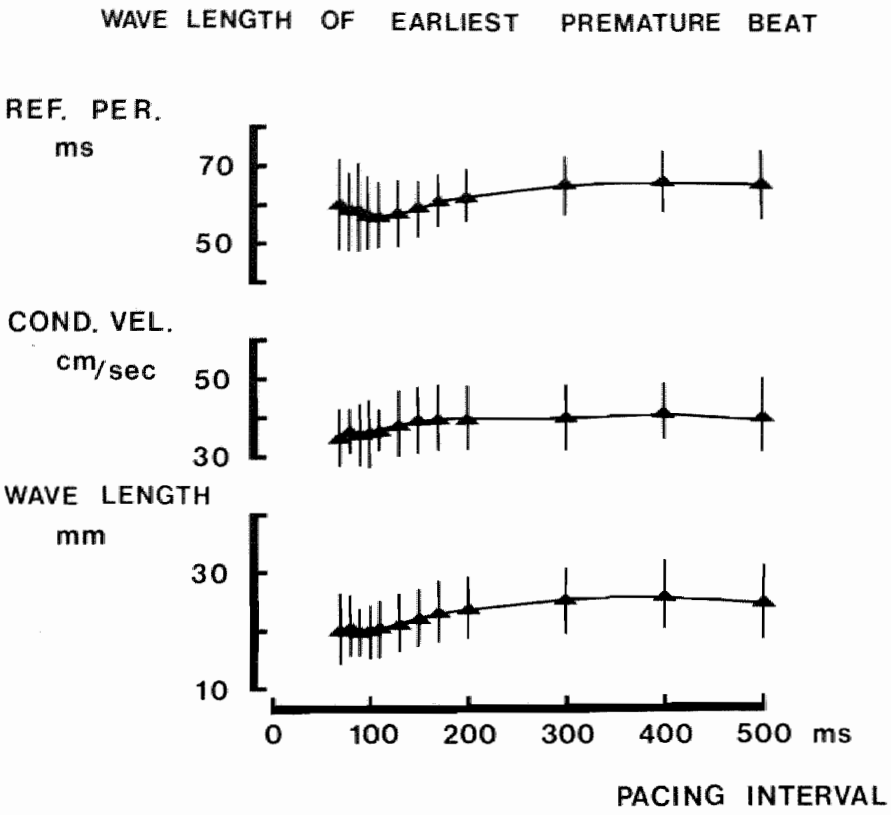

FIGURE 3.5 The effects of incremental pacing on the wave length of the earliest premature beat. The mean value and standard deviation (n=30) of the refractory period, the conduction velocity and the wave length of the earliest premature impulse are shown. The length of the excitation wave of the earliest premature beat shortens slightly at pacing intervals shorter than 170 msec. The shortening can be attributed to a slight shortening of the refractory period and a slight depression of the conduction.

in pacing rate above 5 Hz always resulted in a shortening of the length of the excitation wave. This is true for regular pacing, the earliest premature beat and also for premature beats coming later in the cycle. At all pacing rates the length of the earliest premature impulse was about half the wave length during the underlying regular rhythm. The wave length of a premature beat prolongs steadily at decreasing prematurity. Premature beats coming 20 msec later than the earliest premature beat have a wave length which is almost similar to the wave length of a basic impulse. In other words only very early premature beats have a shortened wave length.

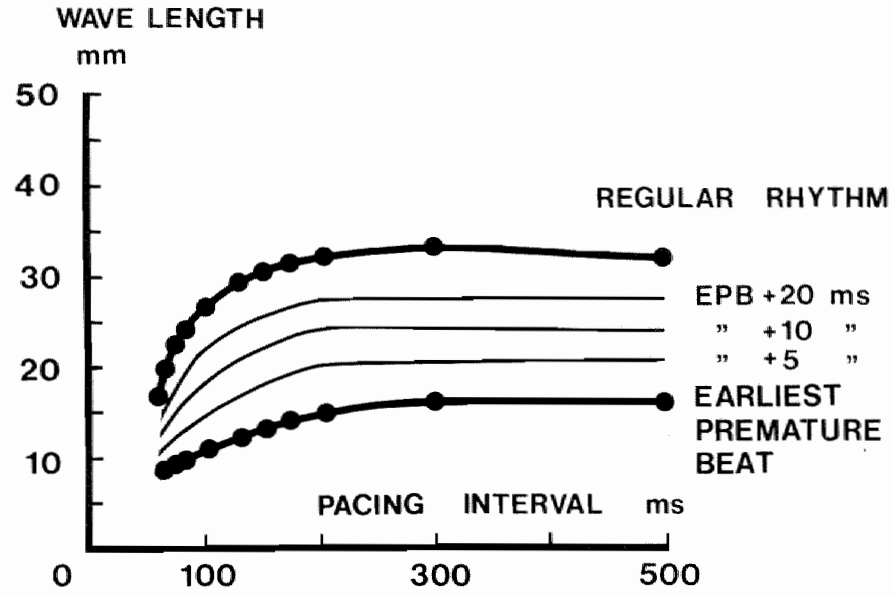

FIGURE 3.6 Effects of rate and rhythm on the length of the excitation wave. The wave length of a regular impulse (top thick curve), of the earliest premature beat (lower thick curve) and of a premature beat coming 5, 10, 20 msec later than the earliest premature beat are plotted as a function of the pacing interval. It is clear that the wave length of a regular impulse and of the earliest premature beat shorten markedly at pacing cycle length's of less than 200 msec. Also premature beats elicited later in the cycle have a shortened wave length at increasing pacing rates. Note that the wave length of the earliest premature beat is almost half the wave length of a basic impulse.

3.3 DISCUSSION

Effects of changes in rate on refractory period and conduction velocity.

An increase in heart rate causes a shortening of the refractory period both in the atrium (Hoffman and Cranefield 1960) as well as in the ventricle (Janse 1971). However, the amount of shortening differs from tissue to tissue, and from species to species (Hoffman and Cranefield 1960). In our experiments an increase in pacing rate in the rabbit atrium initially results in a slight prolongation of the refractory period, followed by a marked shortening at higher pacing rates. The slight prolongation of the refractory period at relatively slow rates, as found in our studies, is probably

specific for the rabbit atrium. This phenomenon was no longer observed after the induction of a single premature beat.

The conduction was depressed at increasing pacing rates. Here again the slowing is initially moderate, but at pacing intervals shorter than 200 msec the speed of propagation is depressed progressively until conduction block occurs. The conduction velocity at the highest pacing rate was about 60% of the value at 2 Hz.

Relation to reentrant arrhythmias.

The role of slow conduction for the occurrence of reentry has been stressed by many authors (Schmitt and Erlanger 1928, Hoffman and Cranefield 1960, Wit et al. 1972a, Wit et al. 1972b, Cranefield 1975). Another condition which can facilitate the occurrence of reentry is a short refractory period (Moe and Abildskov 1959, Hoffman and Cranefield 1960). The interplay of both the speed of propagation and the rate of recovery of excitability is expressed in the length of the excitation wave. Initiation and continuation of a reentrant circuit can be considered to be related to the length of the excitation wave. Normally the length of the excitation wave is relatively long, preventing reentrant excitation within the myocardium. However if the wave length of the impulse is short the chances for initiation of reentry become higher.

Measurement of the wave length of premature impulses showed that early premature impulses have a markedly shortened length of their excitation wave. However the zone of coupling intervals in which the wave length is shortened was rather narrow. The shortest premature beat had an average wave length of 23 mm. The wave length of a premature impulse coming 10 msec later was 28 mm long, whereas premature impulses induced 25 and 45 msec after the refractory period had a length of 33 and 34 mm. It was shown by Allessie et al.(1973) that initiation of a reentrant circuit in the isolated left atrium of the rabbit was only possible with premature stimuli given within about 10 msec after the refractory period. There is a striking correlation between this narrow echo-zone and the range of prematurity in which the excitation wave was shortened. If one accepts the hypothesis that the dimension of reentrant circuits in the myocardium is equal to the length of the circulating excitation wave, the size of the first reentrant circuit must be between 20 and 30 mm.

An alternative way to induce reentrant arrhythmias is rapid pacing. We found that the wave length during regular rhythm is shortened at pacing rates higher than 5 Hz. At the highest pacing rate the length of the impulse was shortened to 28 mm. Here again there is a good correlation between the inducibility of reentrant arrhythmias and the shortening of the wave length. It may be noted that the shortening of the wave length due to incremental pacing is more gradual and takes place over a larger range of pacing intervals than the shortening of the wave length of a premature beat. This indicates that the coupling interval of a premature beat is more critical than the cycle length of rapid pacing.

Allessie et al. (1973) observed that a reentrant tachycardia initiated with a single premature stimulus gradually increased in cycle length during the first 100 revolutions before a steady state was reached. However, very often the tachycardia stopped spontaneously during this initial phase. Our studies on the influence of heart rate on the length of the impulse offers a possible explanation for this phenomenon. The length of the excitation wave of the earliest premature impulse, equal to the size of the first reentrant loop, is smaller (23 mm) than the wave length at the highest pacing rate (28 mm). One can imagine that the size of the reentrant circuits, gradually increases after initiation of the tachycardia. This enlargement of the reentrant loop may be of significance for termination of the arrhythmia.

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4. THE EFFECTS OF TEMPERATURE ON THE LENGTH OF THE EXCITATION WAVE.

It is generally agreed that hypothermia increases the chance on fibrillation. When patients are cooled during open heart surgery ventricular fibrillation frequently develops. Also experimental animal studies report an increased incidence of fibrillation during hypothermia (Covino and D'Amato 1962).

The electrophysiological changes which at low temperatures make the heart more susceptible to fibrillation are not completely understood. An increased dispersion in refractoriness (Han and Moe 1964), as well as slowed conduction (Covino and D'Amato 1962, Yamagishi and Sano 1967) may favor the occurrence of reentrant arrhythmias. But on the other hand the prolongation of the refractory period (Angelakos et al. 1957, Covino and D'Amato 1962) may diminish the chance of fibrillation.

It has been proposed that fibrillation is based on the existence of multiple reentrant circuits within the myocardium (Moe and Abildskov 1959, Allessie et al. 1982). Furthermore Allessie et al. (1977) introduced the hypothesis that the size of such intra-myocardial circuit has to be equal to the wave length of the circulating impulse. A possible explanation for the increased risk of fibrillation at lower temperatures is that, as a result of depressed conduction, the wave length of the impulse shortens. To test this hypothesis we measured the length of the excitation wave during rapid pacing at temperatures from 38 to 21 ^{o}C.

To correlate changes in wave length directly with changes in size of a reentrant circuit we mapped the activation pattern of a circusmovement tachycardia in the left atrium during a decrease in temperature.

4.1 EXPERIMENTAL PROTOCOL.

The effects of changes in temperature of the Tyrode solution were studied in 13 left atrial preparations. The temperature was varied in steps of 2 ^{o}C between 38 and 26 ^{o}C in all preparations and in 2 experiments the temperature was further lowered to 21 ^{o}C. The temperature changes were achieved by switching from one heating system (Tamson TC 9) to another which was prewarmed cq. precooled to the desired temperature. By switching forth and back between the two separate heating systems any temperature step could be selected. The preparation was allowed to accomodate to the new temperature

for at least 5 minutes before the measurements were made. Besides the conduction velocity at 2 Hz, the conduction velocity and the wave length at the shortest possible pacing interval were measured. The potassium concentration in the Tyrode solution was 5.6 mmol.

In additional experiments we studied the effects of changes in temperature on intra-atrial reentry. We isolated pieces of atrial myocardium consisting of the roof of the left atrium and left atrial appendage. After addition of carbamylcholine (4×10^{-7} g/ml), long lasting periods of atrial flutter can be induced in these pieces of atrium (20x20 mm). The sequence of excitation during this arrhythmia was mapped by simultaneously recording of 192 electrograms. The recording equipment is described in detail by Allessie et al. (1982) and Wit et al. (1982). In the present study we used a multiple recording device, in which the recording electrodes (teflon coated silver wire, diameter 0.3 mm) were arranged in a regular array (14x14 electrodes (4 electrodes were not connected), interelectrode distance 1.4 mm). With this mapping technique it was possible to visualize the reentrant circuit responsible for the atrial flutter, and to study directly the effects of changes in temperature on cycle length and size of these intra-atrial circuits.

4.2 RESULTS.

The effects of temperature on conduction velocity, maximum pacing rate and wave length of all experiments are given in table 4.I and figure 4.1. It is clear that changes in temperature have pronounced effects on both conduction velocity and maximum pacing rate. A minor decrease in temperature of the superfusing solution from 37 to 35 ^{o}C already caused a statistically significant ($P<0.05$) slowing of the conduction velocity both at 2 Hz and at the highest pacing rate (table 4.I). The shortest possible pacing interval is even more sensitive for changes in temperature, significant changes occurring already at temperature steps of 1 ^{o}C. At a temperature range from 37 to 27 ^{o}C the slowing in conduction and the prolongation of the minimum pacing interval were almost linear with changes in temperature (figure 4.1). The shortest possible pacing interval was doubled from 86 msec at 37 ^{o}C to 167 msec at 27 ^{o}C. The conduction velocity at the highest possible pacing rate was also markedly depressed (21 cm/sec at 27 ^{o}C versus 34 cm/sec at 37 ^{o}C). Cooling

TABLE 4.1

TEMPERATURE	CONDUCTION VELOCITY 2Hz	INTERVAL FMAX	CONDUCTION VELOCITY FMAX	WAVE LENGTH FMAX	n
^{o}C	cm/sec	ms	cm/sec	mm	
37	62.1	85.9	34.5	29.5	13
	7.0	9.5	4.5	4.4	
36	58.7	97.5*	31.8	31.3	6
	5.3	7.6	4.3	5.4	
35	58.6*	96.9*	30.0*	28.7	8
	6.9	12.8	5.1	3.1	
34	55.6**	101.8***	30.7**	31.4	10
	6.0	11.4	4.4	4.7	
33	55.9**	107.2***	30.4*	32.6	8
	5.5	13.7	4.5	5.0	
32	49.4**	113.9***	27.6**	31.3	8
	5.2	9.9	3.0	4.6	
31	50.6***	127.1***	26.7**	33.8	6
	7.1	24.3	3.8	7.8	
30	48.4***	137.6***	23.8***	34.1*	9
	5.5	10.8	6.6	4.1	
29	46.4***	141.9***	24.8***	34.5*	7
	5.7	18.7	4.2	3.8	
28	44.4***	151.9***	22.0***	33.1*	8
	6.8	20.2	4.0	6.2	
27	42.9***	167.1***	21.1***	35.0*	6
	4.9	18.2	2.7	2.8	
26	38.3***	186.9***	18.4***	33.9*	7
	5.3	16.5	2.3	3.0	
25	37.7	195.0	17.3	32.0	3
	9.3	59.0	5.0	0.9	
22	30.0	310.0	13.0	41.4	2
	1.4	14.1	--	1.8	
21	28.0	315.0	13.5	41.9	2
	1.4	17.7	2.1	5.8	

*=$p<0.05$
**=$p<0.01$
***=$p<0.001$

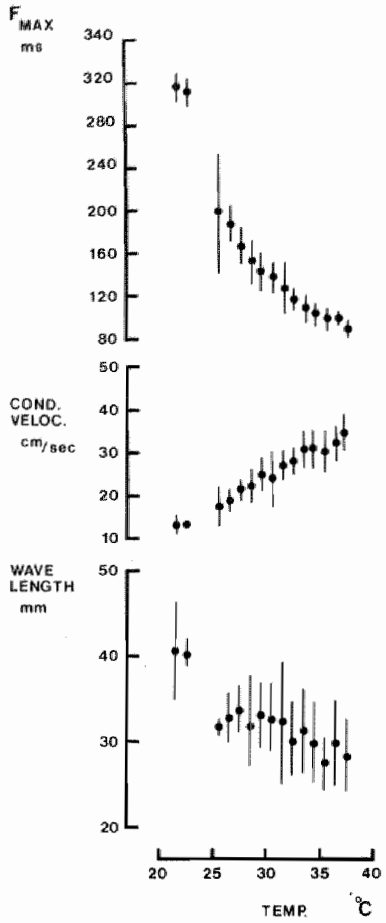

FIGURE 4.1 The effects of temperature on the shortest possible pacing interval (top panel), the conduction velocity at this rate (middle panel) and the wave length (bottom panel). Lowering of the temperature from 37 to 27 caused a marked prolongation of the shortest possible pacing interval from 85 to 170 msec. Concomitantly the conduction velocity was slowed from 34 to 21 cm/sec. The length of the excitation wave prolonged from 30 to 35 mm. If the temperature was lowered from 27 to 21 °C the prolongation of the minimum pacing interval was larger than the depression in conduction. As a consequence the wave length was markedly prolonged.

the heart from 37 to 27 °C caused a prolongation of the length of the excitation wave from 30 to 35 mm. If the temperature was further decreased below 27 °C the minimum pacing interval increased steeply. Since this prolongation was more pronounced than the concomitant depression in conduction velocity, below 27 °C the wave length prolonged markedly. Cooling from 27 to 22 °C prolonged the wave length from 35 to 41 mm.

In figure 4.2 the wave length at the highest pacing rate is plotted as a function of the highest pacing rate at different temperatures. It is clear that lowering the temperature from 37 to 32, 28, 26, and 22 °C causes a marked and progressive prolongation of the shortest possible pacing interval.

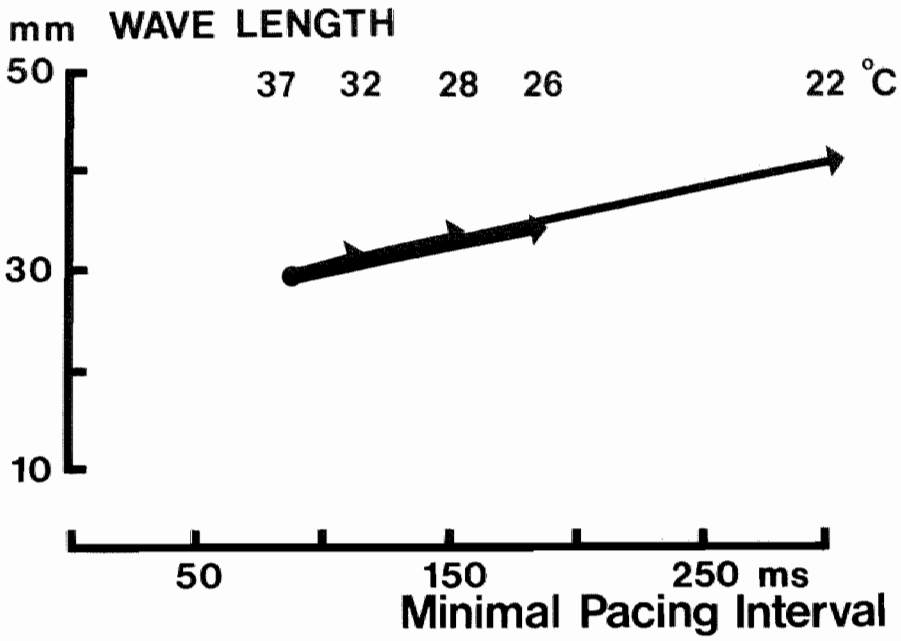

FIGURE 4.2. The changes in wave length in relation to changes in Fmax. The wave length at various temperatures is plotted as a function of the shortest possible pacing interval. If the temperature is lowered to 32, 28, 26 and 22 °C the shortest pacing interval prolongs progressively, reaching a threefold increase at 22 °C. However the wave length is only prolonged with about 30 %.

Simultaneously the wave length at the highest pacing rate is moderately prolonged, as indicated by the slope of the arrows.

The effects of temperature on a reentrant circuit.

In 3 experiments we initiated long lasting reentrant arrhythmias in isolated pieces of rabbit atrium under the influence of carbachol. After the fast reentrant rhythm has stabilized for 500 to 600 beats the temperature of the perfusion solution was quickly lowered, while the cycle length of the arrhythmia was monitored. An example of such an experiment is shown in figure 4.3. Temperature changes are given by the dashed line (left ordinate), whereas the solid line shows the changes in cycle length of the tachycardia (right ordinate). In this example at 37 °C the cycle length was about 100 msec. A lowering in temperature from 37 to 29 °C was accomplished in about 4 minutes. During this gradual decrease in temperature the cycle length of the

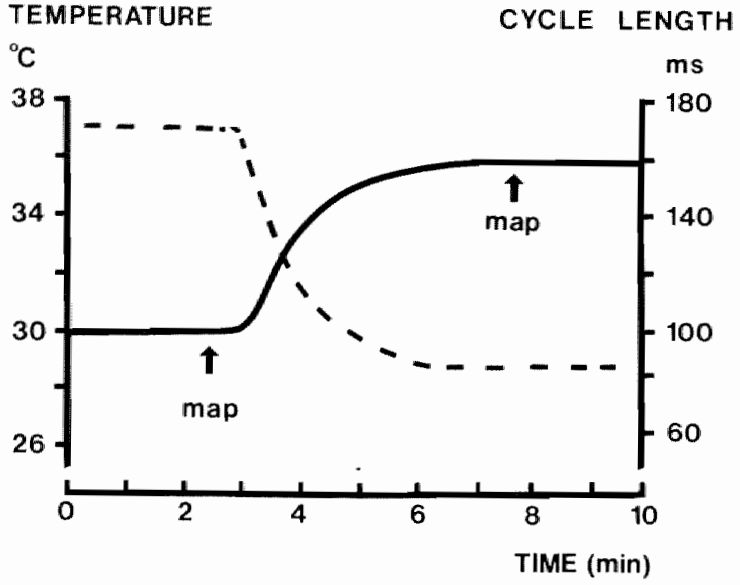

FIGURE 4.3. The effects of cooling on the cycle length of a reentrant arrhythmia. The changes in cycle length of an atrial flutter are shown during a lowering of the temperature from 37 to 29 °C. The cycle length (right ordinate) gradually prolongs from 100 msec to 160 msec if the temperature is lowered from 37 to 29 °C (left ordinate). Although the flutter rate slowed considerably, the arrhythmia continued. Activation maps shown in figure 4.4 were made at the moments indicated by the arrows.

atrial flutter increased gradually. At 29 °C the cycle length had increased from 100 to 160 msec. Although the rate of the reentrant rhythm slowed markedly, it was regular all the time and did not terminate spontaneously. During this period of cooling simultaneously recording of 192 electrograms was performed continuously. The activation sequence at 37 and 29 °C were reconstructed from these recordings and are shown in figure 4.4. At the left the activation map and a schematic representation of the reentrant circuit are shown at 37 °C, whereas at the right the map recorded at 29 °C is given. All activation times are grouped in isochrones of 10 msec. It could be shown that at 37 °C the impulse circulated around in a clockwise direction in about 100 msec. This revolution time was exactly equal to the cycle length of the arrhythmia. The hatched part in the map indicates the area of functional

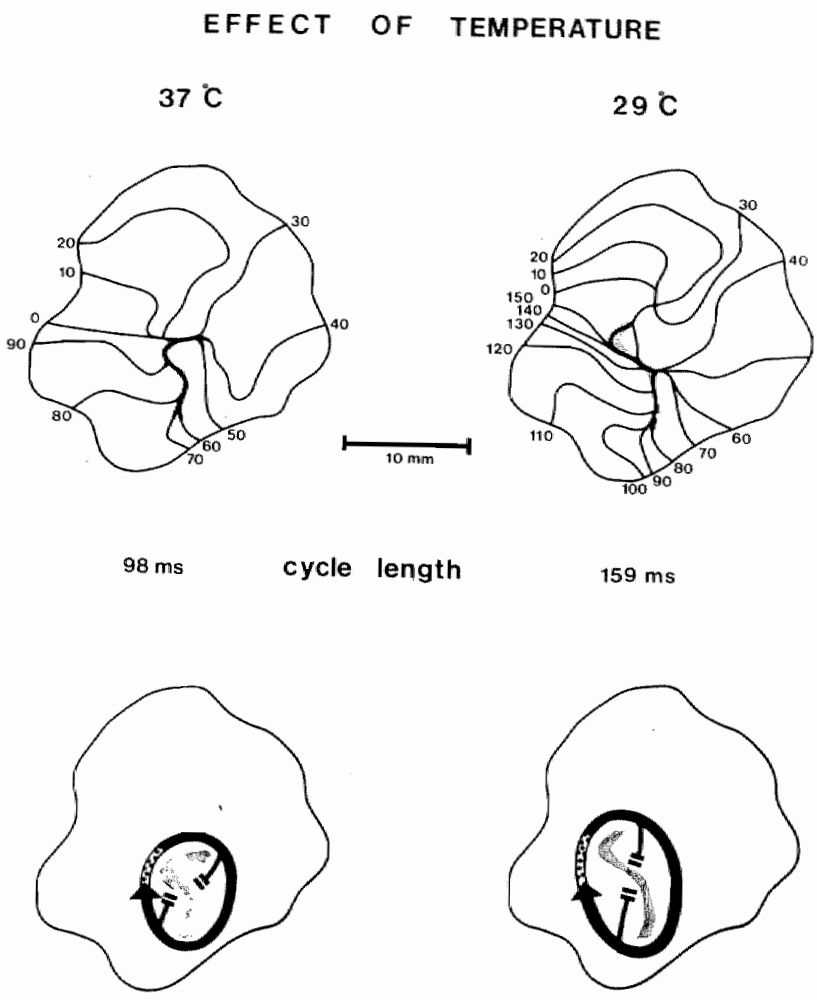

FIGURE 4.4 The effects of temperature on intra-atrial reentry. Activation maps are shown at 37 and 29 °C. The bottom panels give a schematic representation of the circuits. All activation times are grouped in isochrones of 10 msec. At 37 °C the revolution time of the circuit was 98 msec. Cooling to 29 °C caused a change in the the functionally determined circuit in two aspects: the cycle length slowed to 159 msec and the size of the reentrant circuit increased slightly (bottom panel).

conduction block around which the activation wave revolved. The bottom panel gives a schematic representation of the dimensions of this functionally determined circuit. Lowering the temperature to 29 ^{o}C caused a prolongation of the revolution time to 160 msec. The impulse was still circulating in a clockwise direction, although much slower than it did at 37 ^{o}C. It can be clearly seen that the number of isochrones has increased and that the isochrones are closer together, indicating that the speed of propagation of the activation wave was slowed uniformally along the circuitous pathway. Furthermore it must be noted that as a result of cooling the central area of functional block has been somewhat enlarged. As a consequence the size of the reentrant circuit is increased (bottom panel). Despite this increase in the size of the reentrant circuit the impulse continued to revolve regularly and did not stop. However when the temperature was further lowered the flutter was suddenly interrupted at a temperature of 27 ^{o}C.

4.3 DISCUSSION

The effects of temperature on the wave length.

Cooling of the heart causes a pronounced slowing in conduction velocity of the impulse (Covino and D'Amato 1962, Yamagishi and Sano 1967) and a marked prolongation of refractoriness (Angelakos et al. 1957, Covino and D'Amato 1962). In the isolated rabbit atrium we observed a slowing in conduction of 31 % during slow pacing and of 39 % during maximal pacing when the heart was cooled from 37 to 27 ^{o}C. Simultaneously the shortest possible pacing interval was prolonged in excess of 90 %. Because the effect on the minimal pacing interval was greater than the slowing in conduction, the wave length prolonged in this temperature range although this was not a very marked change, namely 19 %. Progressive cooling below 27 ^{o}C caused a strong prolongation of the wave length because at these low temperatures the shortest possible pacing interval is influenced strongly.

Temperature and reentrant arrhythmias.

The observation that the refractory period prolongs markedly if the temperature of the heart is decreased was confirmed in these experiments. Lowering the temperature from 37 to 27 ^{o}C resulted in a prolongation of the shortest possible pacing interval with almost 100 %. Whether this prolongation in refractoriness affects an intra-atrial reentrant arrhythmia

was investigated by mapping a fast flutter in an isolated rabbit atrium while the temperature was lowered. It was observed that the cycle length of this fast intra-atrial reentry prolonged markedly. Allessie et al. (1977) suggested that the cycle length of such a reentrant arrhythmia was proportional to the refractory period. The analogy between the observed decrease in flutter rate and the slowing in the maximal pacing rate support this concept.

Cooling the atrium during a reentrant rhythm not only resulted in a marked slowing of the rate of the tachycardia but also in a slight increase in the size of the reentrant circuit. At very low temperatures (below 25 ^{o}C) the reentrant rhythm suddenly stopped. Measurement of the wave length in isolated strips of atrial myocardium showed identical results. Moderate lowering of the temperature resulted in a moderate increase in the length of the excitation wave, whereas a marked prolongation was observed at temperatures below 27 ^{o}C. Thus the effects of changes in temperature on the shortest possible pacing interval and the wave length are quite similar to the changes in rate and size of a functionally determined intra-atrial circuit.

The observed prolongation in wave length and the resulting increase in size of a reentrant circuit do <u>not</u> fit with the increased incidence of ventricular fibrillation during hypothermia. On the contrary, these results indicate that induction of fibrillation should be more difficult. A possible explanation might be that the ventricular response to hypothermia is different from the atrial response. However the existence of such a difference is unlikely. So if we assume that the wave length in ventricular muscle is prolonged during hypothermia, we must expect that hypothermia impedes the induction of reentrant arrhythmias. The observation during intra-operative mapping studies that the induction of ventricular arrhythmias is more difficult when the thorax is opened and the heart is cooled, fits with this assumption. The increased incidence of ventricular fibrillation therefore cannot be explained by the changes in the wave length; other factors must play a role in the induction of fibrillation during hypothermia. Most probably increased spatial dispersion of refractoriness (Han and Moe 1964) and increased inhomogeneity in conduction are involved. It may also be that increased adrenergic activity induced by hypothermia (Nielsen and Owman 1968) is of importance.

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5.THE EFFECTS OF NEUROTRANSMITTORS ON THE LENGTH OF THE EXCITATION WAVE.

The autonomic nervous system plays an important role in the genesis of cardiac arrhythmias. Both increase in parasympathetic as well as sympathetic activity are associated with a higher incidence of rhythm disturbances. An increased sympathetic activity is reported to favor the occurrence of ventricular arrhythmias (Lown and Verrier 1976), whereas a high parasympathetic tone increases the risk of atrial flutter and fibrillation (Coumel et al. 1978).

It is already an old observation (Rothberger and Winterberg 1910) that atrial fibrillation can easily be provoked during vagal stimulation. In 1921 Lewis et al. showed that atrial flutter can degenerate into fibrillation if the vagal nerve is stimulated. If we consider fibrillation to be a state in which multiple reentrant circuits are simultaneousy present in the myocardium (Moe and Abildskov 1959, Allessie et al. 1982), the chance for initiation and continuation of fibrillation will depend on the number of wavelets which can exist. Many circuits can be present simultaneously if either the heart is large or the circuits are small. A possible explanation for the occurrence of fibrillation during vagal stimulation is that the size of the reentrant circuits is diminished due to the action of acetylcholine. An experimental proof of this hypothesis was given by Allessie and coworkers (1977), who showed that the size of a reentrant circuit, in an isolated left atrium of the rabbit, gets smaller if exposed to acetylcholine. This means that stimulation of the vagus may increase the number of wavelets which can be present in the heart.

In this chapter we will describe the effects of neurotransmittors on the length of the excitation wave both during regular pacing and the application of premature stimuli. We found that carbamylcholine strongly shortens the length of the depolarization wave in the atrium. In our opinion this gives a satisfactory explanation for the arrhythmogenic action of acetylcholine on the atrium. The effects of epinephrine on the wave length turned out to be strongly dependent on the pacing rate.

5.1 MATERIALS AND EXPERIMENTAL PROTOCOL

The effects of neurotransmittors were investigated in 13 left atrial preparations. We used carbamylcholine (Carbacholum, ACF Chemiefarma) as

parasympathicomimetic agent. This derivative of acetylcholine exerts identical electrophysiological effects as acetylcholine, but is not inactivated by cholinesterase. This guarantees that the carbamylcholine concentration is constant at the myocardial receptor throughout the experiment. Epinephrine was used as a sympathicomimetic agent.

The drugs were administered to the superfusing solution either by means of a continuous infusion to the tissue bath or simply by adding the desired dosage directly to the 10 liter reservoir.

The protocol of administration of neurotransmittors was as follows: control period (1 hour), epinephrine ($6x10^{-7}$ M, 1 hour), washout (1 hour), carbamylcholine ($4x10^{-7}$ g/ml, 1 hour), washout (1 hour).

At these concentration of the drugs clear electrophysiological changes can be expected. Epinephrine in this dosage shortens the intrinsic sinus node cycle length with about 100 msec (Bonke et al. 1982), whereas at this concentration of carbamylcholine generally the sinus node activity ceases.

During each of these protocol steps the wave length of the regularly driven impulse and the earliest premature impulse were measured at various pacing rates.

5.2 RESULTS

5.2.1 The effects of carbamylcholine

An example of the effects of carbamylcholine on the wave length during maximal pacing is shown in figure 5.1. The shortest possible pacing interval, the conduction velocity and the wave length during the highest pacing rate are plotted before, during and after administration of carbamylcholine ($4x10^{-7}$ g/ml). After a control period of 1 hour during which the measured parameters were constant, carbamylcholine was added to the Tyrode solution in a final concentration of 4×10^{-7} g/ml. The shortest possible pacing interval, at which every impulse was still conducted, shortened markedly due to carbamylcholine (from 80 to 55 msec). Despite this increase in maximum pacing rate, the conduction velocity at this high rate during carbamylcholine administration was equal to the control value. As a consequence the length of the excitation wave during maximal pacing shortened from 27 mm to 17 mm. After termination of infusion of carbamylcholine, all parameters returned to their control values.

In figure 5.2 the effects of carbamylcholine on the wave length of a regular rhythm (top panel) are compared with the wave length of the earliest premature impulse (bottom panel). The effects of pacing rate on the wave length are similar as described in chapter 3. At relatively slow pacing rates the wave length is almost constant, but a marked shortening occurs at pacing rates faster than 5 Hz. Administration of carbamylcholine to the solution

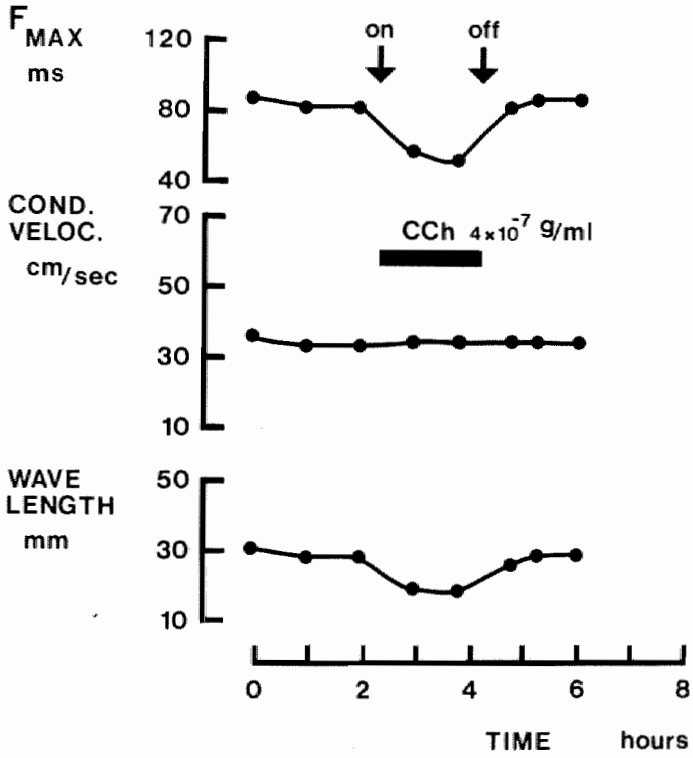

FIGURE 5.1 The effects of carbamylcholine on the wave length during maximal pacing. The shortest possible pacing interval (top panel), the conduction velocity (middle panel) and the wave length (bottom panel) at the highest pacing rate are shown. After a control period of 1 hour, during which all variables were constant, carbamylcholine was added to the perfusion solution in a final concentration of 4 x 10^{-7} g/ml. The administration period is indicated by the arrows. Carbamylcholine shortened the minimal pacing interval from 80 msec to 55 msec, whereas the conduction velocity at this rate was not affected. As a consequence the wave length shortened from 27 to 17 mm. All the variables returned to their control value after termination of the administration indicating a complete wash out of the drug.

(filled squares) shifted this curve downwards and to the left. At all pacing intervals the wave length was shorter than during control. At a pacing interval of 500 msec administration of carbachol shortened the wave length from about 50 mm to about 20 mm. However this effect was less outspoken at higher rates. At a pacing interval of 80 msec the wave length was shortened

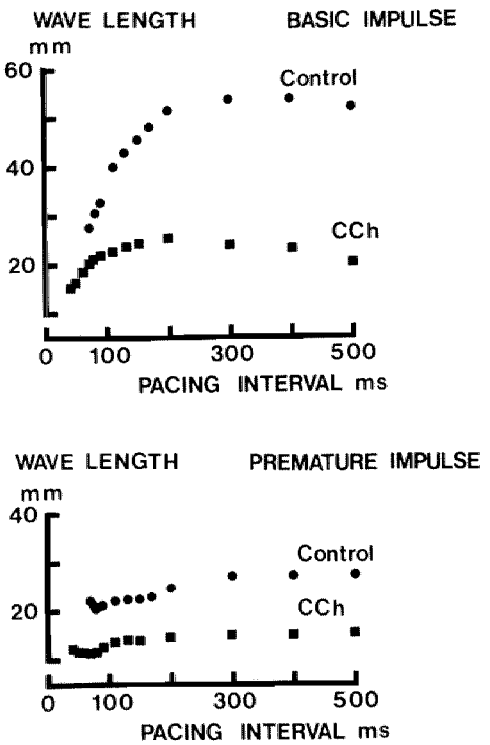

FIGURE 5.2 The effect of carbamylcholine on the wave length of a basic and a premature impulse during incremental pacing. The wave length of a regularly driven impulse as a function of the pacing interval during control and carbamylcholine administration are plotted in the top panel, whereas the bottom panel gives the effects on the wave length of the earliest premature beat. During administration of carbamylcholine (filled squares) the wave length of a regularly driven impulse is shortened at all pacing intervals. However this effect is less at higher pacing rates. Furthermore the minimum pacing interval is shortened under the influence of carbamylcholine (45 versus 70 msec). The wave length of the earliest premature impulse during carbamylcholine infusion is shortened at all pacing intervals to almost an equal extent (bottom panel).

from 28 to 20 mm. The second major change is a shift of the curve to the left and this indicates that the minimum pacing interval under carbamylcholine is shorter than during control. The bottom panel gives the wave length of the earliest premature impulse at different pacing intervals during control and carbamylcholine administration. In contrast with the impulse during regular rhythm the wave length of the earliest premature beat is shortened to an equal extent at all pacing rates. This is indicated by the almost parallel shift of the curve downwards.

A summary of the effects of carbamylcholine on the wave length during slow pacing (2 Hz), the earliest premature impulse and at the highest pacing rate is given in table 5.I. For each parameter, the left column gives the mean and standard deviation during control whereas the right column indicates these values during administration of carbamylcholine (n=12). The major effect of carbamylcholine is a shortening of the refractory period, as can be seen in the first two vertical columns. The refractory period at a pacing rate of 2 Hz shortens from 70 to 38 msec, a decrease of almost 50%. In an

TABLE 5.I

THE EFFECTS OF CARBAMYLCHOLINE

	REFRACTORY PERIOD ms		CONDUCTION VELOCITY cm/sec		WAVE LENGTH mm	
	control	carbamyl-choline	control	carbamyl-choline	control	carbamyl-choline
REGULAR RHYTHM (2 Hz)	70.2 ±11.0	37.6 ±6.7 ***	59.5 ±11.6	57.4 ±11.0 **	41.5 ±8.4	21.4 ±4.4 ***
EARLIEST PREMATURE BEAT	65.7 ±9.8	37.5 ±6.4 ***	34.6 ±8.6	42.3 ±7.8 ***	22.7 ±5.8	16.1 ±5.0 ***
SHORTEST PACING INTERVAL	79.6 ±8.1	54.2 ±6.0 ***	33.3 ±6.1	34.0 ±6.1 ns	26.6 ±5.1	18.0 ±3.0 ***

ns =not significant
** =p<0.01
*** =p<0.001

analogous way the time needed to restore the excitability after the earliest premature impulse is also shortened; the shortest A_2-A_3 interval is 66 msec during control and 38 msec under the influence of carbamylcholine. The faster recovery of excitability during carbamylcholine infusion is also expressed in a higher maximum pacing rate . The average minimum pacing interval was shortened from 80 msec to 54 msec. The conduction velocity was not altered by carbamylcholine, with the exception of the earliest premature impulse, which was conducted somewhat faster during carbamylcholine infusion (42 vs 35 cm/sec). These changes in electrophysiological properties by carbamylcholine resulted in a shortening of the length of the excitation wave of 48 % during slow pacing, of 32 % during rapid pacing and of 29 % during a very early premature beat.

The relation of the changes in wave length with the shortening of the minimum pacing interval is shown in figure 5.3. It is clear that together with a shortening of the minimum pacing interval, also the length of the excitation wave is shortened markedly.

5.2.2 The effects of epinephrine.

Figure 5.4 gives an example of the effects of epinephrine on the shortest possible pacing interval, the conduction velocity and the length of the excitation wave during rapid pacing. After a control period of 1 hour, during which these parameters were constant, administration of epinephrine (6×10^{-7} M) was started. This did not result in any significant change in the measured electrophysiological parameters. The shortest possible pacing interval remained 80 msec and also the conduction velocity was unchanged. As a consequence the wave length at the shortest pacing interval was unchanged too. This lack of electrophysiologic effect was not caused by an insufficient dosage of epinephrine. When applied to the sinus node the same dosage has a strong chronotropic effect (Bonke et al. 1982). The atrial myocardium is also affected by this concentration of epinephrine, as illustrated in figure 5.5. In the top panel the wave length of a regularly driven impulse is plotted as a function of the pacing interval both during control and during epinephrine administration. It is clear that the effect of epinephrine strongly depends on the pacing rate. After addition of epinephrine to the perfusion fluid the wave length of the basic impulse at 500 msec interval clearly prolonged from

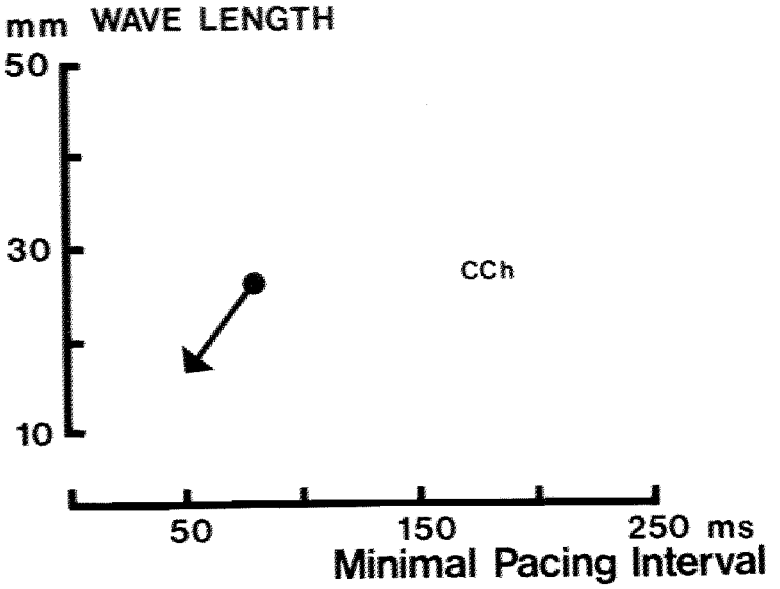

FIGURE 5.3 Relation between wave length and highest pacing rate under the influence of carbamylcholine. A clear shortening of the wave length is observed when the minimum pacing interval is shortened by carbamylcholine.

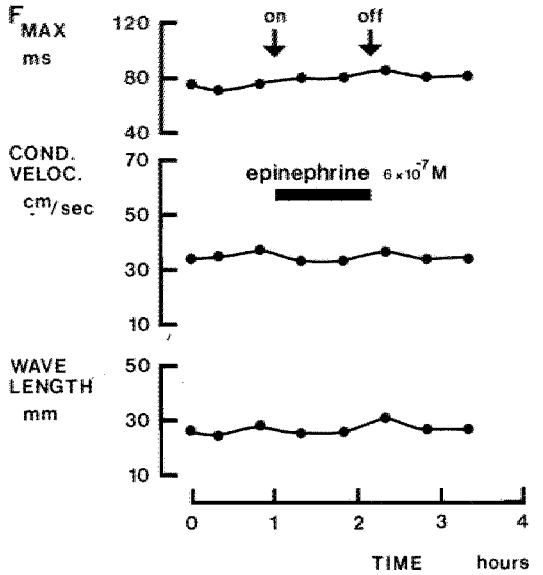

FIGURE 5.4 The shortest possible pacing interval, the conduction velocity at this rate and the wave length during the highest pacing rate are shown during adminstration of epinephrine. After a control period of 1 hour epinephrine was added to the perfusion solution in a concentration of 6 x 10^{-7} M. It is clear that neither of the measured parameters was affected by epinephrine.

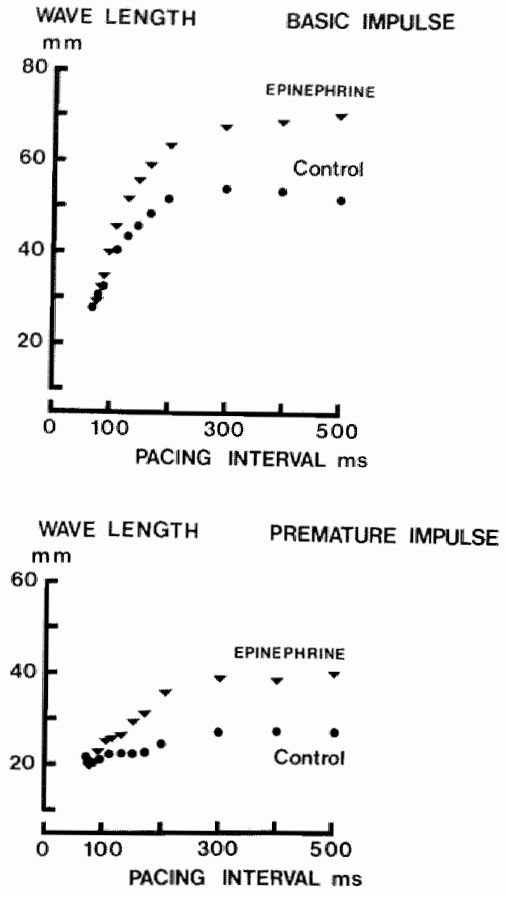

FIGURE 5.5 The effect of epinephrine on the wave length during incremental pacing. The length of the excitation wave of a regularly driven impulse is shown in the top panel, whereas the bottom panel gives the wave length of the earliest possible premature beat. Epinephrine causes a prolongation of the wave length of both a regularly driven impulse and the earliest premature impulse. However the effect is clearly rate dependant. If the pacing interval is shortened, the effect of epinephrine is diminished.

50 to about 70 mm. At an interval range from 500 to 200 msec this prolongation was constant. However if the pacing interval was shortened beyond 200 msec the effect of epinephrine on the wave length gradually diminished. Finally at the highest pacing rate the wave length became almost equal to the control value. This explains why in figure 5.4 no effect was seen. In the bottom panel of figure 5.5 the wave length of the earliest premature impulse is shown as a function of the pacing interval. Here the effects of epinephrine are quite similar as during a regular rhythm. In this example during control the wave length of the earliest premature impulse at a pacing interval of 500 msec was 27 mm. Under the influence of epinephrine this prolonged to 40 mm. However at faster pacing rates this epinephrine effect gradually diminished.

The effects of epinephrine are summarized in table 5.II. The left vertical columns of the three pairs give the control values, the right columns give the measurements during epinephrine infusion. The refractory

TABLE 5.II

THE EFFECTS OF EPINEPHRINE

	REFRACTORY PERIOD ms		CONDUCTION VELOCITY cm/sec		WAVE LENGTH mm	
	control	epine-phrine	control	epine-nephrine	control	epine-phrine
REGULAR RHYTHM (2 Hz)	66.5 ±8.1	98.4 +9.9 ***	56.5 ±10.4	58.3 +10.9 **	37.6 ±8.3	58.3 +15.8 ***
EARLIEST PREMATURE BEAT	61.7 ±7.4	93.5 +13.3 ***	33.2 ±9.7	31.2 +9.1 ns	20.5 ±6.6	28.8 +8.4 ***
SHORTEST PACING INTERVAL	80.4 ±8.3	87.3 +12.4 *	33.9 ±5.9	32.2 +5.2 *	27.3 ±6.1	28.0 +5.4 ns

ns =not significant
* =p<0.05
** =p<0.01
*** =p<0.001

period during regular pacing at 2 Hz prolonged from 67 to 98 msec, whereas the refractory period of the earliest premature impulse prolonged from 62 to 94 msec. Epinephrine only caused a slight increase in the shortest possible pacing interval from 80 to 87 msec. It is interesting to see that under the influence of epinephrine the refractory period at a slow pacing rate is longer than the shortest possible pacing interval (98 msec vs 87 msec). During control the refractory period at 2 Hz is always shorter than the minimal pacing interval. The conduction velocity of the earliest premature impulse, during slow pacing and during the highest possible pacing rate were almost identical to control. As a consequence the wave length during slow pacing (2 Hz) prolonged from 38 to 58 mm, whereas the wave length of the earliest premature impulse prolonged from 20 to 29 mm. Since the shortest possible pacing interval and the conduction velocity at this rate were hardly affected by epinephrine, also the wave length at the highest pacing rate was unchanged (27 compared to 28 mm). Thus the changes in refractoriness and

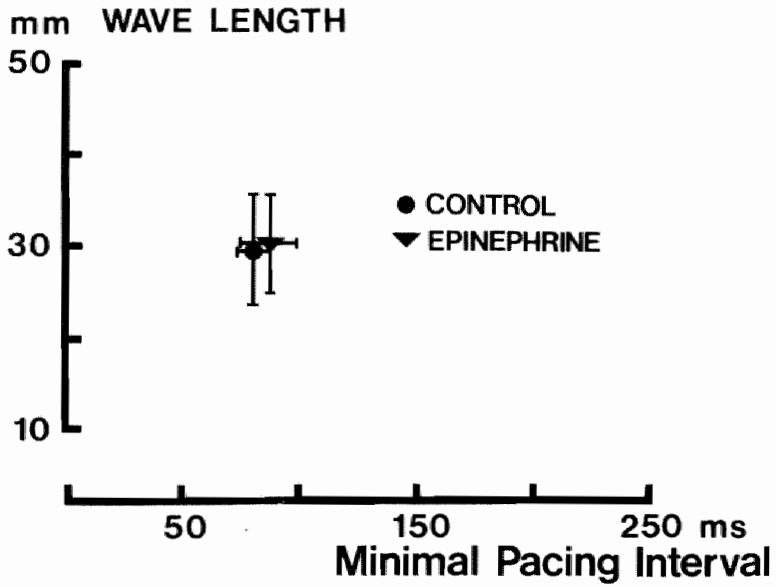

FIGURE 5.6 Relation between wave length and highest pacing rate under the influence of epinephrine. The filled circle gives the mean of the control measurements, the filled triangle gives the average of the measurements during epinephrine infusion. Both the shortest pacing interval and the wave length during rapid pacing are hardly affected.

conduction by epinephrine resulted in a prolongation of the wave length of 55 % during slow pacing, a lengthening of 40 % during an early premature beat whereas at the highest pacing rate the wave length hardly was affected (3 %).

In figure 5.6 the mean value of the wave length during maximal pacing is plotted as a function of the shortest pacing interval during control (filled circles) and during epinephrine (filled squares). It is obvious that both wave length and minimal pacing interval only show minor changes.

5.3 DISCUSSION

Acetylcholine, either released from nerve terminals after vagal stimulation or directly administered, causes a dramatic shortening of the refractory period of the atrium (Hoffman and Cranefield 1960). This is true both for a regularly driven impulse (shortest A_1-A_2 interval), as well as for the refractory period of a premature impulse (shortest A_2-A_3 interval). The shortest possible pacing interval is also markedly decreased. The conduction velocity was not affected, with exception of the speed of propagation of the earliest premature impulse, which was somewhat faster. It was shown in this study that the length of the excitation wave is markedly shortened by the action of carbamylcholine. This shortening of the wave length was observed at all heart rates and at all degrees of prematurities. It is primarily caused by a shortening in the refractory period, while the conduction velocity is unaltered. In case of premature beats the shortening of the wave length is attenuated by a relative increase in conduction velocity.

Epinephrine and norepinephrine have variable effects on the electrophysiological properties of atrial muscle. A shortening of the action potential duration has been reported in canine and cat atria (Brooks et al. 1955), whereas in rats (Webb and Hollander 1956) and rabbits (Hoffman and Cranefield 1960) a prolongation was found. In the isolated rabbit atrium we observed a prolongation of the refractory period with almost 50 % at slow pacing rates. However at higher pacing rates this prolongation disappeared.

The effects of acetylcholine and epinephrine in relation to reentrant arrhythmias.

Fibrillation induced by rapid pacing in the in situ atrium usually terminates spontaneously within seconds. However during stimulation of the vagal nerve fibrillation becomes self-sustained and no spontaneous

termination occurs. Also in the isolated canine atrium fibrillation is long lasting during acetylcholine administration and stops as soon as acetylcholine administration is interrupted. Our results show that acetylcholine may exert its fibrillatory action by shortening of the wave length. This shortening of the length of the impulse will lead to a smaller dimension of circuitous pathways within the atrium and consequently to a larger number of wavelets being present simultaneously during fibrillation. This hypothesis is supported by the observation of Allessie et al. (1977) that the size of the functionally determined reentrant circuit in an isolated rabbit atrium gets smaller when acetylcholine is added during the tachycardia. Thus, a decrease in the size of the reentrant circuits will make that more wavelets can be simultaneously present in the atrium making the chance of spontaneous termination small. In this study we found that the length of the excitation wave, being identical to the size of a functionally determined circuit, is markedly shortened by the action of carbamylcholine. The wave length during the highest pacing rate is shortened with 32 %, whereas the wave length of the earliest premature impulse is diminished with about 30 %. Obviously such a shortening of the wave length with about 30% is sufficient to maintain atrial fibrillation.

The action of epinephrine on the atrial myocardium is less prominent. Allessie et al. (1977) showed that the cycle length of a reentry within an isolated piece of rabbit atrium was slightly prolonged by epinephrine. Although the rate of this fast reentrant rhythm slowed slightly, it remained regular and did not stop. The results in isolated strips of atrial myocardium reported in this chapter, support these findings. Epinephrine did not affect the wave length during maximal pacing, whereas the shortest possible pacing interval increased about 7 msec. Although epinephrine did not influence the continuation of intra-atrial reentry, we might expect that induction of these reentrant circuits is more difficult during exposure to epinephrine. The wave length of the earliest premature beat was significantly prolonged over a wide range of pacing rates. This means that the premature impulse only can be trapped in a circuitous route, if a large arc of conduction block is present. Thus epinephrine would protect the atrial myocardium for the initiation of reentrant arrhythmias. However it must be realized that the effects of epinephrine on the ventricular myocardium are not only stronger but also qualitatively different. In general the refractory period of the ventricular

myocardium is shortened under the influence of epinephrine (Hoffman and Cranefield 1960). Whether epinephrine also changes the wave length in ventricular myocardium needs to be further investigated.

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6.THE EFFECTS OF CHANGES IN EXTRACELLULAR POTASSIUM CONCENTRATION.

It is well known that the risk of rhythm disturbances in the heart is increased if the extracellular potassium concentration is either too low or too high. Hyperkalemia results in a progressive slowing of conduction and a decrease in excitability. Hypokalemia precipitates ectopic beats and rhythms due to enhanced automatic activity in quiescent Purkinje fibers (Vassalle 1965, Gettes and Surawicz 1968). It was reported by Saito et al. (1978) that triggered activity could be elicited in isolated rabbit atrium in a low-potassium environment. Besides such arrhythmias based on abnormal impulse formation, hypokalemia may also facilitate reentry. Using detailed electrophysiological mapping, Lammers et al. (1981) showed that intra-atrial reentry was the underlying mechanism of tachy-arrhythmias during exposure to low potassium. Perfusion of isolated hearts with potassium-deficient solutions produces ventricular fibrillation (Grumbach et al. 1954, Surawicz et al. 1959). In patients with severe hypokalemia, serious ventricular tachy-arrhythmias, including ventricular tachycardia and fibrillation have been reported (Curry et al. 1976).

In the present study we investigated whether the increased incidence of reentrant arrhythmias during hypokalemia is related to changes in the wave length. We measured the length of the excitation wave in the atrium at different extracellular potassium concentrations both during slow and rapid pacing. It could be shown that the wave length is significantly shortened when the heart is exposed to low potassium. On the other hand moderate hyperkalemia did not affect the length of the excitation wave, whereas a significant prolongation was observed in severe hyperkalemia. These observations are in agreement with the hypothesis that shortening of the wave length of the cardiac impulse favors the occurrence of intra-myocardial reentry.

6.1 EXPERIMENTAL PROTOCOL.

The potassium concentration in the surperfusing solution was varied stepwise between 2.0 and 9.0 mM in 13 experiments. The heart was allowed to equilibrate at each potassium concentration for at least one hour. A typical sequence of changes in potassium concentrations is as follows: 4.5 mM, 2.0

mM, 4.5 mM, 7.0 mM, 4.5 mM, 9.0 mM. At each potassium concentration the wave length at a rate of 2 Hz and at the highest pacing rate were measured in all preparations. In addition the wave length was measured in another 13 experiments at a potassium concentration of 5.6 mM, but in these experiments potassium concentration was not changed during the experiment.

Statistical analysis was done using the student-t test. The results at the different potassium concentrations were compared with the values at 4.5 mM.

6.2 RESULTS.

The effects of potassium during slow pacing.

In table 6.I and figure 6.1 the mean values and standard deviation of refractory period, conduction velocity and wave length during slow pacing (2 Hz) are plotted at different potassium concentrations.

The refractory period at this slow rate is clearly affected by changes in the potassium concentration of the superfusing solution. Lowering the potassium concentration from 4.5 to 2.0 mM caused a significant shortening of

TABLE 6.I

THE EFFECTS OF POTASSIUM DURING A SLOW RATE.(2Hz)

Potassium concentration	N	Refractory period	conduction velocity	wave length of a basic impulse
mM		ms	cm/sec	mm
2.0	13	49.6 ±7.0 ***	51.7 ±9.2 ***	24.8 ±6.6 ***
4.5	13	69.8 ±10.0	60.2 ±10.1	41.8 ±9.1
5.6	13	-	62.1 ±7.0 ns	-
7.0	9	76.0 ±10.3 ***	50.9 ±8.3 ***	38.9 ±7.7 ns
9.0	8	-	33.0 ±10.2 ***	-

* =p<0.05
*** =p<0.001

the refractory period from 70 to 50 msec. On the other hand an elevation of the potassium concentration to 7.0 mM resulted in a significant prolongation from 70 to 76 msec.

The shortening of the refractory period at low potassium was accompanied by a depression in conduction velocity from 60 to 52 cm/sec. At a potassium concentration of 5.6 mM the speed of propagation was identical to the value at 4.5 mM, but a significant slowing of the conduction from 60 to 51 cm/sec was found at 7.0 mM. Further elevation of the potassium concentration to 9.0 mM resulted in a marked depression of the conduction velocity to 33 cm/sec.

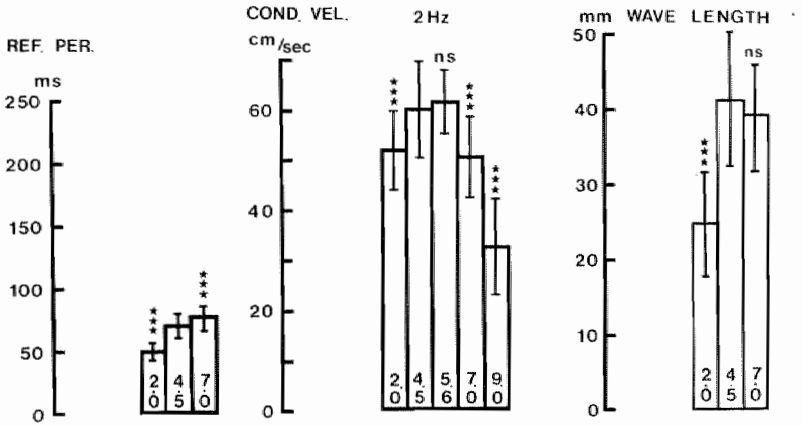

*FIGURE 6.1 The effects of the extracellular potassium concentration on the wave length during slow pacing (2 Hz). A block diagram is shown of the mean values of refractory period, conduction velocity and wave length at different potassium concentrations, as indicated at the bottom of the bars. The refractory period is shortest at the lowest potassium concentration (2.0 mM). At increasing extracellular potassium concentrations the refractoriness prolonged progressively. However the conduction velocity showed a biphasic pattern. The speed of propagation was highest at a potassium range of 4.5 to 5.6 mM, whereas a depression was noted both at low potassium concentrations (2.0 mM) and at high potassium levels (7.0 and 9.0 mM). Due to both the slowing in conduction and the shortening of refractory period, the wave length was significantly shortened if the potassium concentration was lowered from 4.5 to 2.0 mM. An increase of the potassium concentration from 4.5 to 7.0 mM did not cause a significant change of the wave length. Statistical analysis was done using the student-t test (ns = not significant, ***=$p<0.001$).*

Obviously the propagation of the electrical impulse in isolated rabbit atrium is optimal at potassium concentrations between 4.0 and 6.0 mM. Both at lower and higher concentrations conduction is depressed.

The changes in electrophysiological properties at low extracellular potassium resulted in a shortening of the wave length from 42 to 25 mm. This decrease in wave length during slow pacing (2 Hz) with 40 % was caused both by a shortening of the refractory period and by a decrease of conduction velocity. No change in the length of the excitation wave was found when the potassium concentration was increased from 4.5 to 7.0 mM. At this concentration the effect of depressed conduction on the wave length was completely neutralized by a concomitant prolongation in refractory period.

The effects of potassium during rapid pacing.

The effects of different extracellular potassium concentrations on conduction velocity and wave length during maximal pacing are illustrated in figure 6.2. After a control period of about 1 hour, during which the electrophysiological properties were constant we switched to a superfusing solution containing 2.0 mM potassium. Analogous to the shortening of the refractory period during slow pacing, also the shortest possible pacing interval decreased considerably in hypokalemia. Because the conduction velocity at the highest pacing rate was not different from control, the wave length was shortened proportional to the decrease in minimum pacing interval. After switching back to the superfusing solution containing 4.5 mM potassium all values returned to their control values. Increasing the potassium concentration to 7.0 mM caused a slight prolongation of the shortest possible pacing interval, whereas the conduction was depressed. Because these changes exerted an opposite effect on the wave length, the length of the excitation wave was unchanged. After return to control the potassium concentration was further elevated to 9.0 mM. At this concentration we observed strong changes in the electrophysiological properties of the myocardium. The shortest possible pacing interval rapidly prolonged from about 70 to 125, 160 msec and even longer intervals. Also the conduction was depressed markedly, reaching a minimum value after about 20 minutes. As a result at this high potassium level the wave length prolonged markedly, because the effect of the prolongation of the shortest possible pacing interval overruled the depression of conduction velocity. No complete washout

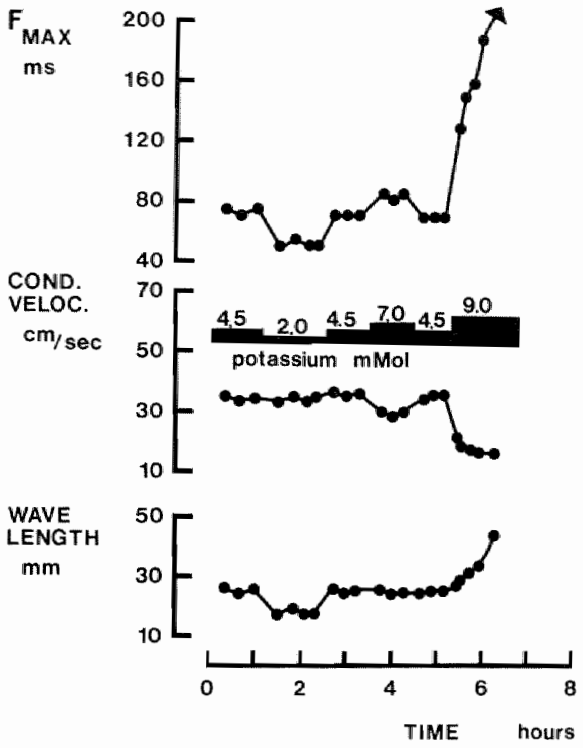

FIGURE 6.2 The effects of the extracellular potassium concentration on the shortest possible pacing interval, the conduction velocity and the wave length. Lowering of the potassium concentration from 4.5 to 2.0 mM caused a marked shortening of the minimal pacing interval, whereas the conduction velocity at this rate was not affected. As a consequence the wave length shortened proportional to the shortest pacing interval. When the potassium concentration was raised to 7.0 mM, the shortest possible pacing interval prolonged and simultaneously propagation was slowed. However the length of the excitation wave was unchanged. At a potassium level of 9.0 mM the shortest possible pacing interval prolonged markedly. Since this prolongation was larger than the concomitant depression in conduction, the length of the excitation wave prolonged.

of the effects of this potassium concentration could be achieved within 30-60 minutes.

In table 6.II and figure 6.3 the mean values and standard deviation of the conduction velocity and wave length during the highest pacing rate are given for all experiments.

The preparation can be paced fastest at a potassium concentration of 2.0 mM. An increase in potassium concentration caused a progressive prolongation of the shortest possible pacing interval from 66 to 78, 86, and 97 msec at 2.0, 4.5, 5.6, and 7.0 mM respectively. At 9.0 mM the shortest possible pacing interval was even prolonged to 235 msec.

In contrast with our findings during slow pacing, during rapid pacing propagation was not depressed at a low potassium concentration. However a significant lowering of conduction velocity from 37 cm/sec to 30 and 21 cm/sec was found at 7.0 and 9.0 mM respectively.

During rapid pacing lowering the potassium concentration caused a shortening of the wave length from 29 to 24 mm (17 %) and this was solely based on the fact that the preparation could be paced at a higher rate compared to control. No change in the length of the excitation wave was

TABLE 6.II

THE EFFECTS OF POTASSIUM DURING RAPID PACING

Potassium concentration	N	Interval Fmax	Conduction velocity Fmax	Wave length Fmax
mM		ms	cm/sec	mm
2.0	13	65.8 ±13.4 **	36.6 ±6.3 ns	23.7 ±4.3 **
4.5	13	77.9 ±7.6	37.0 ±6.1	28.7 ±4.4
5.6	13	85.9 ±9.5 *	34.5 ±4.5 ns	29.5 ±4.4 ns
7.0	9	96.7 ±10.3 ***	30.0 ±3.9 ***	28.8 ±4.8 ns
9.0	8	235.6 ±131.9 *	20.9 ±5.0 ***	45.5 ±19.9 *

* =p<0.05
** =p<0.01
*** =p<0.001

observed when the potassium concentration was increased to 5.6 and 7.0 mM. At these concentrations the effect of the prolongation of the shortest possible pacing interval is counteracted by an equal depression of conduction. However at a potassium concentration of 9.0 mM, the prolongation of the shortest possible pacing interval becomes larger than the depression in conduction velocity. Now the wave length is prolonged from 29 to 46 mm.

In figure 6.4 the relation between the wave length and the shortest possible pacing interval is given at different potassium concentrations. It is clear that at 2.0 mM both the minimum cycle length and the wave length are shortened, as indicated by the arrow pointing to the left and downwards. At 7.0 mM the arrow is horizontal, indicating that the prolongation of the shortest possible pacing interval did not affect the wave length. However at

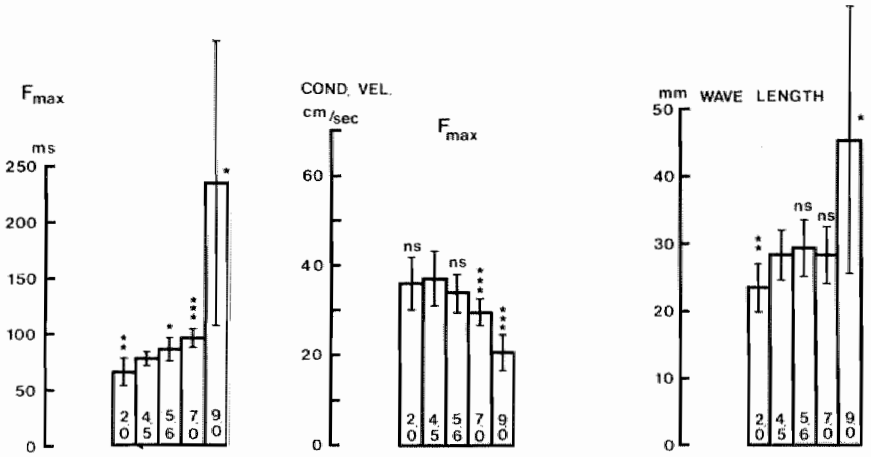

FIGURE 6.3 The wave length during rapid pacing at different extracellular potassium concentrations. The mean values of the shortest possible pacing interval, conduction velocity and wave length are plotted as a block diagram. Lowering the extracellular potassium level from 4.5 to 2.0 mM caused a significant shortening of the minimal pacing interval, whereas a elevation of the potassium concentration to 5.6, 7.0, 9.0 mM caused a significant prolongation of the shortest pacing interval. The conduction velocity at the highest rate was not changed at potassium concentrations between 2.0 and 5.6 mM. A significant slowing was observed at 7.0 and 9.0 mM. The length of the excitation wave was constant at potassium levels between 4.5 and 7.0 mM, whereas at 2.0 mM a significant shortening and at 9.0 mM a significant prolongation was observed.

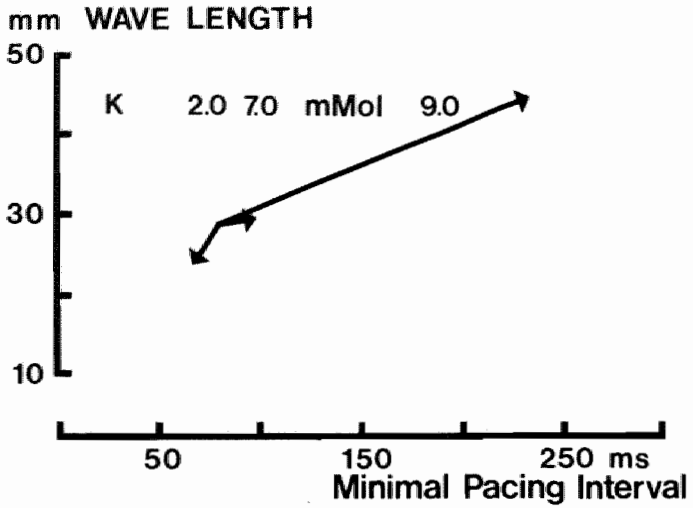

FIGURE 6.4 The relation between wave length and minimal pacing interval (= cycle length during fastest rate) at different potassium concentrations. The wave length at the shortest possible pacing interval is plotted as a function of the shortest pacing interval at potassium concentration of 2.0, 7.0 and 9.0 mM. At 2.0 mM the shortening of the pacing interval was accompanied by a shortening of the wave length, as indicated by the arrow pointing to the left and downwards. At 7.0 mM the arrow points almost horizontally to the right, indicating that the wave length was hardly changed although the preparation could not be paced as fast as at a concentration of 4.5 mM. Increasing the potassium concetration to 9.0 mM caused a prolongation of both the wave length and the shortest possible pacing interval.

9.0 mM the wave length is also prolonged, although still to a lesser extent than the shortest possible pacing interval.

6.3 DISCUSSION

The effects of low potassium on refractoriness and conduction velocity.

Both Teiger et al. (1969) and Lammers et al. (1981) showed that in the rabbit atrium the refractory period was shortened in low potassium. The shortening of the functional refractory period with 29 % during slow pacing (2 Hz) and during fast pacing with 17 % in our experiments is in agreement with these references. At low extracellular potassium concentration the conduction velocity is lowered (Antoni and Zerweck 1967). In our experiments the

conduction velocity decreased significantly during slow drive but was not altered during fast pacing; in the latter case conduction velocity even did not change although the maximum driving rate was higher, namely 66 msec versus 78 msec as shortest possible driving interval (a change of about 15 %).

The effects of high potassium on refractoriness and conduction velocity.

If the potassium concentration is increased from 4.5 to 7.0 mM the conduction is slowed with about 15 % both during slow and rapid pacing. This is in agreement with the literature as presented by Hoffmann and Cranefield (1960), Dominiguez and Fozzard (1970) and Sperelakis et al. (1970). We found in our experiments an increase of the functional refractory period during slow pacing (about 10 %) and more outspoken during fast pacing (more than 20 %) if the potassium concentration was increased from 4.5 to 7.0 mM. If the potassium concentration was increased to 9.0 mM the effects on conduction and refractoriness are more pronounced.

Changes in potassium concentration and wave length of the impulse.

Since in our experiments the reduced conduction velocity is counteracted by the increased refractoriness, there is almost no change in wave length in case of changes of the extracellular potassium concentration between 4.5 and 7.0 mM. However at higher potassium concentrations refractoriness increased considerably and this caused an outspoken increase of the length of the excitation wave. On the other hand a reduction of the extracellular potassium concentration to 2.0 mM induces a clear reduction of the wave length of the impulse.

Potassium and reentrant arrhythmias.

According to the concept of Allessie et al. (1977a) the size of a functionally determined circuit should be equal to the length of the excitation wave. We may expect that initiation of intra-atrial and intra-ventricular reentry will be facilitated by lowering the extracellular potassium concentration, since under these conditions the wave length of the impulse is considerably shortened. Recently Lammers and coworkers (1981) have shown that initiation of reentry within the atrium was indeed highly favored

by lowering the potassium concentration of the superfusing fluid to 2.0 mM. It has been postulated that low extracellular potassium concentrations increases the inhomogeneity in refractoriness. Gettes and Surawicz (1968) have shown that hypokalemia increases the difference in action potential duration of the Purkinje and ventricular fibers. The increased sensitivity of patients with severe hypokalemia to serious ventricular tachy-arrhythmias like ventricular tachycardia and fibrillation may be due to a combination of increased inhomogeneity in excitability and a marked shortening of the length of the excitation wave.

If the potassium concentration was raised moderately (7.0 mM), we did not find a difference in the length of the excitation wave compared to control. This indicates that initiation or termination of a reentrant circuit in the atrium is not influenced by moderate hyperkalemia. However a slowing in rate of an existing reentrant rhythm can be expected because the refractory period and the shortest possible pacing interval were both prolonged at 7.0 mM. This is in agreement with the observation that atrial flutter and fibrillation do not revert to sinus rhythm after potassium administration, probably because the dosage used in the therapy of the arrhythmia does not reach the apropriate concentration. Clinical and laboratory observations suggest that defibrillation of the atria can be expected when the potassium concentration exceeds 7.0 mM (Brooks et al. 1955, Bettinger et al. 1956, Burn et al. 1956). Also in patients with chronic atrial fibrillation, defibrillation has been reported by inducing severe hyperkalemia (Brown et al. 1951). The observation that during severe hyperkalemia the length of the excitation wave is markedly prolonged gives again a good explanation for this clinical experience. Our studies on the electrophysiological effects of changes in the extracellular potassium concentrations support the concept that changes in the wave length of the impulse can be used as a marker for the risk of intra-myocardial reentry.

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7. THE EFFECTS OF LIDOCAINE.

The local anesthetic lidocaine is an antiarrhythmic drug which is especially useful in the treatment of acute life threatening arrhythmias. It is used to treat ventricular arrhythmias in the setting of acute myocardial infarction to prevent ventricular fibrillation and sudden death (Lie et al. 1974). Lidocaine is also used in the treatment of arrhythmias caused by digitalis intoxication, and arrhythmias occurring after open heart surgery (Bigger and Hoffmann 1980). However lidocaine is less effective in the treatment of supraventricular rhythm disturbances. Although Danahy and Aronow (1978) reported a slight slowing of the rate of atrial flutter by lidocaine, it seldomly reverts atrial flutter and fibrillation to sinus rhythm.

In this chapter the effects of different dosages of lidocaine are studied on conduction velocity and refractoriness of the electrical impulse in the atrium. From these electrophysiologial properties the wave length was calculated. Special attention was paid to changes in the electrophysiological properties at high heart rate. It was speculated that in the light of the poor antifibrillatory properties of lidocaine in the atrium, it should not alter the length of the excitation wave.

7.1 EXPERIMENTAL PROTOCOL

To study the effect of lidocaine we selected three different dosages: 2, 5 and 10×10^{-6} g/ml. The concentrations of 2 and 5×10^{-6} g/ml are within the range of normal therapeutic plasma levels (1 to 5×10^{-6} g/ml, Hoffman and Bigger 1971, Koch-Weser 1975). The highest concentration of 10×10^{-6} g/ml has to be considered as a toxic dosage. After administration of lidocaine to the perfusion solution the electrophysiological effects are attained within 10 minutes. After three reproducable measurements were made the concentration of lidocaine in the superfusing fluid was increased, without an intermittent return to control. After the highest concentration has been reached, lidocaine was washed out. The effects of lidocaine were completely reversible. All electrophysiologic parameters returned to control within 30 minutes of washout with normal Tyrode solution. The effects of lidocaine were studied in 18 experiments.

7.2 RESULTS.

The effects of lidocaine during slow pacing.

In table 7.I the effects of different lidocaine concentrations on refractoriness, conduction velocity and wave length are given during slow pacing.

At a concentration of $2x10^{-6}$ g/ml the refractory period is hardly affected (78 versus 75 msec). However at higher concentrations of lidocaine recovery of excitability is clearly delayed. A prolongation to 99 and 158 msec is observed at concentrations of 5x and $10x10^{-6}$ g/ml.

Simultaneously with this prolongation of the refractory period, the

TABLE 7.I

THE EFFECTS OF LIDOCAINE DURING SLOW PACING (2Hz)

Concentration of Lidocaine g/ml	N	Refractory period ms	Conduction velocity cm/sec	Wave length mm
control	18	75.3 ±14.2	59.6 ±12.9	44.6 ±11.5
$2x10^{-6}$	15	78.5 ±13.6 ns	53.9 ±12.4 **	42.0 ±11.0 ns
$5x10^{-6}$	18	99.3 ±22.4 ***	51.2 ±11.8 ***	49.5 ±11.6 ns
$10x10^{-6}$	17	158.4 ±59.4 ***	45.1 ±12.8 ***	65.9 ±12.5 ***

ns = not significant
** = $p<0.01$
*** = $p<0.001$

conduction velocity is depressed, being lowered from 60 cm/sec to 54, 51 and 45 cm/sec at 2x, 5x and $10x10^{-6}$ g/ml lidocaine.

The length of the excitation wave at a pacing rate of 2 Hz was not significantly changed at "therapeutic" lidocaine concentrations. However a significant prolongation of the wave length was found at a concentration of $10x10^{-6}$ g/ml. This was caused by the fact that at this high level of lidocaine the prolongation of the refractory period clearly exceeded the slowing in conduction. As a result the length of the depolarization wave at a regular rhythm of 2 Hz prolonged from 45 to 66 mm.

The effects of lidocaine during rapid pacing.

In figure 7.1 an example of the effects of lidocaine on conduction and wave length during maximum pacing is given. After a control period of one hour during which the measured parameters were constant, lidocaine in a concentration of $2x10^{-6}$ g/ml was administered to the superfusing solution. At this concentration the shortest possible pacing interval slightly prolonged. Increasing the lidocaine concentration to $5x10^{-6}$ or $10x10^{-6}$ g/ml caused a progressive prolongation of the shortest possible pacing interval. Simultaneously the conduction velocity during the maximum pacing rate was depressed. The wave length remained unchanged or was only slightly prolonged. In table 7.II the effects of lidocaine on the shortest possible pacing interval, the conduction velocity and the wave length during rapid pacing are summarized.

If we compare table 7.I and 7.II, it is clear that the effects of lidocaine as seen during slow rate are exaggerated if the atrium is paced rapidly. Already at a concentration of $2x10^{-6}$ g/ml the shortest possible pacing interval was prolonged from 86 to 105 msec. A further prolongation to 130 and 175 msec was observed at lidocaine concentrations of 5 and $10x10^{-6}$ g/ml. The speed of propagation at the highest pacing rate was slowed from 32 cm/sec to 24, 21, and 19 cm/sec at 2x, 5x, $10x10^{-6}$ g/ml of lidocaine.

The length of the excitation wave during maximal pacing was not significantly influenced by lidocaine. At "therapeutic" lidocaine concentrations the wave length was 24 and 26 mm, compared to 27 mm during control. At these concentrations the effect of slowing of conduction on the wave length was completely neutralized by the concomitant prolongation of the minimal pacing interval. Although not statistically significant at "toxic"

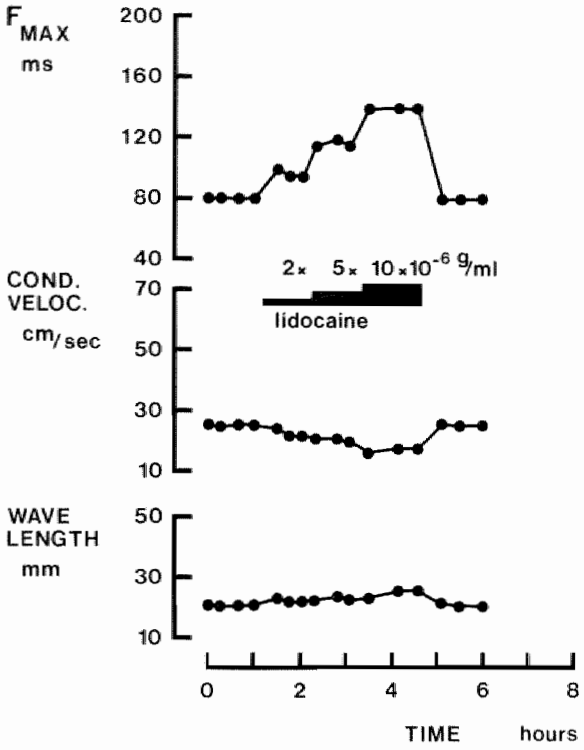

FIGURE 7.1 The effects of lidocaine on the shortest possible pacing interval, conduction velocity and wave length. The shortest possible pacing interval (top panel) is prolonged progressively at increasing lidocaine concentrations whereas simultaneously the conduction velocity at this rate (middle panel) is depressed during increasing lidocaine concentrations. As a consequence the length of the excitation wave at the highest pacing rate remained almost unchanged at 2 and $5x10^{-6}$ g/ml. At the highest concentration ($10x10^{-6}$ g/ml) a slight prolongation of the wave length was observed. Wash out of all effects of lidocaine was achieved within 30 min.

TABLE 7.II

THE EFFECTS OF LIDOCAINE DURING RAPID PACING

concentration of Lidocaine g/ml	N	Interval of Fmax ms	Conduction velocity at Fmax cm/sec	Wave length at Fmax mm
control	18	86.4 ±15.7	31.8 ±7.3	26.7 ±5.4
$2x10^{-6}$	15	105.2 ±15.2 ***	23.8 ±5.6 ***	24.5 ±4.5 ns
$5x10^{-6}$	18	130.3 ±24.3 ***	20.8 ±5.7 ***	26.5 ±6.5 ns
$10x10^{-6}$	17	175.7 ±52.9 ***	18.8 ±4.3 ***	32.1 ±10.0 ns

ns =not significant
*** =$p<0.001$

lidocaine concentration ($10x10^{-6}$ g/ml) the prolongation of the shortest possible pacing interval exceeded the slowing in conduction, resulting in a slight prolongation of the wave length to 32 mm.

In figure 7.2 the wave length at the highest pacing rate (ordinate) is plotted as a function of the shortest possible pacing interval (abscissa) at different lidocaine concentrations. At low concentrations of lidocaine the arrow points slightly downwards, indicating that the prolongation of the shortest possible pacing interval is accompanied by a slight shortening of the wave length. At high therapeutic lidocaine concentrations the arrow is pointing almost horizontally. This means that although the shortest possible pacing interval is markedly prolonged, the length of the excitation wave remains constant. Toxic concentration of lidocaine ($10x10^{-6}$ g/ml) caused more than a doubling of the shortest possible pacing interval and only a slight prolongation of the wave length.

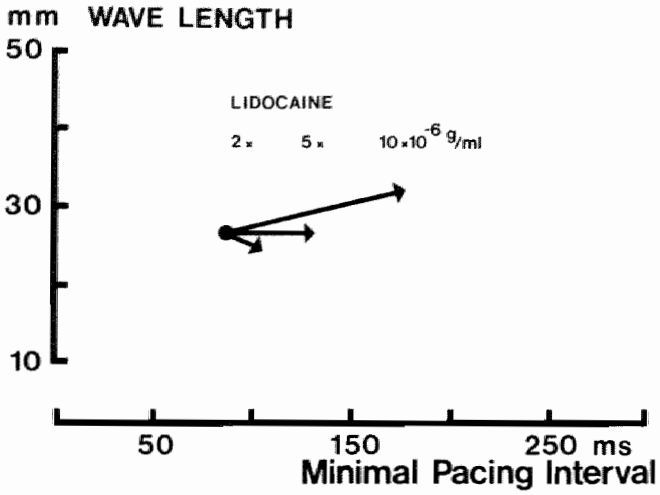

FIGURE 7.2 The relation of the minimal pacing interval and wave length at different lidocaine concentrations. The shortest possible pacing interval and the wave length at this rate are plotted at 2x, 5x, and $10x10^{-6}$ g/ml lidocaine. At the lowest concentration lidocaine the short arrow points to the right and downwards, indicating that the shortest possible pacing interval prolonged whereas the wave length was slightly reduced. The middle arrow (5×10^{-6} g/ml) is almost horizontal which means that the prolongation of the minimal cycle length is not accompanied by a change in wave length. At the highest concentration (10×10^{-6} g/ml) both the shortest possible pacing interval and wave length are prolonged, as indicated by the large upward pointing arrow.

7.3 DISCUSSION

The action of lidocaine, being a class 1 antiarrhythmic drug (Vaughan Williams 1970) consists mainly of a reduction of the maximal rate of depolarization in cardiac muscle. Changes in the rate of depolarization are associated with an increase in threshold of excitability and depression of conduction velocity. The effect of lidocaine is different in ischemic and normal tissue. Depression of the maximum rate of depolarization occurs in ischemic tissue at a much lower concentration of lidocaine than in normal tissue (Allen et al. 1978, Lazarra et al. 1978).

In this study, on healthy atrial tissue under normokalemic conditions, we observed during slow pacing (2Hz) a depression of the conduction velocity with 10-14 % at therapeutic lidocaine concentrations. At the highest possible

pacing rate the propagation was depressed with 25-35 %. These findings support the observation that the effect of lidocaine is enhanced at higher heart rates (Tritthart et al. 1978).

Although the conduction is slowed under influence of lidocaine the wave length is not significantly changed at therapeutic concentrations. Simultaneously with the slowing in conduction, the refractory period and the shortest possible pacing interval prolonged to an equal extent, completely neutralizing the effect of the slowing in conduction on the wave length. Only at toxic concentrations of lidocaine the prolongation in refractoriness and shortest pacing interval become larger than the slowing in conduction, resulting in a prolongation of the wave length.

The clinical observation that lidocaine seldomly restores the sinus rhythm in patients with atrial fibrillation is in good accordance with this finding. It supports the possibility that direct measurement of the length of the cardiac impulse may be used as a marker for the fibrillatory and antifibrillatory action of a drug.

In contrast to its action on the atria, lidocaine is very effective in protecting the ventricles against fibrillation (Lie et al. 1974, Cardinal et al. 1981). Cardinal et al. (1981) showed that lidocaine did not prevent the occurrence of large reentrant circuits in an acute ischemic porcine heart, but protected it from degenerating into ventricular fibrillation. It has been shown by Kabella (1973) that the effects of lidocaine on atrial and ventricular myocardium are very different. Direct measurement of the wave length in ventricular muscle has not been done. To further evaluate the validity of our hypothesis measurement of the effect of lidocaine on the length of the cardiac impulse in both healthy and ischemic ventricular myocardium seems worthwhile.

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8. THE EFFECTS OF QUINIDINE

In 1914 Wenckebach discovered that quinine, used in the treatment of malaria, was also effective against atrial fibrillation. In 1918 Frey reported that in this respect quinidine was even more effective than quinine. This observation was followed by a number of papers (Frey 1921, Viko et al. 1923) reporting conversion of atrial fibrillation to sinus rhythm in 50 to 70 % of the cases after administration of quinidine. According to Lewis and Drury (1921) this beneficial effect of quinidine on atrial fibrillation should be attributed to a prolongation of the effective refractory period of atrial myocardium. On the other hand it has been reported that quinidine also depresses the conduction velocity of the impulse. Wallace et al. (1966) even suggested that a possible mechanism of action of quinidine could be a lowering of the stimulating efficacy of the wave front, resulting in conduction block.

It has been proposed that fibrillation is based on multiple reentrant circuits and that continuation of fibrillation is related to the number of simultaneously present wavelets (Moe and Abildskov 1959, Allessie et al. 1982). Any intervention which increases the size of intra-atrial reentrant pathways will result in a decrease of the number of impulses circulating around during fibrillation. Because of this decrease in number of wavelets the fibrillatory process may terminate spontaneously.

In this chapter we investigated whether the antifibrillatory action of quinidine can be attributed to such an increase in the dimensions of intra-atrial reentry. The effect of quinidine on the length of the excitation wave was measured at different pacing rates.

8.1 EXPERIMENTAL PROTOCOL.

The effects of quinidine on the electrophysiological properties of the atrium were studied in 14 experiments. The dosages of quinidine used (between 1 and 7 mg/l) are within the therapeutic range (3 to 6 mg/l, Sokolow and Edgar 1949, Hoffman et al. 1975). After addition of quinidine to the superfusing solution it took a rather long time before the first effects could be noticed. During the first hour the major changes occurred, but in the second

hour the effects of quinidine still progressed. Even after 3 hours of administration of quinidine no steady state was reached. This slow uptake of quinidine in the in vitro heart prevented us from making a full quantitative analysis of the effects of quinidine on the length of the excitation wave. In designing the experimental protocol a compromise had to be found between the limited total duration of in vitro experiments and the condition that the measurement of the electrophysiologic properties should be done in a steady state. The following protocol turned out to be the most appropriate. Since in the experimental protocol no steady state measurement could be made, the results could not be plotted in dose-response curves. Instead we used the effect of quinidine on the shortest possible pacing interval as parameter to compare the different experiments. The measurements during quinidine administration were divided in two groups. In group I the electrophysiological properties are given at the moment that quinidine prolonged the shortest possible pacing interval to 150 msec. Group II represents all results when the shortest possible pacing interval was increased to 200 msec due to quinidine. If the electrophysiological properties of the preparation were constant, quinidine administration was started with a concentration of $3x10^{-6}$ g/ml (in one preparation the starting dosage was $1x10^{-6}$ g/ml, see figure 8.1). After a period of at least 90 minutes the concentration of quinidine was stepwise increased to $5x10^{-6}$ g/ml and 1 hour later to $7x10^{-6}$ g/ml. In some experiments, however this latter change was not performed and the quinidine concentration was kept during the rest of the experiment at $5x10^{-6}$ g/ml or was lowered to the original concentration of $3x10^{-6}$ g/ml. The length of the excitation wave was measured in all preparations during slow and rapid pacing (Fmax).

8.2 RESULTS.

Figure 8.1 and figure 8.2 illustrate the great differences in sensitivity to quinidine from preparation to preparation. Figure 8.1 gives an example of a preparation which was very sensitive to quinidine. Administration of $1x10^{-6}$ g/ml quinidine started to prolong the shortest possible pacing interval already after half an hour. Simultaneously the conduction velocity at this pacing rate was depressed. Because the effect of quinidine on the conduction velocity was less than the change in the maximum pacing rate, the length of the excitation wave was clearly prolonged. After about 90 minutes of exposure

to this concentration of quinidine, electrophysiological changes progressed rapidly, finally leading to conduction block during regular driving (2 Hz).

In figure 8.2 the effects of quinidine in another experiment are shown. The arrangements of the panels is identical as in figure 8.1. After a control period of 1 hour, quinidine was administered to the Tyrode solution at a concentration of $3x10^{-6}$ g/ml. Here again the shortest possible pacing interval prolonged whereas the conduction velocity at this rate was

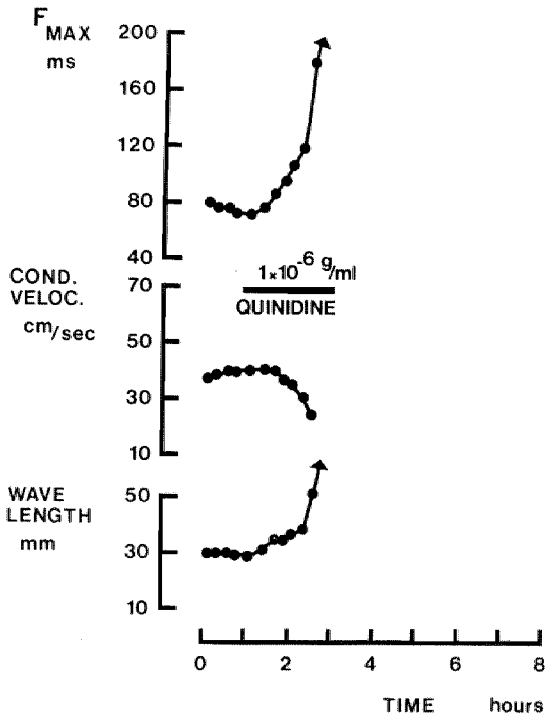

FIGURE 8.1 The effects of a low concentration of quinidine on the shortest possible pacing interval, conduction velocity and the wave length. After a control period of about 1 hour quinidine (10^{-6} g/ml) was administered to the Tyrode solution. After an exposure of 30 minutes the first effects were noticed. The shortest possible pacing interval prolonged, whereas the conduction velocity was depressed. The length of the excitation wave prolonged markedly because the increase in the minimal pacing interval was larger that the concomitant depression in conduction velocity. After about 90 minutes the electrophysiological changes progressed rapidly, resulting in conduction block during slow pacing.

depressed. The wave length at the highest pacing rate prolonged gradually during superfusion with 3 x 10^{-6} g/ml. This prolongation was caused by the fact that the prolongation of the shortest possible pacing interval was larger than the concomitant depression in conduction velocity. After 150 minutes the concentration of quinidine was increased to $5x10^{-6}$ g/ml. At this concentration of quinidine the shortest possible pacing interval prolonged further and the slowing in conduction increased. Since the prolongation of the minimum pacing interval exceeded the depression in conduction velocity

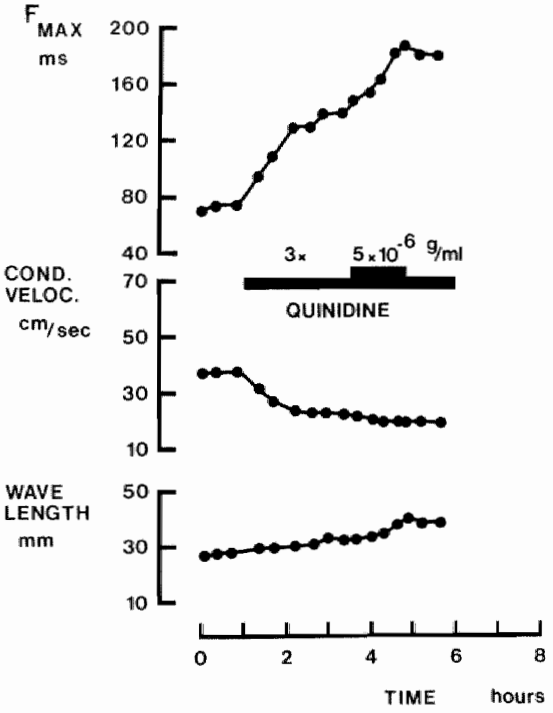

FIGURE 8.2 The effect of quinidine on the shortest possible pacing interval, conduction velocity and wave length. After a control period of 60 minutes, quinidine was administered in a concentration of $3x10^{-6}$ g/ml (150 minutes) and $5x10^{-6}$ g/ml (80 minutes). Quinidine caused a marked prolongation of the shortest possible pacing interval and simultaneously the speed of propagation was slowed. Since the increase in shortest possible pacing interval exceeded the depression in conduction, the wave length prolonged. After lowering the quinidine concentration to $3x10^{-6}$ g/ml the interval at Fmax, the conduction velocity were more or less constant.

the length of the excitation wave prolonged further. After 80 minutes the concentration was again lowered to $3x10^{-6}$ g/ml, and the electrophysiological properties remained more or less constant.

In tabel 8.I and 8.II the effects of quinidine are given in two groups. In group I all data are gathered at the moment quinidine had prolonged the shortest possible pacing interval to 150 msec. Group II represents the values at the moment the shortest possible pacing cycle was prolonged to 200 msec. The effects of quinidine on refractoriness, conduction velocity and wave length during slow (2Hz) and rapid pacing are given. Quinidine exerted clear changes in the electrophysiological properties of the atrial myocardium during slow pacing (2 Hz). The refractory period prolonged significantly from 73 msec to 104 and 123 msec in group I and group II respectively. Also the conduction velocity is markedly lowered during administration of quinidine. The speed of propagation was slowed from 61 cm/sec during control to 43 and 38 cm/sec in group I and II. The length of the excitation wave during this slow rate (2 Hz) was not significantly changed, since the prolongation of the refractory period was equal but opposite to the slowing in the conduction velocity.

TABLE 8.I

THE EFFECTS OF QUINIDINE DURING SLOW PACING (2Hz)

Quinidine	N	Refractory period	Conduction velocity	Wave length
		ms	cm/sec	mm
control	14	72.6 ±11.6	60.8 ±9.4	43.5 ±5.7
group I	13	104.5 ±15.6 ***	43.2 ±8.7 ***	45.0 ±9.9 ns
group II	12	123.1 ±11.7 ***	38.2 ±7.4 ***	46.7 ±8.1 ns

ns = not significant
*** = $p<0.001$
group I = results at the moment the interval of Fmax increased to 150 msec during quinidine administration.
group II = results at the moment the interval of Fmax prolonged to 200 msec during quinidine administration.

TABLE 8.II

THE EFFECTS OF QUINIDINE DURING RAPID PACING (Fmax)

Quinidine	N	Interval of Fmax	Conduction velocity at Fmax	Wave length at Fmax
		ms	cm/sec	mm
control	14	80.7 ±10.4	33.9 ±5.7	27.1 ±4.2
group I	13	150	21.2 ±4.0 ***	32.0 ±6.9 **
group II	12	200	20.3 ±4.5 ***	40.3 ±8.8 ***

** =p<0.01
*** =p<0.001
group I = results at the moment the interval of Fmax increased to 150 msec during quinidine administration.
group II = results at the moment the interval of Fmax prolonged to 200 msec during quinidine administration.

The changes during rapid pacing are shown in table 8.II. It must be noted that the changes in the shortest possible pacing interval (150, and 200 msec, being the selection criterium) were almost twice as large as the prolongation of the refractory period. The conduction velocity during maximum pacing was depressed from 34 to 21 and 20 cm/sec in group I and group II respectively. This slowing in conduction velocity with 37 and 40% was almost equal to the depression in conduction during slow pacing (29 and 37%). Obviously quinidine did not enhance the slowing in conduction at higher pacing rates.

The wave length during maximum pacing was significantly prolonged from 27 mm to 32 mm in group I and to 40 mm in group II. This prolongation of the wave length is caused by the fact that the increase in the shortest possible pacing interval was larger than the concomitant slowing in conduction.

In figure 8.3 the relation between the prolongation of the shortest possible pacing interval and the associated wave length is given for quinidine group I and II. The shorter arrow indicates that a prolongation of the shortest possible pacing interval to 150 msec was already accompanied by

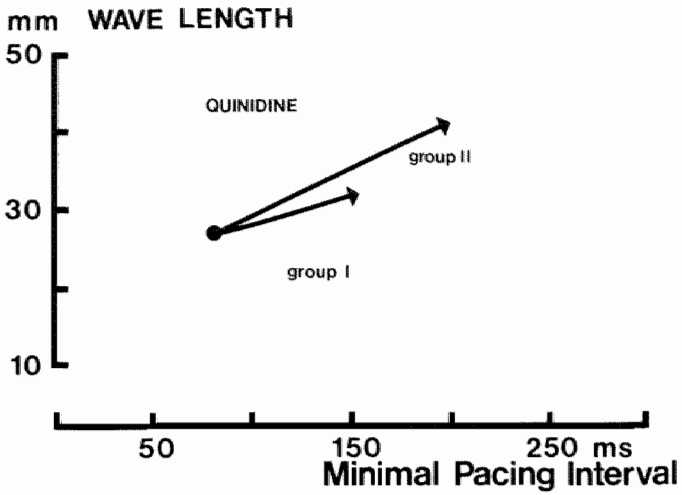

FIGURE 8.3 The effects of quinidine on the relation of shortest possible pacing interval and wave length. If due to the effect of quinidine the shortest possible pacing interval prolonged to 150 msec, the length of the excitation wave was also prolonged, as indicated by the short arrow. This prolongation of the wave length was even more pronounced if the minimum pacing interval was prolonged to 200 msec under the influence of quinidine (the arrow is longer and points more upwards).

an increase in the length of the excitation wave. This prolongation of the wave length was even more pronounced when the pacing interval was increased to 200 msec (group II, large arrow).

8.3 DISCUSSION

Quinidine and quinidine like drugs cause marked changes in the electrophysiological properties of cardiac tissue. The maximum upstroke velocity of the transmembrane potential is progressively decreased at increasing quinidine concentrations. The action potential duration is hardly affected by quinidine (Vaughan Williams 1958). In spite of this the refractory period is markedly prolonged (Lewis and Drury 1926). Also the highest possible pacing rate is considerably decreased under the influence of quinidine (Dawes 1946). We found a prolongation of the refractory period with 40 to 70 % during slow pacing. At higher pacing rates the refractory period prolonged even more, as is also reflected in the pronounced prolongation of the shortest possible pacing interval. A marked slowing in the conduction velocity of the impulse

with about 40 % both during slow and rapid pacing was found. In contrast with lidocaine (see chapter 7) the depression in conduction was not rate dependent. Although the effects of quinidine on the conduction velocity and refractoriness exerted opposite effects on the length of the excitation wave, the lengthening in refractory period was far more outspoken than the slowing in conduction of the impulse. As a result quinidine caused a marked prolongation of the length of the excitation wave in the atrium.

Although these changes in electrophysiological properties are pronounced, the experimental conditions were not strictly constant, because the effect of quinidine progressed slowly in time. These measurements of refractoriness, conduction velocity and wave length should actually be repeated in a steady state condition. This probably could be achieved by chronic administration of quinidine to the animal, before the heart is isolated.

Quinidine, wave length and reentrant arrhythmias.

Although quinidine is a relatively old drug, it is still of great clinical value in terminating atrial flutter and fibrillation. Furthermore it is used to reduce the incidence of relapse of atrial fibrillation after electrical cardioversion (Sodermark et al. 1975). Administration of quinidine during intra-atrial reentry in rabbits resulted in a gradual slowing of the rate and eventually in termination of the arrhythmia (Allessie et al 1977b). Such slowing in the rate of a reentrant arrhythmia can be understood by the prolongation of the refractory period by quinidine, which is also reflected in the decrease of the maximum pacing rate.

However this does not yet explain why quinidine may terminate atrial fibrillation and prevent its recurrence. We consider the prolongation of the length of the excitation wave by quinidine, as reported in this chapter, to be responsible for the antifibrillatory action of quinidine. There is evidence that the susceptibility for initiation and maintenance of fibrillation is associated with a large atrial mass (Garrey 1914, Moore and Spear 1982). Moe an Abildskov (1959) demonstrated that atrial fibrillation can persist in a stable state if a sufficient number of wavelets are randomly wandering around. According to the multiple wavelet hypothesis the number of wavelets should be directly related to the mass of tissue involved and inversely related to the size of the intra-atrial reentrant circuits. If the number of circulating wavelets within the myocardium gets lower than a

critical level, the fibrillatory proces gets unstable and may terminate spontaneously. The marked prolongation of the wave length by quinidine must decrease the number of wave fronts which can be present simultaneously in atria of a certain size. The clinical observation that quinidine can change "fine" fibrillation into "coarse" and more regular fibrillation (Hart 1922) is consistent with a decrease in the number of wavelets.

Although the present in vitro studies did not allow quantitative analysis of the effects of quinidine, we conclude that the observed lengthening of the activation wave offers a good explanation for the antifibrillatory action of the drug.

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9.THE EFFECTS OF OUABAIN

Digitalis is frequently used in the treatment of two groups of cardiac patients. First of all it is used in patients with congestive heart failure to increase the contractility of the heart. Secondly digitalis is used in atrial flutter and fibrillation to slow the venticular response by direct and indirect effects on the atrio-ventricular node. The direct action is an increase in effective refractory period of the A-V node (Hoffman and Bigger 1980) and indirectly the conduction through the A-V node is impaired because of the increased parasympathetic effect on the node. In patients with atrial flutter cardiac glycosides not only influence the A-V conduction but also induce a change in the rate of the flutter; the flutter rate increases and can finally degenerate into fibrillation. This action of cardiac glycosides on the behaviour of atrial flutter might be attributed to an increase in parasympathetic influence on the atrial myocardium, as demonstrated in the dog by Farah and Loomis (1950). In the same study it was shown that cardiac glycosides cause an increase of refractoriness and was accompanied by a reversion of atrial flutter into sinus rhythm. Therefore we decided to include a study on the direct effects of a cardiac glycoside, namely ouabain, on the isolated left atrium of the rabbit during regular and fast driving.

9.1 EXPERIMENTAL PROTOCOL

As a representative of cardiac glycosides we have chosen ouabain, because this drug has a rapid onset of action (Strophantin G, British Drug House). The starting dosage was $2x10^{-7}$ M in 6 preparations and $3x10^{-7}$ M in 2 preparations. After this concentration had been given for 2 to 3 hours, the ouabain concentration was increased stepwise to 3 and 4 $x10^{-7}$ M (4 experiments) and $5x10^{-7}$ M (in 2 experiments). We measured the effects of different concentrations of digitalis in 8 experiments.

In 4 of these experiments we isolated also the right atrium of the rabbit heart to monitor direct chronotropic effects of ouabain on the sinus node.

In figure 9.1 an example of the experimental protocol is given. The effects of different concentrations of ouabain on the shortest possible pacing interval, the conduction velocity and the wave length are plotted against time. After a control period of 45 minutes, a concentration of $2x10^{-7}$ M ouabain was administered during 2 hours. The concentration of ouabain was

increased to 3x, 4x and 5 $x10^{-7}$ M. Under the influence of ouabain the shortest possible pacing interval prolonged gradually, whereas the conduction velocity was hardly affected. As a result the length of the excitation wave prolonged. At $5x10^{-7}$ M the conduction rapidly deteriorated and the preparation became inexcitable. Washout did not reverse these effects. Statistical analysis was done using the student-t test (paired samples).

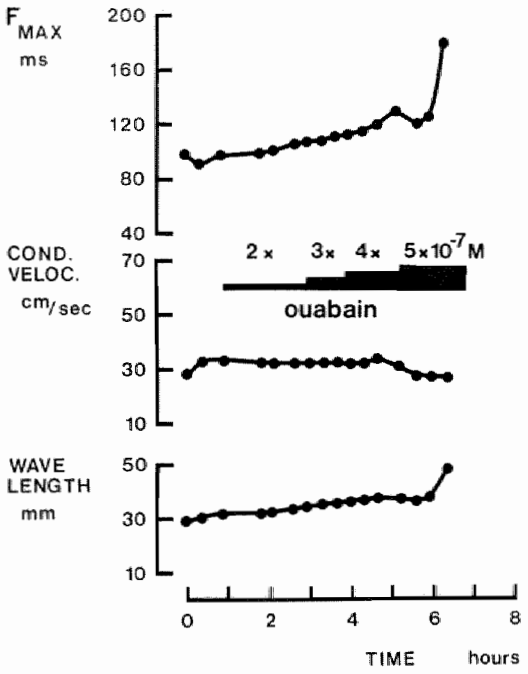

FIGURE 9.1 The effects of different concentrations of ouabain on the shortest possible pacing interval, conduction velocity and wave length. Ouabain was administered in stepwise increasing dosages from $2x10^{-7}$ M (120 minutes) to 3x, 4x, and $5x\ 10^{-7}$ M (60 minutes each). The shortest possible pacing interval prolonged gradually, whereas the conduction velocity at this rate was slightly depressed. Since the prolongation in the minimum pacing interval was somewhat larger than the depression in conduction velocity, the wave length prolonged moderately.

9.2 RESULTS

The effect of ouabain at a slow pacing rate.

In each experiment the refractory period and the conduction velocity were measured during regular driving with 2 Hz at the end of 2 hour administration of ouabain in a concentration of $2x10^{-7}$ M and at the end of a subsequent period of one hour in which the concentration of the drug was raised to $3x10^{-7}$ M. Refractory period did not change in case of the lower concentration but increased significantly from 73 msec to 87 msec when the higher concentration of ouabain was present (table 9.I). Conduction velocity did not change in either of the two concentrations. Therefore the length of the excitation wave could be calculated to be not affected at an ouabain concentration of $2x10^{7}$ M, whereas it was slightly prolonged if ouabain was administered in a concentration of $3x10^{-7}$ M.

The effect of ouabain on the wave length of the earliest premature impulse.

In table 9.II the effects of ouabain on refractoriness, conduction velocity and wave length of the earliest premature impulse are plotted. The

TABLE 9.I

THE EFFECTS OF OUABAIN DURING LOW PACING (2Hz)

	REFRACTORY PERIOD ms	CONDUCTION VELOCITY cm/sec	WAVE LENGTH mm	N
CONTROL	73.0 ±13.5	61.5 ±12.1	46.4 ±7.7	8
OUABAIN $2x10^{-7}$ M (120 min)	73.8 ±16.7 ns	61.2 ±12.3 ns	44.5 ±8.8 ns	5
OUABAIN $3x10^{-7}$ M (60 min)	86.7 ±22.9 *	59.3 ±10.2 ns	50.2 ±10.5 *	6

ns = not significant
* = $p<0.05$

TABLE 9.II

THE EFFECTS OF OUABAIN DURING AN EARLY PREMATURE BEAT.

	REFRACTORY PERIOD ms	CONDUCTION VELOCITY cm/sec	WAVE LENGTH mm	N
CONTROL	66.5 ±12.9	40.6 ±6.7	26.6 ±5.0	8
OUABAIN $2x10^{-7}$ M (120 min)	61.2 ±13.2 ns	36.2 ±8.1 ns	21.7 ±4.8 ns	5
OUABAIN $3x10^{-7}$ M (60 min)	64.5 ±16.3 ns	34.7 ±6.2 *	23.7 ±5.5 ns	6

ns = not significant
* = $p<0.05$

refractory period of the earliest premature impulse, being the shortest possible A_2-A_3 interval, is slightly shortened from 67 msec to 61 and 64 msec at 2x and $3x10^{-7}$ M respectively. However these changes were not statistically significant. The conduction velocity of the earliest premature impulse was slightly depressed by ouabain. At $2x10^{-7}$ M it was slowed from 41 cm/sec to 36 cm/sec, whereas at $3x10^{-7}$ M it was further depressed to 35 cm/sec. The length of the excitation wave was slightly shortened from 27 mm to 22 and 24 mm at 2x and $3x10^{-7}$ M (not statistically significant). This slight shortening was caused by both a slight shortening of the refractory period and a minor reduction in the conduction velocity.

The length of the excitation wave at the highest pacing rate.

In table 9.III the effects of ouabain during maximal pacing are given. As during slow pacing and early premature beats, also during rapid pacing the direct effects of ouabain were not very evident. The shortest possible pacing interval increased from 82 msec to 95 and 102 msec at 2x and $3x\ 10^{-7}$ M ouabain. Simultaneously the conduction velocity at this rate was depressed from 38 cm/sec to 35 and 33 cm/sec during the different ouabain concentrations. Calculation of the length of the excitation wave during fast

TABLE 9.III

THE EFFECTS OF OUABAIN DURING RAPID PACING (FMAX)

	INTERVAL Fmax ms	CONDUCTION VELOCITY at Fmax cm/sec	WAVE LENGTH at Fmax mm	N
CONTROL	81.9 ±12.2	38.0 ±6.7	30.7 ±4.9	8
OUABAIN $2x10^{-7}$ M (120 min)	95.0 ±10.0 **	35.4 ±4.9 *	33.5 ±5.0 *	5
OUABAIN $3x10^{-7}$ M (60 min)	102.5 ±10.8 **	32.8 ±4.6 **	33.7 ±4.7 *	6

ns = not significant
* = $p<0.05$
** = $p<0.01$

pacing rate revealed a slight but significant prolongation from 31 mm to 34 mm at both concentrations of ouabain. This prolongation of the length of the excitation wave was caused by the fact that the prolongation of the shortest possible pacing interval was somewhat larger than the depression in conduction velocity.

In figure 9.2 the effects of ouabain ($3x10^{-7}$ M) on the shortest possible cycle length and the concomitant wave length are given. The prolongation of the shortest possible pacing interval is accompanied by a prolongation of the wave length, as is indicated by the arrow, pointing upwards and to the right.

The effect of ouabain on pacemaker activity.

The relation between concentration of ouabain and its effect can be indicated by monitoring the effect on the intrinsic sinus rate. In general the intrinsic rate of the sinus node is not influenced by "therapeutic" plasma concentrations of ouabain. However, at toxic concentration of ouabain a shortening of the spontaneous cycle length can be noted, followed by

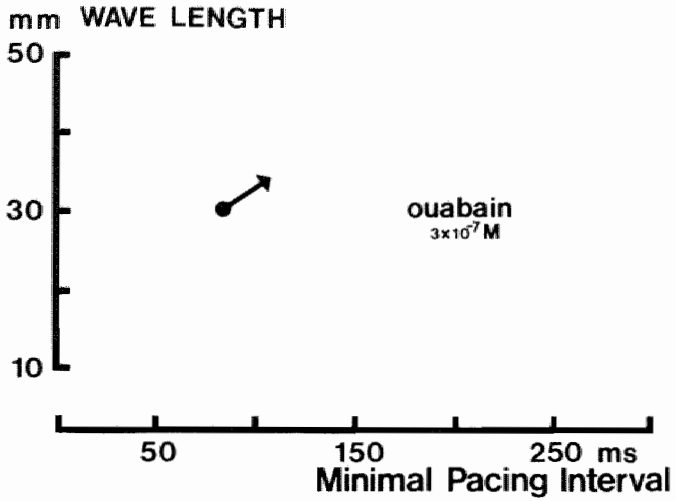

FIGURE 9.2 Relation of wave length and shortest possible pacing interval under the influence of ouabain. Ouabain in a concentration $3x10^{-7}$ M caused a prolongation of the shortest possible pacing interval (arrow points to the right) and together with this increase in minimum pacing interval, the length of the excitation wave prolonged (arrow points upwards).

irregularities and sinus node exit-block (Steinbeck et al. 1980). Irregularities in sinus rhythm were observed in all four preparations, in 2 at a concentration of $2x10^{-7}$ M, and in the other 2 preparations at $5x10^{-7}$ M ouabain. In three sinus node preparations these irregularities were preceded by a shortening of the spontaneous cycle length of the sinus node. These results indicate that the ouabain at the concentrations used in the present study exert toxic effects after long lasting administration.

In four strips of left atrial myocardium, devoid of any nodal tissue, spontaneous activity was observed during the administration of ouabain. The concentration of ouabain at which this spontaneous activity occurred was $2x10^{-7}$ M in 1 preparation, $3x10^{-7}$ M in 2 preparations and $4x10^{-7}$ M in 1 preparation. In general this activity was observed after pacing the preparations at high rates for a few minutes. The duration of this spontaneous activity ranged from 1-2 seconds to 8-10 minutes on one occasion; the cycle length of this spontaneous ectopic pacemaker activity ranged from 220 to 280 msec.

9.3 DISCUSSION

Effect of cardiac glycosides on refractoriness and conduction velocity.

In animal studies as well as in man, cardiac glycosides will cause a slowing of heart rate and this effect is considered to be vagal in origin because it is partly abolished by atropine. To explain this vagal effect several hypotheses have been advanced: 1) sensitization of the heart to vagal activity, 2) central vagal stimulation and 3) sensitization of the carotid sinus (thus an effect via the baroreflex). The first hypothesis is correct as was demonstrated by Gaffney et al. (1958) in the open-chest dog (vagotomized so that no central vagal influences on the heart could be present): they found that stimulation of the peripheral trunk of the vagal nerve led to a more pronounced slowing of the heart rate than under control conditions. Recently it was demonstrated by Bonke et al. (1982) that ouabain in a non toxic concentration (10^{-7} M : causing no effect on impulse formation, but exercising a positive inotropic effect) enhances the sensitivity of the isolated sinus node to cholinergic substances.

Thus in a heart with intact innervation cardiac glycosides can enhance the shortening of the refractoriness of atrial myocardium during parasympathetic stimulation. However it was reported that the direct action of cardiac glycosides on atrial myocardium was a prolongation of the refractory period (Mendez and Mendez 1953). These studies were done in anesthetized dogs with denervated hearts and intraveous injection of cardiac glycosides and this procedure makes it difficult to compare their results with ours. We found no change of refractoriness during regular driving of atrial myocardium during administration of ouabain ($2x10^{-7}$ M), but if the concentration was increased to $3x10^{-7}$ M the prolongation of refractoriness was obvious. The shortest possible pacing rate decreased (15-25 %) under the influence of cardiac glycosides. This is in agreement with the results of Farah and Loomis (1950) in canine atrium. The conduction velocity was not affected by ouabain during slow drive, but at the highest pacing rate a sligth lowering of the conduction was observed.

Effect of cardiac glycosides on wave length of the impulse in atrial myocardium.

Since cardiac glycosides caused a prolongation of refractoriness in

atrial myocardium with a concomitant decrease of the conduction velocity, the wave length of the impulse did not change in an explicit way. There was an increase of the length of the excitation wave of about 10 % during fast pacing.

Cardiac glycosides and reentrant arrhythmias in the atrium.

Farah and Loomis (1950) studied the effects of cardiac glycosides on atrial flutter in the dog heart. They induced a flutter according to the method of Rosenblueth and Garcia Ramos (1947) by making an obstacle in the atrium around which the impulse has to travel. In such a case of circus movement around an anatomical obstacle the flutter rate decreased during the administration of cardiac glycosides and finally reversion to sinus rhtyhm occurred. In their opinion this was caused by the increase in the refractory period. However if the heart was not denervated auricular flutter changed into fibrillation and because atropine or cutting the vagal nerves promptly reverted the fibrillation to a sinus rhythm, they concluded that this change from flutter into fibrillation was mediated through the vagus. In case there is no anatomical obstacle, for instance in the experiments of Allessie and coworkers (1982) inducing fibrillation in the canine atrium, we might expect that cardiac glycosides will have hardly any effect on the fibrillation since the wave length of the circulating impulse is only slightly increased and this will not be enough to really enhance the chance of termination of fibrillation. Further experiments are needed to prove this hypothesis.

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10. THE EFFECTS OF VERAPAMIL.

Verapamil was initially introduced by Haas and Hartfelder in 1962 as a coronary vasodilator in the treatment of ischemic heart disease. It soon became apparent that verapamil also possessed antiarrhythmic properties (Melville at al. 1964, Bender et al. 1966, Schmid and Hanna 1967, Kauman and Armendia 1968). Given intravenously verapamil caused a slowing of the ventricular rate in case of atrial fibrillation (Schamroth 1971, Schamroth et al. 1972). In patients with paroxysmal supraventricular tahycardias intravenous administration of verapamil caused a prompt termination of the arrhythmia and restoration of sinus rhythm (Krikler and Spurrel 1974). In the same study it was shown that in patients with supraventricular tachycardias associated with the Wolff-Parkinson-White syndrome, the arrhythmia could also be stopped by verapamil. These and others studies have demonstrated that verapamil is very effective against tachycardias based on a circus movement either completely within a nodal structure (for instance the A-V- node) or a circuit in which nodal tissue is a part. This is in agreement with the fact that verapamil can be considered as a calcium antagonist or a calcium-entry blocker and therefore will reduce the slow inward current during the action potential (Fleckenstein et al. 1969, Kohlhardt et al. 1975). Since the slow inward current is especially important as depolarizing current in nodal tissue and only of limited value for the depolarization of normal atrial myocardial cells, one might expect that verapamil will act predominantly on nodal tissue. Assuming that atrial fibrillation is based on multiple intra-atrial circuits - as was already stated in the previous chapter - one might expect that verapamil will not influence atrial fibrillation. This hypothesis seems to be supported by the fact that only in 16 % of the patients with atrial fibrillation the arrhythmia could be reverted into sinus rhythm using verapamil (Zipes and Troup 1978). Therefore if our hypothesis about the importance of the wave length as determinant of circular excitation in atrial myocardium is correct we expect that verapamil will not effect the electrophysiological properties of atrial myocardium in such a way that a change in wave length will occur.

10.1 EXPERIMENTAL PROTOCOL

To test the effect of verapamil on the length of the excitation wave in

the isolated atrium we selected 3 dosages of the drug: 0.1 mg/l, 0.5 mg/l and 1 mg/l (Isoptin, Knoll). It has been previously described that verapamil in this concentration range influences the electrophysiological properties of the isolated rabbit sinus node (Wit and Cranefield 1974). After a control period of 1 hour the administration of verapamil was started in a concentration of 0.1 mg/l. After about 1 hour the concentration of verapamil was stepwise increased to 0.5 mg/l. After this dosage had been given for another hour, the concentration of verapamil was further increased to 1.0 mg/l. No

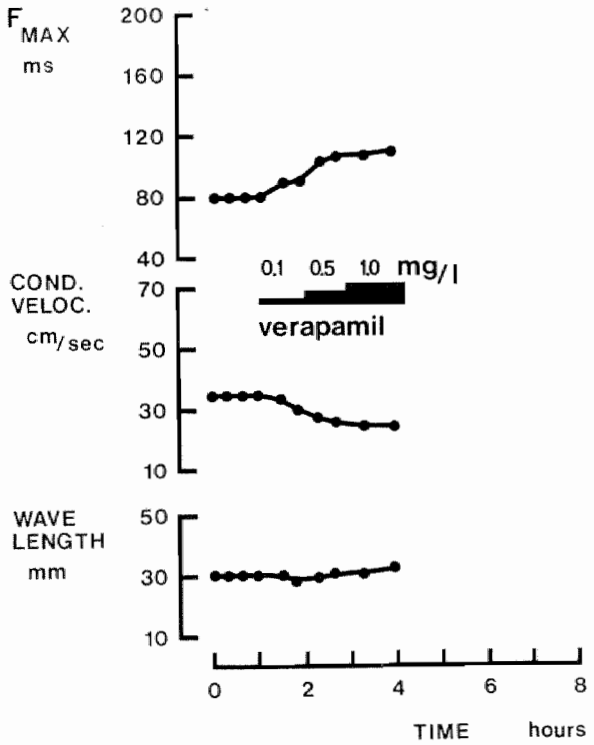

FIGURE 10.1 The effects of different concentration of verapamil on the shortest possible pacing interval, conduction velocity and wave length. After a control period of 1 hour verapamil was given at a concentration of 0.1, 0.5, 1.0 mg/l, without an intermittent return to control. The shortest possible pacing interval prolonged, and simultaneously the conduction velocity was depressed. Since the prolongation of the minimum pacing interval was equal but opposite to the depression in conduction velocity, the wave length was not changed.

attempts were made to washout the drug effects. In a few preliminary experiments we had superfused the preparation after verapamil administration, during a period of more than 1 hour with normal verapamil-free solution, but we were not able to washout the effects of verapamil completely. At each concentration at least two complete sets of measurements were made. An example of the experimental protocol is given in figure 10.1.

10.2 RESULTS

In figure 10.1 the results of a representative experiment are given. Under the influence of verapamil the shortest possible pacing interval prolonged, whereas the conduction velocity at the highest pacing rate was depressed. However the length of the excitation wave was not changed because the changes in conduction velocity and shortest pacing interval were opposite and about equal to each other.

In table 10.I the effects of different concentrations of verapamil on the refractory period, the conduction velocity and the wave length are given during pacing at 2 Hz. The restoration of the excitabilty was prolonged by verapamil. At a concentration of 0.1 mg/l the refractory period prolonged from 66 msec to 69 msec, and was further increased to 78 and 77 msec at 0.5 and 1.0 mg/l. The speed of propagation was slightly depressed from 56 cm/sec

TABLE 10.I

THE EFFECTS OF VERAPAMIL DURING SLOW PACING (2Hz)

Concentration of Verapamil mg/l	N	Refractory period ms	Conduction velocity cm/sec	Wave length mm
control	7	66.4 ±12.3	56.4 ±8.2	37.0 ±5.1
0.1	5	69.0 ±14.1 ns	52.6 ±9.9 ns	35.5 ±5.4 ns
0.5	6	77.8 ±19.2 ns	51.2 ±11.1 ns	38.4 ±5.0 ns
1.0	7	76.8 ±28.0 ns	53.0 ±13.9 ns	37.7 ±2.1 ns

ns = not significant

to 53, 51 and 53 cm/sec at different concentrations of verapamil. None of the changes in refractory period and conduction velocity were significant. Consequently there was no significant alteration in the calculated wave length at different verapamil concentrations, being 36, 38 and 38 mm versus 37 mm during control.

In table 10.II the effects of verapamil on the refractory period, conduction velocity and wave length of the earliest possible premature beat during slow pacing are given. The refractory period of the earliest premature beat was somewhat prolonged by verapamil. It increased from 61 msec during control to 63, 64 and 69 msec at 0.1, 0.5 and 1 mg/l verapamil. The speed of propagation of the earliest premature impulse was hardly influenced since conduction velocity changed from 34 cm/sec during control to 32, 30 and 33 cm/sec at 0.1, 0.5 and 1.0 mg/l verapamil. Neither the changes in refractory period nor the changes of the conduction velocity were significant, and therefore the wave length of the premature impulse remained unchanged by verapamil.

The measurements during maximal pacing are given in table 10.III. Also during rapid pacing the effects on refractoriness and conduction velocity are not prominent. The shortest possible pacing interval prolonged slightly from

TABLE 10.II

THE EFFECTS OF VERAPAMIL DURING PREMATURE STIMULATION

concentration Verapamil mg/l	N	Refractory period ms	Conduction velocity cm/sec	Wave length mm
control	7	60.9 ±11.8	33.9 ±7.2	20.3 ±3.2
0.1	5	62.8 ±13.5 ns	32.4 ±7.7 ns	19.8 ±3.5 ns
0.5	6	64.0 ±13.2 ns	30.3 ±10.7 ns	20.5 ±5.6 ns
1.0	6	68.7 ±20.0 ns	33.2 ±8.1 *	22.2 ±3.8 ns

ns =not significant
* =p<0.05

TABLE 10.III

THE EFFECTS OF VERAPAMIL DURING RAPID PACING (Fmax)

concentration of Verapamil mg/l	N	Interval of Fmax ms	Conduction velocity at Fmax cm/sec	Wave length at Fmax mm
control	7	85.0 ±17.8	32.6 ±4.4	27.1 ±2.3
0.1	5	89.0 ±21.9 ns	30.6 ±5.5 ns	26.3 ±2.6 ns
0.5	6	100.8 ±33.4 ns	28.2 ±5.0 *	27.0 ±4.2 ns
1.0	6	95.8 ±31.4 ns	26.2 ±5.3 **	24.2 ±5.8 ns

ns =not significant
* =p<0.05
** =p<0.01

85 msec during control, to 89, 101 and 96 msec at 0.1, 0.5 and 1.0 mg/l verapamil. These differences did not reach a statistically significant level. Simultaneously the conduction velocity during maximal pacing was diminished from 33 cm/sec to 31, 28 and 26 cm/sec at the different verapamil concentrations. Since again the slight prolongation of the shortest possible pacing interval was equal and opposite to the depression in conduction, the length of the excitation wave was not changed by verapamil. At 0.1, 0.5 and 1 mg/l the values were 26, 27 and 24 mm compared to 27 mm during control.

10.3 DISCUSSION

The effects of verapamil on refractoriness and conduction velocity of normal atrial myocardium.

Wit and Cranefield (1974) showed that the maximal upstroke velocity of the action potential in atrial fibers of the rabbit was not changed by verapamil even at a concentration of 2 mg/l. Verapamil also did not change the action potential amplitude and the action potential duration at 50 % repolarization (at high concentrations of verapamil). From these results one might expect

that impulse conduction in atrial fibers is not influenced by verapamil. Our study is in agreement with this statement as far as it concerns the conduction of the impulse during slow pacing or during a single premature beat; however during fast pacing conduction velocity in atrial tissue was slightly depressed by verapamil in a concentration of 1 mg/l. Studies on the effect of verapamil on refractoriness are very limited. Rosen et al. (1974) reported that the effective refractory period of Purkinje fibers is only slightly altered by verapamil. Indirect support for this is given by the studies by Singh and Vaughan Williams (1972) and Wit and Cranefield (1974) demonstrating that the duration of the action potential of rabbit atrial tissue is hardly affected by verapamil.

Verapamil and reentrant arrhythmias.

Both the literature on the effect of verapamil on the electrophysiology of atrial myocardium as discussed above and the results of our study, point out that verapamil has no major effect on the electrophysiological properties of healthy atrial myocardium. It is therefore not surprising that the wave length of the impulse is not influenced by the drug. However verapamil may suppres electrical activity from diseased atrial or ventricular muscle fibers having reduced resting membrane potentials; in such fibers activity largely depends on the calcium flux through the "slow" channel. Therefore we postulate that verapamil will not influence reentrant arrhytmias occurring in healthy myocardium, whereas in case nodal tissue or diseased myocardium is part of the circuit the arrhythmia will be influenced and probably terminated by verapamil. This is in agreement with the literature reviewed by Zipes and Troup (1978) who found that conversion to sinus rhythm occurred after intravenous administration of verapamil in 80 % of episodes of supraventricular tachycardia, 30 % of episodes of atrial flutter and only 16 % of episodes of atrial fibrillation.

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11. THE EFFECTS OF AMIODARONE

Amiodarone, a benzofuran derivative, was developed in the laboratories of Labaz Brussels in 1961. Initially it was used as an antianginal agent, but later the antiarrhythmic effects were recognised. Beneficial effects have been noted in the Wolff-Parkinson-White syndrome, both to prevent supraventricular tachycardias (Rosenbaum et al. 1974) and to reduce a high ventricular rate when atrial fibrillation occurs in a patient with WPW (Wellens et al. 1976). It has also been shown to be effective against atrial flutter and fibrillation (Rosenbaum et al. 1974, Rowland and Krikler 1980, Santos et al. 1979, Wheeler et al. 1979), and against recurrent ventricular tachycardias (Rosenbaum et al. 1974, Wheeler et al. 1979). However the electrophysiological mechanisms by which the drug exerts its antiarrhythmic effects are poorly understood. A prolongation of the refractory period has been reported as one of the most important electrophysiological effects of amiodarone, while the conduction velocity should not be affected or only slightly depressed (Singh and Vaughan Williams 1970).

Recent investigations indicate that atrial fibrillation is based on multiple local reentrant circuits (Moe and Abildskov 1959, Allessie et al. 1982). The number of these functionally determined circuits, which are present simultaneously, depends on the size of the circuits relative to the size of the heart. If the dimension of the circuits is small relative to the size of the atria there is room for many circuits. In this situation the statistical chance on termination of fibrillation is small. On the other hand if the size of the atria is small or the minimal size of a circuit is large, only a limited number of wave fronts can be present at the same time and termination of fibrillation becomes more likely. A drug which prolongs the length of the excitation wave can be expected to act as an antifibrillatory agent. The primary purpose of this study was to verify whether amiodarone -except for its prolongation of the refractory period- also prolonged the length of the excitation wave. If so, this might explain the antifibrillatory action of amiodarone.

11.1 METHODS

Since the solubility of amiodarone in Tyrode solution is limited and only allows low concentrations to be studied, we investigated the effects of

amiodarone after chronic administration to the rabbit. Because the binding of amiodarone after longterm treatment is very strong the heart can be isolated and studied in vitro without further addition of amiodarone to the perfusion solution. Amiodarone (Cordarone, Labaz) was injected intraperitoneally during a period of 4 weeks. 27 Rabbits (New Zealand, both sexes, 1.5 kg) were included in the study. Three groups of 9 rabbits were selected at random. During 4 weeks all animals were given one intraperitoneal injection daily; in group 1 only the solvent (0.8 ml/kg) was injected, group 2 was given 20 mg/kg amiodarone, whereas group 3 received 40 mg/kg amiodarone daily. Both amiodarone groups were loaded with 65 mg/kg amiodarone during the first 3 days.

After 4 weeks of treatment two preparations were isolated: the right atrium to measure the intrinsic rate of the sinus node, and a strip of left atrium to measure the functional refractory period and the conduction velocity of the myocardium. The two preparations were put together in a tissue bath which was perfused at a rate of 100 ml/min. No amiodarone was added to the perfusion solution. There was no evidence that amiodarone or its metabolites were washed-out during the superfusing period after isolation of the heart. The first measurement, done about 25 min after the operation, and the last measurement, one and a half hour later, gave the same results.

Statistical analysis was done using the student t-test.

11.2 RESULTS

To evaluate whether the injected amiodarone was absorbed from the peritoneal fluid we determined the amiodarone plasma concentration prior to the operation. The group receiving 20 mg/kg had a mean plasma level of 0.43 mg/l ($\pm$ 0.45). The group with the high concentration of amiodarone (40 mg/kg) had a mean plasma level of 1.44 mg/l ($\pm$ 1.02). It may be noted (table 11.I, 11.II and 11. III) that the individual plasma levels varied considerably in both groups. It is not clear whether this is caused by differences in absorption or in metabolism.

The weight of the rabbits at the first day of treatment was 1.48 ($\pm$ 0.06) kg.; there were no differences between the three groups. However at the day

vof operation the weight of the rabbits having received amiodarone was significantly less than in the placebo group. Obviously the growth of young rabbits is retarded by amiodarone.

The effects of amiodarone on the intrinsic rate of the sinus node.

Figure 11.1 shows the intrinsic rate of the sinus node after 4 weeks of treatment in the 3 groups. The open bar represents the intrinsic rate of the placebo group, the bar with the large circles the group with the low concentration amiodarone, while the bar with the small circles indicates the group treated with the high concentration of amiodarone. In the placebo group (n=9) the mean cycle length was 426 msec (± 41.2 msec). Essentially the same value was found in the group (n=8) treated with 20 mg/kg amiodarone (429 msec ± 48.1 msec). A significant prolongation of the cycle length of the sinus node was observed in the group (n=9) with a high concentration of amiodarone (495 msec ± 75.6 msec).

TABEL 11.I

PLACEBO

	REGULAR RHYTHM 2Hz			PREMATURE IMPULSE			RAPID PACING					
	CV	FRP	W	CV	FRP	W	CV	Fmax	W	PLam	Kg	SR
1	53	62	33	33	54	18	37	80	29	ND	2.2	446
2	49	49	23	26	50	13	25	85	21	ND	2.5	394
3	53	81	43	38	68	26	33	80	26	ND	2.2	468
4	43	65	27	26	59	15	23	90	20	ND	2.0	438
5	46	66	30	23	67	15	29	80	23	ND	2.6	474
6	41	68	28	25	71	17	30	70	20	ND	2.1	350
7	56	67	37	25	65	16	34	85	28	ND	2.3	420
8	58	68	39	23	61	14	35	70	24	ND	2.6	390
9	70	68	47	29	60	19	36	80	28	ND	2.8	455
MEAN	52.1	66.0	34.4	27.6	61.7	17.3	31.3	80.0	24.9		2.4	426.1
S.D.	8.8	8.3	7.7	5.0	6.8	3.9	4.9	6.6	3.4		.27	41.2

CV conduction velocity (cm/sec)
FRP functional refractory period (msec)
W wave length (mm)
Fmax minimal pacing cycle length (msec)
Plam plasmalevel of amiodarone (mg/l)
KG weight on the day of operation (kg)
SR sinus node cycle length (msec)
ND non detectable

Tabel 11.II

AMIODARONE
20 mg/kg

	REGULAR RHYTHM 2Hz			PREMATURE IMPULSE			RAPID PACING					
	CV	FRP	W	CV	FRP	W	CV	Fmax	W	PLam	Kg	SR
1	46	58	26	32	55	17	30	85	25	1.4	2.0	492
2	47	71	33	26	68	17	27	100	26	0.4	2.0	422
3	50	51	25	30	54	16	32	85	27	0.1	1.7	436
4	46	62	28	27	61	16	27	80	21	0.3	2.0	---
5	47	72	33	34	67	22	31	80	24	0.1	2.1	340
6	40	71	28	29	64	18	24	100	23	0.5	2.0	420
7	66	53	34	30	52	15	36	75	27	0.1	2.0	464
8	73	69	50	38	67	25	32	90	28	0.9	2.1	466
9	49	73	36	28	68	18	20	90	18	0.1	2.0	390
MEAN	51.6	64.4	33.2	30.4	61.8	19.2	28.8	87.2	25.0	0.43	2.0	428.8
S.D.	10.7	8.6	7.4	3.8	6.5	3.2	4.8	8.7	3.3	0.45	.12	48.1

Tabel 11.III

AMIODARONE
40 mg/kg

	REGULAR RHYTHM 2Hz			PREMATURE IMPULSE			RAPID PACING					
	CV	FRP	W	CV	FRP	W	CV	Fmax	W	PLam	Kg	SR
1	48	64	30	31	54	16	30	85	25	1.5	2.0	496
2	39	68	26	29	61	17	20	105	21	3.1	2.0	490
3	38	100	37	23	107	24	20	160	31	1.1	1.9	550
4	45	79	35	25	79	19	26	100	25	0.1	2.5	398
5	43	64	27	27	58	15	28	110	30	0.9	2.2	406
6	42	84	35	27	77	20	20	120	23	1.0	2.3	620
7	31	70	21	23	65	15	20	90	18	1.4	2.0	420
8	48	72	34	34	64	21	21	110	23	3.1	1.7	550
9	56	81	45	34	67	22	34	90	30	0.8	2.0	528
MEAN	43.3	75.8	32.9	28.1	70.2	19.7	24.3	107.8	25.7	1.44	2.1	495.3
S.D.	7.1	11.6	6.9	4.2	16.0	3.4	5.3	22.7	4.7	1.02	.23	75.6

CV	conduction velocity (cm/sec)
FRP	functional refractory period (msec)
W	wave length (mm)
Fmax	minimal pacing cycle length (msec)
Plam	plasmalevel of amiodarone (mg/l)
KG	weight on the day of operation (kg)
SR	sinus node cycle length (msec)
ND	non detectable

The effects of amiodarone during slow rate.

In figure 11.2 the effects of amiodarone on conduction velocity, functional refractory period and wave length of the atrial impulse during a regular rhythm of 2 Hz are plotted. In the placebo group the conduction velocity at a rate of 2 Hz was 52 cm/sec (± 8.8). The functional refractory period was 66 msec (± 8.3); thus the length of the excitation wave during this slow rhythm was calculated to be 34 mm (± 7.7). Treatment during 4 weeks with a low concentration of amiodarone (20 mg/kg daily) did not affect the conduction velocity (52 cm/sec ± 10.7), nor the functional refractory period (64 msec ± 8.6). Therefore the wave length of the impulse remained the same (33 mm ± 7.4). However when the rabbits were treated for 4 weeks with a higher dosage of amiodarone (40 mg/kg daily), clear electrophysiological changes occurred. During slow pacing with 2 Hz the functional refractory period prolonged from 66 msec (± 8.3) to 76 msec (± 11.6). If such a prolongation in refractory period was not accompanied by a depression in

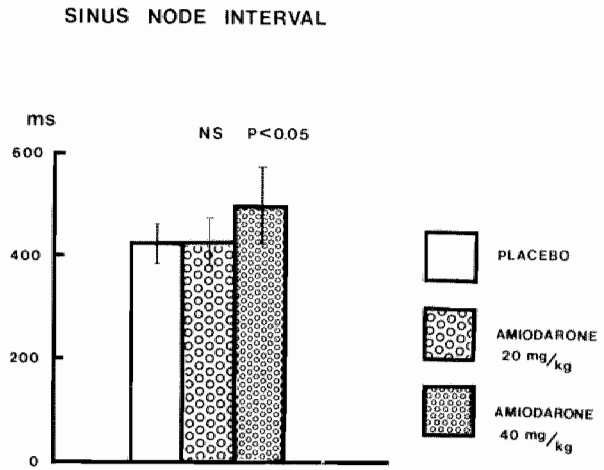

FIGURE 11.1 The effects of amiodarone on the intrinsic rate of the sinus node. the open bar represents the placebo group (n=9), the bar with the large circles is the group with the low concentration amiodarone (20 mg/kg, n=8) and the bar with the small circles the group with the high concentration of amiodarone (40 mg/kg, n=9). there is no change of the cycle length in the low concentration group if compared with the placebo group, but the group with the high concentration shows a significant increase in the cycle length from 426 (±41.2) to 495 msec (±75.6).

conduction velocity, this would result in a lengthening of the excitation wave. However amiodarone in the high concentration did slow down the speed of propagation from 52 cm/sec (± 8.8) to 43 cm/sec (±7.1, P < 0.05). This slowing of the conduction velocity completely neutralized the effect of the prolongation of the refractory period on the length of the excitation wave.

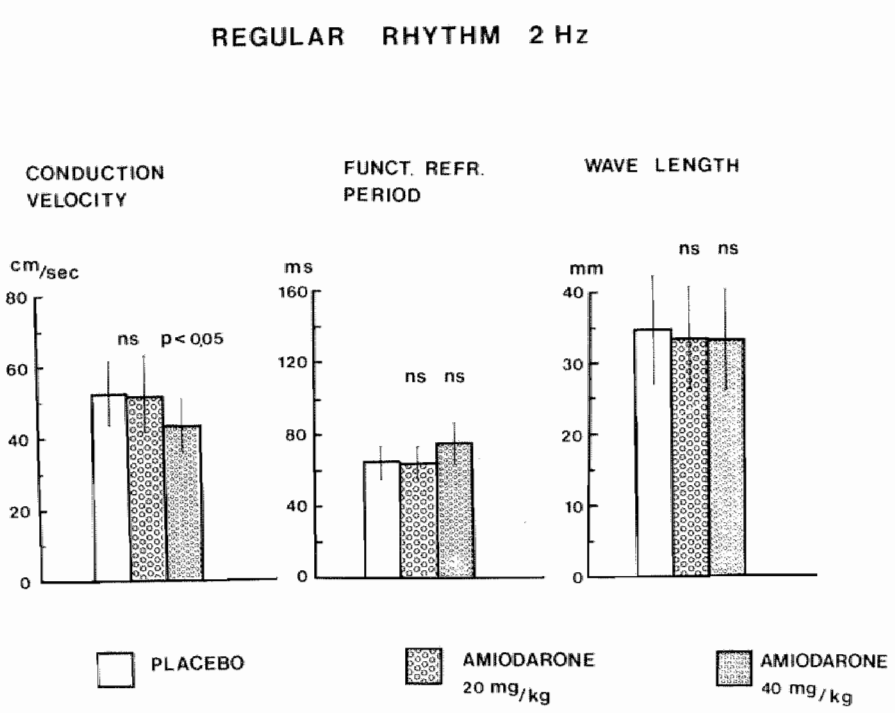

FIGURE 11.2 The effects of amiodarone on the wave length of the regular impulse. The conduction velocity in the placebo group (n=9, open bar) is 52 cm/sec (±8.8). The mean refractory period is 66 msec (±8.3). The mean wave length of the impulse at 2 hz is 34 mm (±7.7). After chronic treatment with a low concentration of amiodarone (bar with the large circles), there is no alteration in the conduction velocity (52 cm/sec±10.7) or in the refractory period (64 msec (±8.6)). The calculated wave length is 33 mm (±7.4). After treatment for 4 weeks with a high concentration of amiodarone (40 mg/kg) both the conduction velocity and the refractory period are affected. The conduction velocity at 2 hz is depressed to 43 cm/sec (±7.1). This would imply that the wave length would be smaller if the refractory period was constant. However this is not the case. The refractory period increased from 66 (±8.3) to 76 msec (±11.6). Because of the fact that these changes are almost equal and have an opposite effect on the length of the excitation wave, the product of the two remains unchanged: 33 mm (±6.9).

As a result amiodarone had no effect on the wave length of the impulse during a regular rhythm of 2 Hz. The values were 34 mm, 33 mm and 33 mm for the placebo group, low amiodarone group and high amiodarone group respectively.

The effects of amiodarone on premature beats.

In figure 11.3 the electrophysiological influence of amiodarone on early premature beats is depicted. As can be expected the conduction velocity of a

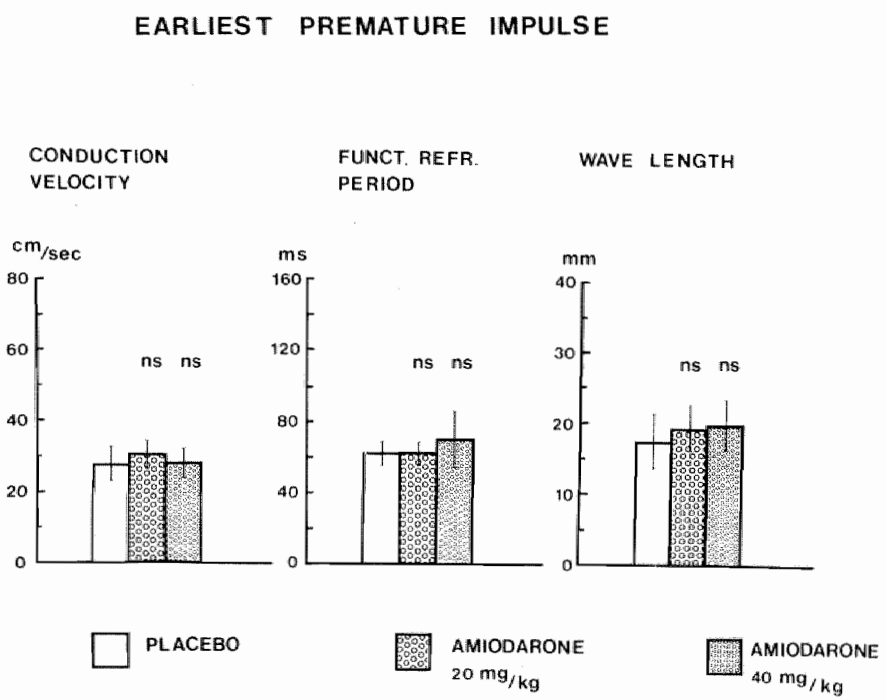

FIGURE 11.3 The effects of amiodarone on the wave length of the earliest premature impulse. The open bars represent the placebo group (n=9), the bars with the large circles the group with the low concentration amiodarone (20 mg/kg, n=8), the bars with the small circles the group with the high concentration amiodarone (40 mg/kg, n=9). The conduction velocity of the earliest premature impulse in the placebo group is 28 cm/sec (±5.0). The excitability is recovered after 62 msec (±6.8). The mean wave length of the premature impulse is 17 mm (±3.9), half of the length of the excitation wave at 2 Hz. At a low concentration of amiodarone the wave length increases sligthly to 19 mm (±3.2). At a high concentration the wave length has increased to 20 mm (±3.4), due to a slight prolongation of the refractory period (70 msec ±16.0), the conduction velocity remaining the same (28 cm/sec ±4.2).

premature impulse is slower than the speed of propagation during a slow rhythm. In the placebo group the mean conduction velocity of the earliest premature beat was 28 cm/sec (± 5.0 cm/sec), compared to 52 cm/sec•of the regular impulse. The mean functional refractory period of the earliest premature beat was slightly shorter than during basic rhythm (62 msec ± 6.8 versus 66 msec ± 8.3). This marked depression of the conduction velocity and the shortening of the refractory period both shortened the excitation wave. As a consequence the length of the excitation wave of the early premature beat was not more than 17 mm (± 3.9).

After chronic administration of 20 mg amiodarone per kg daily, the conduction velocity and the functional refractory period of the earliest premature impulse were not changed: 30 cm/sec (±3.8) and 62 msec (± 6.5) respectively. The length of the excitation wave was 19 mm (±3.2). These values were not statistically different ($P > 0.05$) from the placebo group. At the high concentration of amiodarone there was some prolongation of the mean functional refractory period, from 62 msec to 70 msec (±16.0). The mean conduction velocity was the same as in the placebo group (28 cm/sec ±4.2). Due to the slight increase in refractory period, the wave length of the early premature beat was also slightly lengthened (from 17 to 20 mm). However these differences did not reach a statistically significant level.

The effects of amiodarone during rapid rate.

Figure 11.4 gives the mean values of the conduction velocity, refractory period and the wave length during the highest possible pacing rate in the three groups. The conduction velocity during the highest pacing rate is much slower than during a regular rhythm of 2 Hz (31 cm/sec compared to 52 cm/sec). This distinct slowing of the speed of propagation did cause a marked shortening of the excitation wave (25 versus 34 mm). The length of the excitation wave of the earliest premature impulse is shorter than the wave length at the highest pacing rate (17 versus 25 mm), because the functional refractory period of a premature impulse is shorter than the minimal pacing cycle length.

After 4 weeks of treatment with amiodarone (20 mg/kg/day) none of the measured electrophysiological parameters were significantly changed. The shortest possible pacing interval prolonged slightly from 80.0 ± 6.6 to 87.2 ± 8.7 msec. The conduction velocities at these rates decreased from

31.3 + 4.9 to 28.8 ± 4.8 cm/sec. The resulting wave length was 24.9 ± 3.4 mm during control whereas after treatment with this low dosage of amiodarone the wave length was 25.0 ± 3.3 mm.

After 4 weeks of treatment with amiodarone (40 mg/kg/day) the maximum possible pacing rate of the atrium was clearly diminished; the shortest possible pacing interval prolonged from 80 to 108 msec. This increase in the minimum time required for recovery of excitability between two succesive

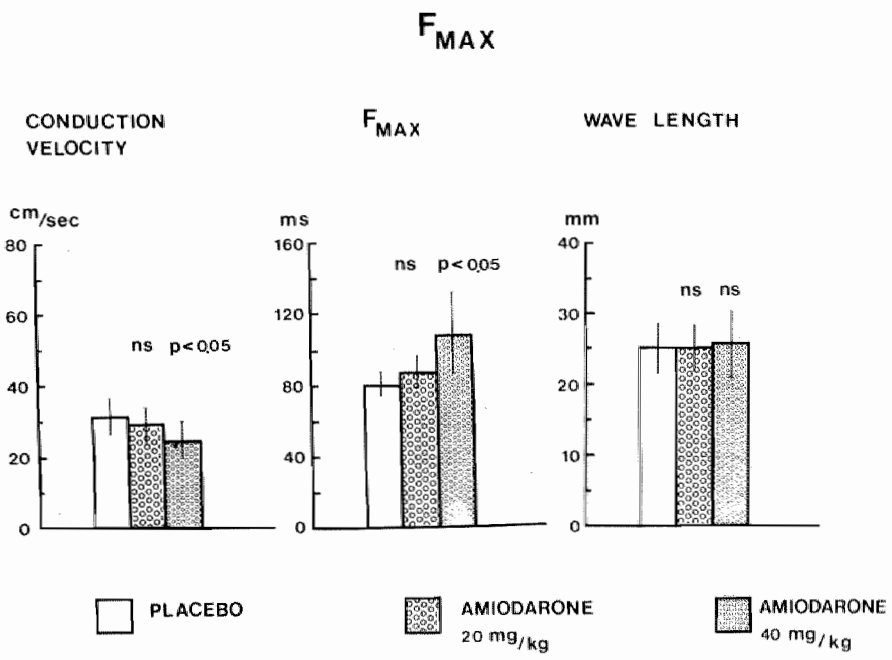

FIGURE 11.4 The wave length at the highest pacing rate. The format is the same as in figure 11.2 and 11.3. The shortest possible pacing interval (Fmax) is in the placebo group 80 msec(±6.6), the conduction velocity at this pacing rate being 31 cm/sec (±4.9). The mean length of the excitation wave is 25 mm (±3.4). The wave length is still 25 mm (±3.3) at a low concentration of amiodarone. In this situation there is only a minor increase in the minimum pacing interval (87 msec ±8.7). This effect on the wave length is counter acted by the decrease in conduction velocity (29 cm/sec ±4.8). In the group with the high amiodarone concentration the interval of Fmax increased further to 108 msec (±22.7) while the conduction velocity was depressed (24 cm/sec ±5.3). Because of the fact that these changes were about equal but opposite amiodarone had no effect on the wave length of the impulse during rapid pacing.

impulses should have prolonged the length of the excitation wave considerably. However such prolongation of the wave length was not found. In contrast to what was expected, amiodarone did not alter the length of the excitation wave in the atrium. This was caused by the fact that the prolongation of the time for recovery of excitability was accompanied by a concomitant depression in conduction velocity. Thus the slowing of the speed of propagation completely neutralized the effect of the prolongation of the refractory period on the wave length.

11.3 DISCUSSION

Effects of amiodarone on heart rate.

One of the beneficial effects of amiodarone in patients with angina pectoris is the slowing of heart rate. In animal studies this bradycardia is found both after chronic and acute administration (Singh and Vaughan Williams 1970, Rosenbaum et al. 1974, Goupil and Lenfant 1976, Lubbe et al. 1979). In clinical studies the bradycardia is also observed after chronic oral treatment, but acute intravenous administration of amiodarone gave controversial results. When amiodarone is injected intravenously a transient tachycardia occurs during the first 3-4 min. This is probably due to the solvent (Tween 80) because Sicart et al. (1977) could show that the same tachycardia occurred after injection of the solvent alone. Apart from this short initial tachycardia, some investigators did not observe a change in heart rate (Sicart et al. 1977, Benaim and Uzan 1978, Cote et al. 1979) while others found a bradycardia (Ourbak et al. 1976, Touboul et al. 1979). One of the explanations for the bradycardia is the non competitive beta-blocking properties of amiodarone (Charlier 1970, Polster and Broekhuysen 1976). Besides these beta-blocking effects amiodarone also has a direct suppressive effect on the sinus node. In the present study we confirmed the observations of Singh and Vaughan Williams (1970) and Goupil and Lenfant (1976), that the intrinsic rate of the isolated, denervated sinus node is slowed down under the influence of amiodarone. Thus it seems likely that the bradycardia observed in patients is caused both by a direct suppression of the intrinsic rate of the sinus node and by an indirect effect mediated by the betablocking properties of amiodarone.

Effects of amiodarone on refractory period, maximum pacing frequency and conduction velocity.

Singh and Vaughan Williams (1970) measured the transmembrane action potential in rabbit atrium after chronic treatment with 20 mg/kg amiodarone. They observed a prolongation of the action potential duration of 30 %, suggesting that the refractory period increased by amiodarone. Olsson et al. (1973) showed that the monophasic action potential in the right atrium of humans was lengthened with about 30 % after chronic administration of amiodarone. Direct measurement of the refractory period of the atrium in patients by programmed electrical stimulation revealed a prolongation varying from 17 % (Wellens et al. 1976) to 28 % (Rowland and Krikler 1980). We found in the rabbit atrium after 4 weeks of treatment with 40 mg/kg a prolongation of the functional refractory period from 66 to 76 msec (+15%). The maximum pacing frequency of the atrium was also clearly diminished by amiodarone. The shortest possible interval of a train of stimuli which still showed a 1:1 response increased from 80 to 108 msec (+35 %). This means a decrease of the maximum pacing rate from 750 to 555 beats per min.

Less uniform are the results about the effects of amiodarone on the conduction velocity. Singh and Vaughan Williams (1970) found in the rabbit atrium a small but significant decrease of the maximum rate of rise of the action potential. Nevertheless they did not measure a difference in conduction velocity in the atrium between the amiodarone treated and control rabbits. In clinical studies no change was found in the intra-atrial and intra-ventricular conduction (Benaim and Uzan 1978, Rosenbaum et al. 1974). In our studies the conduction velocity in the atrium was clearly depressed after 4 weeks of treatment with 40 mg/kg amiodarone. During a slow regular rhythm of 2 Hz the speed of propagation was diminished by 18 %. Pacing the atrium with the highest possible rate caused a further depression of the conduction velocity with 22 %. Also the conduction velocity of premature beats after treatment with amiodarone was less than in the placebo group.

Effects of amiodarone on the wave length.

In figures 11.2, 11.3 and 11.4 the wave length during a rate of 2 Hz, the wave length of the earliest premature beat and the wave length during the highest pacing rate are depicted. During the highest possible pacing

frequency the wave length was 26 % shorter than the wave length at 2 Hz. Treatment for 4 weeks with amiodarone (40 mg/kg/day) only caused minor changes in this parameter. The wave length of the earliest premature beat prolonged by 14 % compared to the placebo group. However this was not statistically significant. The wave length during a regular rhythm of 2 Hz and during the highest pacing rate were not changed under influence of amiodarone. This finding can be explained by the fact that the effect of the prolongation of the refractory period on the wave length was totally neutralized by the concomitant depression of the conduction velocity.

The antiarrhythmic effects of amiodarone in relation to reentrant arrhythmias.

Clinical experience with amiodarone in the treatment of atrial fibrillation looks promising. Especially in paroxysmal atrial fibrillation the success rate is high during chronic oral treatment. Rosenbaum et al. (1974) reported reversion to sinus rhythm in 29 out of 30 patients, Rowland and Krikler (1980) 7 out of 8 and Wheeler et al. (1979) 9 out of 15. This means that the overall success rate is 45 out of 53 patients or 85%. Atrial fibrillation with a recent onset also responds well to chronic oral treatment. Santos et al. (1979) reported that 85% of their patients reverted to sinus rhythm.

Our measurements of the electrophysiological effects of amiodarone in the isolated rabbit atrium do not give a satisfactory explanation for this well documented antifibrillatory action of amiodarone. In contrast with all the other interventions studied in the previous chapters, in case of amiodarone no correlation between changes in the length of the excitation wave and susceptibility to atrial fibrillation could be demonstrated. We have no good explanation for this discrepancy. The most likely explanation is that in case of amiodarone there are marked species differences. Evidence is accumulating that the complex pharmacokinetics of amiodarone are different in the different species. For instance the metabolite desethyl-amiodarone is found in plasma of humans in concentrations as high as amiodarone itself. It is likely that this metabolite has antiarrhythmic properties itself. In the plasma of the rabbit this metabolite is not found suggesting that differences in pharmacokinetics may indeed play an important role as far as the anti-

arrhythmic properties are concerned. In the light of the growing clinical application of amiodarone as an antiarrhythmic drug and because of the marked species differences it might be usefull to measure the effects of amiodarone on the length of the excitation wave in the human heart.

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SUMMARY AND CONCLUSIONS.

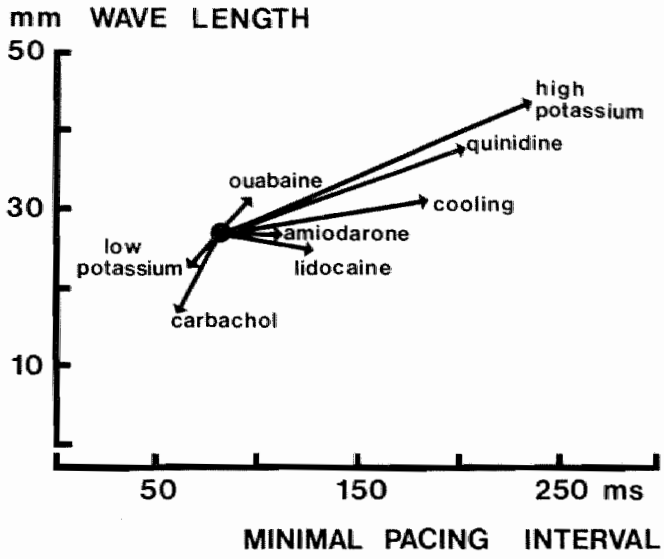
The aim of this thesis was to investigate whether the length of the excitation wave in cardiac muscle can be used as an indicator for the risk of occurrence of reentrant arrhythmias. Whether an impulse can be trapped in a circuitous route depends both on the inhomogeneity in excitability and conduction and on the size of the reentrant circuit. The delicate interplay between these two electrophysiological properties actually determines the chance of initiation of a functionally determined circuit. If the size of a circuit is small, a small area of conduction block will be sufficient to set the stage for a reentrant arrhythmia. On the other hand if the dimensions of a circuit are large, a large area of conduction block is required for the occurrence of reentry. The size of a functionally determined reentrant circuit is equal to the length of the excitation wave, because a tight fit exists between head and tail of the wave front. Interventions which shorten the wave length of the cardiac impulse will decrease the size of the reentrant circuit. As a consequence the number of circuits which can be simultaneously present in a given mass of heart tissue increases. The chance of spontaneous termination of all circuits at the same time will be low. However if an intervention prolongs the wave length, the size of the reentrant circuit will increase. As a result only a limited number of circuits can be present at the same time, making spontaneous termination likely. If our hypothesis is correct that the length of the excitation wave indicates the risk of occurrence of reentrant arrhythmias we should expect that interventions which are known to facilitate the initiation of reentrant arrhythmias will cause a shortening of the wave length of the cardiac impulse. Interventions which terminate reentrant arrhythmias should be expected to increase the length of the excitation wave.

In chapter 2 a simple method to measure the length of the excitation wave in an isolated strip of left atrial tissue is described. By simultaneously measuring conduction velocity and refractory period of the activation wave under conditions comparable to those during a reentrant tachycardia the length of the excitation wave can be calculated from these two basic electrophysiological parameters.

A well known method to initiate reentrant arrhythmias is programmed electrical stimulation of the heart. By either applying a properly timed premature stimulus or by pacing the heart at a rapid rate, reentrant arrhythmias can be initiated as well as terminated. In chapter 3 a description is given of the measurement of the length of the excitation wave during this kind of programmed electrical stimulation. It turned out that the length of the excitation wave of the earliest possible premature impulse was the shortest. At progressively longer coupling intervals the wave length of the premature beat rapidly prolonged. It was observed that the wave length was only shortened in a narrow zone of prematurity (30 - 40 msec after the refractory period). This is consistent with the finding that the reentrant arrhythmias usually can be initiated by critically timed premature beats. Furthermore it was found that the wave length of the cardiac impulse was also considerably shortened at high pacing rates (faster than 5 Hz). Both the shortening of the wave length of premature impulses and the shortening of the length of the excitation wave during rapid pacing support our hypothesis.

In addition changes in the length of the excitation wave were measured during several interventions which are known to exert an arrhythmogenic or antiarhythmic action. The effects of changes in temperature (chapter 4), in the extracellular potassium concentration (chapter 5), of carbamylcholine and epinephrine (chapter 6) and of antiarrhythmic drugs such as lidocaine (chapter 7), quinidine (chapter 8), ouabain (chapter 9), verapamil (chapter 10) and amiodarone (capter 11) were investigated. In the figure below a summary of the results is presented. In this graph the length of the excitation wave (ordinate) is plotted as a function of the minimal pacing cycle length (abcissa). The effects of various interventions can be divided in three groups: 1) interventions which shorten the wave length - indicated by the arrows pointing downwards -, 2) interventions which do not influence the wave length of the impulse - indicated by the arrows pointing horizontally to the right - and 3) interventions which prolong the wave length - indicated by the arrows pointing upwards and to the right.

Ad 1) Both a lowering of the extracellular potassium concentration and administration of carbamylcholine cause a shortening of the wave length (arrows pointing downwards). These interventions are well known for their arrhythmogenic behavior. The observed shortening of the wave length in the



A summary of the effects of different interventions on the wave length and on the minimal pacing interval. Amiodarone (40 mg/kg/day), carbamylcholine ($4x10^{-7}$ g/ml), lidocaine ($5x10^{-6}$ g/ml), ouabain ($3x10^{-7}$ M), low potassium (2.0 mM), high potassium (9.0mM), quinidine (group II), low temperature (26 ^{o}C).

isolated atrial muscle, as measured in this study, indicates that the size of a functionally determined circuit decreases. This is consistent with the arrhythmogenic behavior of these interventions. Furthermore one can expect that the cycle length of the reentrant tachycardia will shorten, as indicated by the fact that the arrow also points to the left.

Ad 2) The effects of lidocaine, amiodarone and moderate cooling are indicated by the arrows pointing almost horizontally to the right. The length of the excitation wave is hardly affected (horizontal position of the arrows), whereas the minimal pacing interval is prolonged (arrows pointing to the right), indicating that the revolution time of a reentrant circuit will increase. The clinical results of the treatment of atrial fibrillation with lidocaine is in agreement with these findings. Moderate cooling during a circus movement based on a functionally determined reentrant circuit, causes a slowing of the rate of the arrhythmia, without a major change in the size of the reentrant circuit. Not in agreement with our hypothesis is the observation that amiodarone does not prolong the length of the excitation

wave in the atrial myocardium. This discrepancy between the well documented antifibrillatory action of amiodarone in the human atrium and the finding that the length of the excitation wave in the rabbit atrial muscle is not prolonged, could not be explained. It is possible that the pharmacokinetic behavior of amiodarone differs from species to species.

Ad 3) A third category consisting of hyperkalemia and of the anti-arrhythmic drugs ouabain and quinidine is indicated by the arrows pointing upwards and to the right. Ouabain causes a slight prolongation of the minimal pacing cycle length and a small prolongation of the length of the excitation wave. However both quinidine and hyperkalemia caused a pronounced prolongation of the minimal pacing interval, indicated by a large shift to the right. Simultaneously the length of the excitation wave is markedly prolonged. In other words if quinidine is administered or if the potassium concentration is elevated the slowing in the rate of a reentrant arrhythmia is accompanied by a prolongation of the length of the excitation wave - indicating that the reentrant circuit will increase in size. As a consequence the chance of spontaneous termination of the arrhythmia becomes more likely. These findings support our hypothesis, since both hyperkalemia and quinidine administration are classical ways to terminate atrial fibrillation.

In conclusion the measurement of the length of the excitation wave in isolated atrial tissue is a rapid and easy method which can unravel the direct effects of different drugs on the wave length of the cardiac impulse. A shortening of the wave length indicates an increased risk of occurrence of reentrant arrhythmias if the inhomogeneity in excitability and conduction is unchanged, whereas an increase in the wave length will make initiation of a reentrant circuit more difficult and spontaneous termination more likely. The results obtained in this thesis support the hypothesis that the length of the excitation wave can be used as an indicator for the risk of occurrence of reentrant arrhythmias.

SAMENVATTING EN CONCLUSIES

Een regelmatige samentrekking van de hartspier is van evident belang voor het functioneren van het menselijk lichaam. Deze samentrekking wordt veroorzaakt door een electrische activatiegolf, die zich gelijkmatig en met grote snelheid (50 - 100 cm/sec) in de hartspier voortplant. Een dergelijke activatiegolf wordt in rust 70 - 80 keer per minuut gegenereerd. Onder bepaalde omstandigheden echter kan een verstoring in dit ritme plaats vinden waarbij zowel een vertraging als een versnelling van de hartfrekwentie kan ontstaan. Sommige ritmestoornissen kunnen zo snel zijn (600 - 700 "slagen" per minuut) dat ze de normale pompfunctie van het hart verstoren waardoor de bloedvoorziening in het lichaam stagneert.

Deze snelle ritmestoornissen kunnen worden veroorzaakt door het feit dat de hartspier voortdurend geactiveerd wordt. Nader onderzoek heeft aangetoond dat de activatiegolf zich dan in een cirkelvormig pad voortplant. Een van de factoren die het onstaan van cirkelgeleiding kan bepalen is de grootte van het circuit. Als het circuit klein is in verhouding tot de grootte van het hart kunnen meerdere circuits tegelijkertijd aanwezig zijn. De kans dat deze circuits allemaal op hetzelfde moment stoppen is klein. Wanneer het circuit echter groot is kunnen er slechts een beperkt aantal tegelijk aanwezig zijn en neemt de kans op spontaan stoppen evenredig toe. Ook bij het starten van deze ritmestoornis is de grootte van het circuit van belang. Hoe kleiner het circuit des te gemakkelijker dit past in de hartspier, hoe groter het circuit des te moeilijker.

De grootte van een dergelijk circuit is gelijk aan de golflengte van het activatiefront. In hoofdstuk 2 wordt een eenvoudige methode beschreven waarmee de golflengte gemeten kan worden. In deze studie werd de geisoleerde linker hartboezem van het konijn gebruikt. Centraal staat de gedachte dat verkorting van de golflengte de kans op het ontstaan en voortduren van deze cirkelgeleiding verhoogt. Daarentegen zal een langere golflengte de kans op het ontstaan en voortduren verminderen. De golflengte werd gemeten onder omstandigheden, die het optreden van cirkelgeleiding bevorderen en onder omstandigheden die dit tegengaan. Wanneer de golflengte inderdaad een van de factoren is die de kans op het optreden van cirkelgeleiding weergeeft verwachten wij dat deze interventies de golflengte op corresponderende wijze zullen veranderen.

Een van de methoden om cirkelgeleiding in het hart op te wekken is geprogrammeerde electrische stimulatie. Door het toedienen van electrische prikkels op bepaalde tijdstippen of door het snel prikkelen van het hart kan cirkelgeleiding opgewekt worden. Het bleek dat beide vormen van electrische stimulatie een verkorting van de golflengte teweegbrachten (hoofdstuk 3). Deze bevinding is in overeenstemming met de gedachte dat een verkorting van de golflengte het optreden van cirkelgeleiding bevordert.

Ook werden twee andere interventies bestudeerd die bekend staan om hun arrhythmogene (=ritmestoornis bevorderende) eigenschappen; nl. een lage kalium concentratie (hoofdstuk 5) en een hoge concentratie acetylcholine (hoofdstuk 6). Ook onder deze omstandigheden werd de golflengte aanmerkelijk korter, geheel in overeenstemming met bovenvermelde hypothese.

Een tweede methode om de hypothese te toetsen bestond uit het toepassen van interventies die de kans op het optreden van deze vorm van ritmestoornis niet of nauwelijks beinvloeden. Voorbeelden hiervan zijn matige afkoeling (hoofdstuk 4), lichte hyperkalemie (hoofdstuk 5) en de anti-arrhythmica lidocaine (hoofdstuk 7) en verapamil (hoofdstuk 10). De golflengte werd zoals verwacht door deze interventies niet noemenswaardig beinvloed.

Tenslotte werd het effect van een aantal anti-arrhythmica bestudeerd waarvan bekend is dat ze deze vorm van cirkelgeleiding bestrijden. Twee van deze middelen zijn quinidine (hoofdstuk 8) en ouabaine (hoofdstuk 9). Deze veroorzaakten inderdaad een verlenging van de golflengte. Een derde geneesmiddel, amiodarone (hoofdstuk 11), bleek geen effect te hebben op de golflengte. Dit resultaat is niet in overeenstemming met onze hypothese. Een duidelijke verklaring voor dit verschil werd niet gevonden. Een mogelijke verklaring zou kunnen liggen in het feit dat het metabolisme van amiodarone bij het konijn anders verloopt als bij de mens.

Concluderend kunnen we stellen dat meting van de golflengte in het geisoleerde hartspierweefsel een eenvoudige methode is, waarmee de directe effecten van allerlei interventies op de lengte van de activatiegolf gemeten kunnen worden. De resultaten van de in dit proefschrift beschreven interventies ondersteunen de hypothese dat de golflengte een belangrijke factor is bij het onstaan en het voortduren van deze vorm van ritmestoornissen.

NAWOORD

Met zeer veel genoegen kijk ik terug op de afgelopen 4 jaren. Niet alleen de aard van het wetenschappelijk werk, maar ook de kameraadschappelijke sfeer, waarin door de sectie ritme en ritmestoornissen van de capaciteitsgroep Fysiologie wordt gewerkt, is daarbij bepalend geweest.

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Bij het uitvoeren van de experimenten, die in deze studie beschreven staan, heb ik steun gekregen van vele mensen. Met name Jan Hollen heeft aan deze proeven een belangrijke bijdrage geleverd. Zijn nimmer falende technische ondersteuning was van veel betekenis voor het welslagen van menig experiment. Astrid Dingjan, Jan Corsel, Eugene Herens en Victor Kaiser wil ik bedanken voor hun aandeel in een aantal van de experimenten.

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CURRICULUM VITAE:

30 maart 1952	geboren te Heerlen
1964 - 1971	Gymnasium b gevolgd aan het Veldeke College te Maastricht.
1971 - 1978	studie geneeskunde aan de Rijksuniversiteit Groningen.
1978 - 1983	Wetenschappelijk ambtenaar bij de capaciteitsgroep Fysiologie, Rijksuniversiteit Limburg.
1 maart 1983	In dienst getreden als arts-assistent bij de capaciteitsgroep Interne Geneeskunde, Rijksuniversiteit Limburg, in het kader van de opleiding cardiologie.