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Citation for published version (APA):

Schiffelers, S. L. H., van Harmelen, V. J., de Grauw, H. A., Saris, W. H. M., & van Baak, M. A. (1999). Dobutamine as selective beta(1)-adrenoceptor agonist in in vivo studies on human thermogenesis and lipid utilization. *Journal of Applied Physiology*, 87(3), 977-981. <https://doi.org/10.1152/jappl.1999.87.3.977>

Document status and date:

Published: 01/01/1999

DOI:

[10.1152/jappl.1999.87.3.977](https://doi.org/10.1152/jappl.1999.87.3.977)

Document Version:

Publisher's PDF, also known as Version of record

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Dobutamine as selective β_1 -adrenoceptor agonist in in vivo studies on human thermogenesis and lipid utilization

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Schiffelers, S. L. H., V. J. A. van Harmelen, H. A. J. de Grauw, W. H. M. Saris, and M. A. van Baak. Dobutamine as selective β_1 -adrenoceptor agonist in in vivo studies on human thermogenesis and lipid utilization. *J. Appl. Physiol.* 87(3): 977–981, 1999.—The use of dobutamine as selective β_1 -adrenoceptor agonist in in vivo studies on human thermogenesis and lipid utilization was investigated in 20 men. At 2.5, 5, and 10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, dobutamine induced significant increases in energy expenditure, lipid oxidation, and lipolysis. The β_1 -adrenoceptor antagonist atenolol (bolus: 42.5 $\mu\text{g}/\text{kg}$, infusion: 1.02 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) blocked all dobutamine-induced effects on thermogenesis and lipid utilization. All parameters remained at levels comparable to those during saline infusion. The dose of atenolol used did not inhibit β_2 -adrenoceptor-specific changes in energy expenditure, lipid oxidation, and lipolysis during salbutamol infusion (85 $\text{ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). This indicates that atenolol was specific for β_1 -adrenoceptors and did not camouflage concomitant β_2 -adrenoceptor stimulation during dobutamine infusion. Therefore, we conclude that dobutamine can be used as a selective β_1 -adrenoceptor agonist at dosages $\leq 10 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in in vivo studies on human thermogenesis and lipid utilization.

atenolol; salbutamol; lipid oxidation; lipolysis

THE SYMPATHETIC NERVOUS system plays an important role in the regulation of human thermogenesis. Sympathetic nervous system activity is mainly stimulated in response to food digestion and physical exercise but can also be triggered by cold exposure or pathogenic stimuli. In response to these stimuli, catecholamines are released that subsequently induce thermogenesis (14). This increase in energy expenditure is due to stimulation of both β_1 - and β_2 -adrenoceptors of the sympathetic nervous system (4). α_1 -Adrenoceptors probably do not play a role (4, 6, 17). The effect of β_3 -adrenergic stimulation on human thermogenesis is, at the moment, still debatable (4, 11, 23), because the available agonists appear to be only weak partial agonists in humans (1). In rodents β_3 -agonists induce significant effects, but this might be explained by the pharmacological differences between human and rodent β_3 -adrenoceptors (10, 15).

In obese men nonselective β -adrenergic stimulation leads to a reduced increase in thermogenesis and lipid utilization compared with in lean men (3). Therefore, it is interesting to know whether these impaired re-

sponses might be due to a defect in the β_1 - or the β_2 -adrenoceptor. The most selective β_1 -adrenoceptor agonist for in vivo use in humans is dobutamine. In lean healthy volunteers dobutamine increases oxygen consumption, indicating an increase in thermogenesis (2, 7), decreases respiratory exchange ratio (RER), suggesting an increase in lipid oxidation, and increases plasma glycerol and nonesterified fatty acids (NEFA) concentrations, indicating an increase in lipolysis (7).

However, both in vitro (15, 16) and in vivo (12, 13) animal studies have shown that dobutamine also has α_1 - and β_2 -adrenoceptor agonistic properties. Because α_1 -adrenoceptors are not important for human thermogenesis, their role was not further investigated. The selectivity of dobutamine for β_1 - and β_2 -adrenoceptors in studies on human thermogenesis and lipid utilization was elucidated in this study. Therefore, we evaluated the effect of atenolol, a predominantly β_1 -adrenoceptor antagonist, on dobutamine-induced increases in energy expenditure, lipid oxidation, and lipolysis. Addition of atenolol should block all β_1 -adrenoceptor-mediated effects and reveal all other effects of dobutamine. In a control test, the selective β_1 -adrenoceptor-blocking properties of atenolol at the dose used were verified. Addition of atenolol should have no effect on the increases in thermogenesis and lipid utilization induced by the selective β_2 -adrenoceptor agonist salbutamol.

MATERIALS AND METHODS

Subjects. Twenty lean male volunteers participated in this study. Mean age and body mass index were 22.0 yr (range: 18–27 yr) and 21.9 kg/m^2 (range: 19.4–25.3 kg/m^2), respectively. The subjects were healthy and took no medication at the time of the study. They gave written informed consent before participating in the study. The study protocol was reviewed and approved by the Ethics Committee of Maastricht University.

Experimental protocol. The study protocol consisted of four tests. In the dobutamine test, a 30-min baseline period was followed by consecutive infusions of 2.5, 5, and 10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ dobutamine (selective β_1 -adrenoceptor agonist; Dobax, Byk, Zwanenburg, The Netherlands), each dose administered during 30 min. This test intended to measure all dobutamine-mediated effects. The saline test consisted of a 30-min baseline period followed by three times a 30-min period of saline infusion (0.6 ml/min) to study the regular changes in thermogenesis and associated metabolic processes over this period of fasting. In the dobutamine plus atenolol test, dobutamine was given (as described above) in combination with the β_1 -adrenoceptor antagonist atenolol (Tenormin, Zeneca, Ridderkerk, The Netherlands) to reveal possible β_2 -adrenoceptor-mediated effects of dobutamine. Therefore, a priming dose of 42.5 $\mu\text{g}/\text{kg}$ atenolol was administered intravenously within 5 min at the start of the baseline period, after

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which a continuous infusion of atenolol ($1.02 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was started for the remainder of the test. The salbutamol plus atenolol test consisted of a 45-min baseline period, after which the β_2 -adrenoceptor agonist salbutamol (Ventolin, GlaxoWellcome, Zeist, The Netherlands) was given for 90 min at an infusion rate of $85 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. During the last 45 min, atenolol was added to the salbutamol infusion at the same dose as described above to study possible β_2 -adrenoceptor-blocking effects of atenolol. The infusion periods were prolonged during the last test, because thermogenesis did not reach steady state within 30 min during salbutamol infusion, as it did during dobutamine infusion. Twenty subjects participated in the dobutamine test, 10 subjects in the saline test, 14 subjects in the dobutamine plus atenolol test, and 10 subjects underwent the salbutamol plus atenolol test. Each of the 20 subjects participated in 2 or 3 trials. There were no statistically significant differences in subject parameters between tests. The study design was single blind, and the order of tests was randomized.

The subjects came to the laboratory at 8:30 AM, with at least 2 days between tests. All individuals were fasted for at least 10 h (overnight) and came to the laboratory by car or by bus to minimize the amount of physical activity before the tests. At the beginning of each test, a catheter was inserted into a forearm vein for drug infusion and blood sampling. During the tests, energy expenditure and RER were continuously measured, and, at the end of each 30- or 45-min interval, a blood sample was obtained. For safety reasons the infusion was stopped when heart rate had increased >30 beats/min and/or mean blood pressure had risen more than 30 mmHg. After these criteria, one subject was not tested at the highest dose of $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ dobutamine. Room temperature was kept between 23 and 25°C.

Methods. An open-circuit ventilated-hood system was used for measurement of whole body energy expenditure and RER. The volume of air drawn through the hood was measured by a dry-gas meter (Schlumberger, Dordrecht, The Netherlands), and the composition of the inflowing and outflowing air was analyzed by a paramagnetic O_2 analyzer (Servomex, Crowborough, UK) and an infrared CO_2 analyzer (Hartmann and Braun, Frankfurt, Germany). Airflow rate and the O_2 and CO_2 concentrations of the inflowing and outflowing air were used to compute O_2 consumption (coefficient of variation 2.4%) and CO_2 production (coefficient of variation 3.1%) on-line every 2 min through an automatic acquisition system interfaced with a personal computer. Energy expenditure was calculated according to the formula of Weir (21). Energy expenditure and RER values were averaged over the last 10 min of each infusion step, during which their values were stable, and their means were used in the data analysis. Blood pressure was measured by an automated blood pressure device (Tonoprint, Speidel & Keller, Jungingen, Germany, and UA 731, Takeda Medical, Rotterdam, the Netherlands) during the last 10 min of each period. The mean of four measurements per interval was computed and used for further analysis. Heart rate was monitored continuously by conventional electrocardiogram and was recorded at the end of every 5-min period. The values over the last 10 min were averaged and used for further analysis.

Analytic methods. Blood samples for glycerol and NEFA determination were preserved in sodium-EDTA. All samples were immediately centrifuged for 1 min at 7,280 g. Plasma was transferred to microtest tubes, rapidly frozen in liquid nitrogen, and stored at -70°C until further analysis. Plasma glycerol concentrations were measured with a glycerol kit (Boehringer 148270, Mannheim, Germany), and plasma NEFA concentrations were measured with the NEFA C kit (Wako NEFA C kit 99475409, Neuss, Germany), both on a Cobas-

Fara analyzer (Roche Diagnostica, Basel, Switzerland). In each run, standard samples with known concentrations were included for quality control.

Data analysis. All values are presented as means \pm SE. The differences in outcome between the dobutamine, the saline, and the dobutamine plus atenolol tests were analyzed with a split-block incomplete-block factorial ANOVA. In this design, categories are made for treatment and subject to account for missing values. Post hoc testing between studies was done according to Bonferroni's inequalities. The effects within studies were analyzed with repeated-measurements ANOVA. Post hoc testing between time points was done with a paired *t*-test, corrected according to Bonferroni's inequalities. All statistical tests were performed two sided. $P < 0.05$ was regarded as statistically significant.

RESULTS

Dobutamine, saline, and dobutamine plus atenolol tests. Energy expenditure increased significantly during dobutamine infusion ($P < 0.001$) (Fig. 1). During

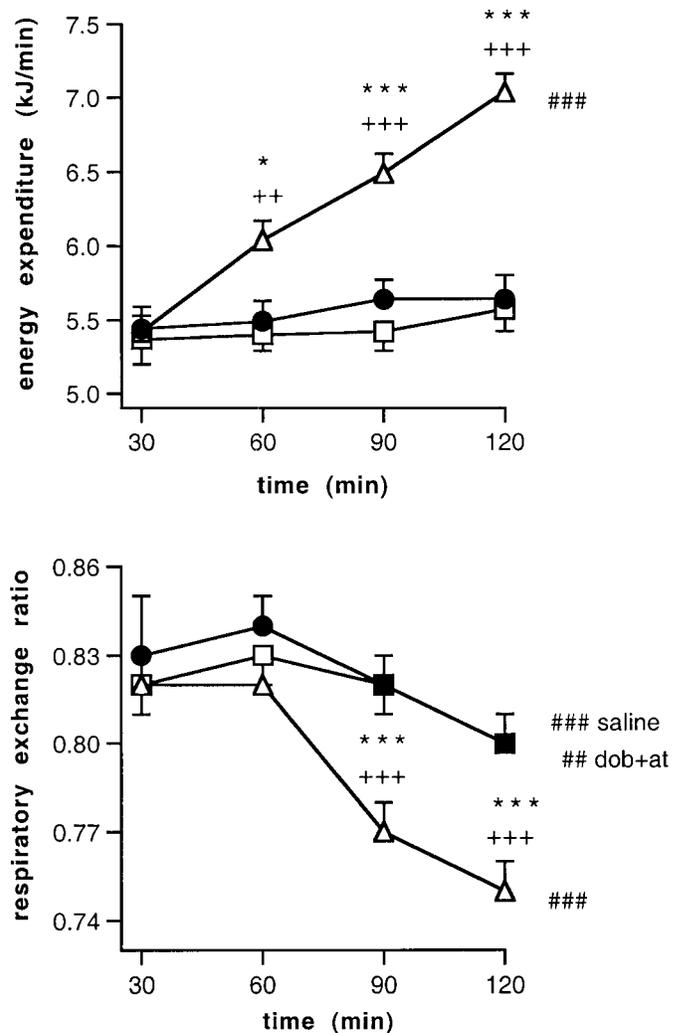


Fig. 1. Energy expenditure (*top*) and respiratory exchange ratio (*bottom*) during infusion of saline (●; $n = 10$ subjects), dobutamine (Δ; $n = 20$ subjects), or dobutamine plus atenolol (dob+at; □; $n = 14$ subjects). Values are means \pm SE. Repeated-measurements ANOVA: ## $P < 0.01$; ### $P < 0.001$. Unpaired *t*-test: dobutamine vs. saline, * $P < 0.05$, ** $P < 0.001$; dobutamine vs. dobutamine plus atenolol: ++ $P < 0.01$, +++ $P < 0.001$.

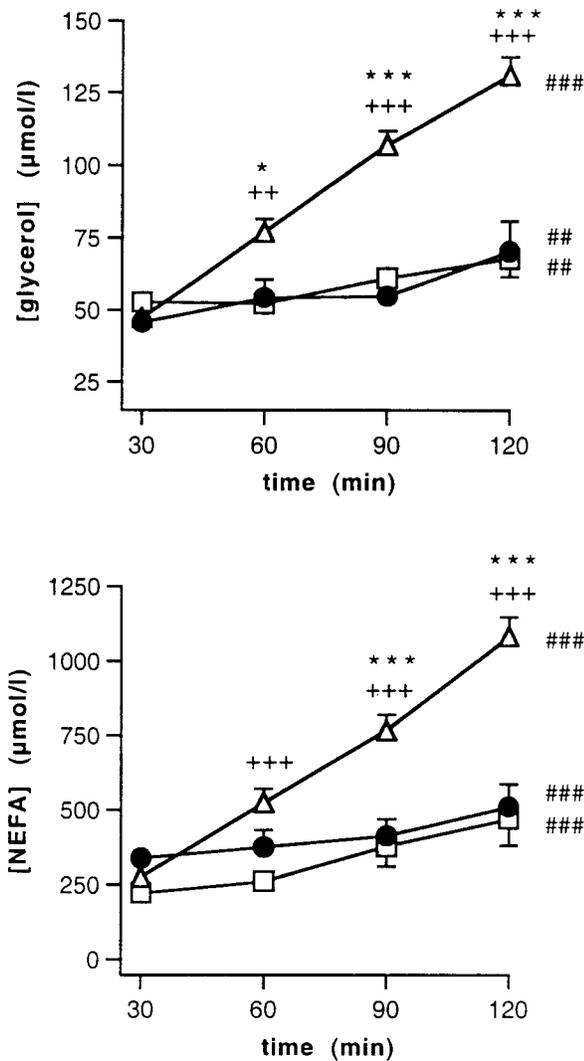


Fig. 2. Plasma glycerol (top) and nonesterified fatty acid (NEFA) concentration (bottom) during infusion of saline (\bullet ; $n = 10$ subjects), dobutamine (Δ ; $n = 20$ subjects), or dobutamine plus atenolol (\square ; $n = 14$ subjects). Values are means \pm SE. Repeated-measurements ANOVA: $\#P < 0.01$, $\#\#\#P < 0.001$. Unpaired t -test: dobutamine vs. saline, $*P < 0.05$, $***P < 0.001$; dobutamine vs. dobutamine plus atenolol: $++P < 0.01$, $++++P < 0.001$.

saline infusion there was no significant change in energy expenditure. Simultaneous administration of atenolol completely prevented the dobutamine-induced increase in energy expenditure. Energy expenditure remained at a similar level as during the saline test. RER decreased significantly in all three tests (dobutamine, saline: $P < 0.001$; dobutamine plus atenolol: $P < 0.01$) (Fig. 1). During the second and third infusion period of dobutamine, RER was significantly lower than during the corresponding infusion periods with saline (both $P < 0.001$). Atenolol infusion prevented the more pronounced reduction in RER at the higher dobutamine dosages. RER decreased to a comparable level as during saline infusion.

Plasma glycerol and NEFA concentrations increased significantly with dobutamine (both $P < 0.001$), saline (glycerol: $P < 0.01$, NEFA: $P < 0.001$), as well as

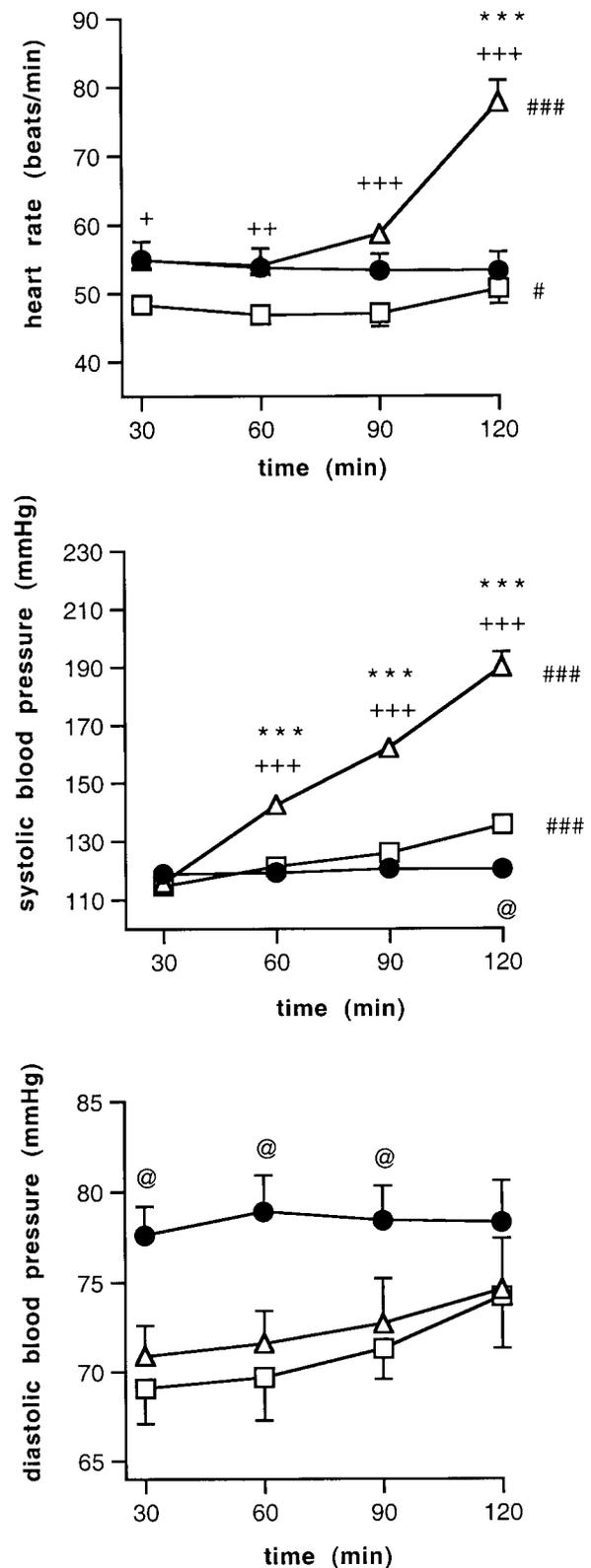


Fig. 3. Heart rate (top) and systolic (middle) and diastolic (bottom) blood pressure during infusion of saline (\bullet ; $n = 10$ subjects), dobutamine (Δ ; $n = 20$ subjects), or dobutamine plus atenolol (\square ; $n = 14$ subjects). Values are means \pm SE. Repeated-measurements ANOVA: $\#P < 0.05$, $\#\#\#P < 0.001$. Unpaired t -test: dobutamine vs. saline, $***P < 0.001$; dobutamine vs. dobutamine plus atenolol: $+P < 0.05$, $++P < 0.01$, $++++P < 0.001$; saline vs. dobutamine plus atenolol: $@P < 0.05$.

dobutamine plus atenolol (glycerol: $P < 0.01$, NEFA: $P < 0.001$) (Fig. 2). In all infusion periods glycerol and NEFA concentrations were significantly higher in the dobutamine test compared with the saline and dobutamine plus atenolol tests. During atenolol administration, glycerol and NEFA levels remained similar to those during saline infusion.

During the last infusion period, heart rate was significantly higher with dobutamine than with saline ($P < 0.001$) (Fig. 3). Heart rate did not change during saline infusion. Atenolol lowered heart rate at baseline and completely inhibited the increase in heart rate by dobutamine. Systolic blood pressure increased significantly ($P < 0.001$), and diastolic blood pressure remained unchanged in the dobutamine test (Fig. 3). Saline infusion caused no changes in systolic and diastolic blood pressure. Atenolol inhibited most of the increase in systolic blood pressure, but there was still a significant increase ($P < 0.001$). Diastolic blood pressure did not change during dobutamine plus atenolol infusion.

Salbutamol test. Energy expenditure significantly increased during salbutamol infusion ($P < 0.001$) and remained at this level after the addition of atenolol (Table 1). RER remained unchanged during the whole test. Plasma glycerol and NEFA concentrations increased significantly during salbutamol infusion (glycerol: $P < 0.01$, NEFA: $P < 0.001$) and decreased after the addition of atenolol (glycerol: $P < 0.05$, NEFA: $P < 0.001$). However, plasma glycerol and NEFA levels remained significantly higher with salbutamol plus atenolol compared with baseline (both $P < 0.01$). Heart rate and systolic blood pressure increased significantly with salbutamol (both $P < 0.001$) and decreased significantly after the addition of atenolol (heart rate: $P < 0.001$, systolic blood pressure: $P < 0.01$). Heart rate remained significantly higher during salbutamol plus atenolol infusion compared with baseline ($P < 0.001$), but systolic blood pressure did not differ from baseline during salbutamol plus atenolol administration. Diastolic blood pressure did not change during the test.

DISCUSSION

This study was performed to examine whether dobutamine can be used as a selective β_1 -adrenoceptor agonist in in vivo studies on human thermogenesis and lipid utilization. Dobutamine induced significant increases in energy expenditure, lipid oxidation, as mea-

sured by a decrease in RER, and lipolysis, as measured by increases in plasma glycerol and NEFA levels. This is in accordance with previous studies (2, 7).

From animal studies it is known that dobutamine has significant α_1 - and β_2 -adrenoceptor-stimulating properties at higher dosages in vitro (12, 13, 15, 16). Furthermore, Daul et al. (5) showed that these adrenoceptors also play a role in the regulation of dobutamine-induced changes in heart rate and blood pressure at dosages of $\geq 6 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in vivo in humans. We intended to study the β_1 -adrenoceptor selectivity of dobutamine for changes in thermogenesis and lipid utilization. For that reason, β_1 -adrenoceptor-mediated effects of dobutamine on energy expenditure, lipid oxidation, and lipolysis were blocked with the selective β_1 -adrenoceptor-antagonist atenolol. This design should reveal all β_2 -adrenoceptor-mediated effects of dobutamine, because α_1 -adrenoceptors play no role in human thermogenesis (4, 6, 17). We found that atenolol completely inhibited the dobutamine-induced increases in energy expenditure and plasma glycerol and NEFA concentrations and the decrease in RER. These parameters remained at levels comparable with those during the saline test, suggesting that dobutamine affects these parameters only via β_1 -adrenoceptor stimulation at the dosages used.

A control test was done to evaluate the selectivity for β_1 - and β_2 -adrenoceptors of the dose of atenolol used. β_2 -Adrenoceptor-mediated effects of salbutamol were compared with those during simultaneous salbutamol plus atenolol infusion. If atenolol blocks β_2 -adrenoceptors, all responses on salbutamol infusion should be impaired after the addition of atenolol. We found that salbutamol-induced changes in energy expenditure and RER were not affected by atenolol. Thus it is unlikely that the diminished increases in thermogenesis and lipid oxidation during simultaneous dobutamine plus atenolol infusion were due to β_2 -adrenoceptor blockade by atenolol at the dosage used. This is also supported by the fact that atenolol has an inhibition constant of 72 ng/ml for β_1 -adrenoceptors and 2,519 ng/ml for β_2 -adrenoceptors (22). Thorne and Wahren (19) reported a plasma atenolol concentration of ~ 300 ng/ml at a dose of $1.67 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. This is comparable with a plasma concentration of ~ 180 ng/ml for the dose of atenolol we used ($1.02 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). The affinity of

Table 1. Parameters at baseline, during salbutamol infusion, and during salbutamol plus atenolol infusion

Parameter	Baseline	Salbutamol ($85 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Salbutamol Plus Atenolol ($1.02 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	ANOVA
Energy expenditure, kJ/min	5.40 ± 0.19	6.20 ± 0.14^b	5.95 ± 0.20^f	<0.001
Respiratory exchange ratio	0.83 ± 0.01	0.82 ± 0.01	0.83 ± 0.01	NS
Glycerol concentration, $\mu\text{mol/l}$	53.6 ± 4.7	115.2 ± 14.2^a	$96.7 \pm 13.1^{c,g}$	<0.001
NEFA concentration, $\mu\text{mol/l}$	249 ± 32	710 ± 75^b	$472 \pm 65^{e,g}$	<0.001
Heart rate, beats/min	56 ± 2	70 ± 3^b	$65 \pm 3^{e,h}$	<0.001
Systolic blood pressure, mmHg	117 ± 3	130 ± 3^b	121 ± 4^d	<0.001
Diastolic blood pressure, mmHg	67 ± 2	61 ± 2	65 ± 3	<0.05

Values are means \pm SE; $n = 10$ subjects. NEFA, nonesterified fatty acids; NS, not significant. Atenolol was bolus administered at $42.5 \mu\text{g/kg}$. Paired t -test: rest vs. salbutamol, ^a $P < 0.01$, ^b $P < 0.001$; salbutamol vs. salbutamol plus atenolol: ^c $P < 0.05$, ^d $P < 0.01$, ^e $P < 0.001$; rest vs. salbutamol plus atenolol: ^f $P < 0.05$, ^g $P < 0.01$, ^h $P < 0.001$.

salbutamol for β_2 - and β_1 -adrenoceptors lies only eight-fold apart (8). This suggests that concomitant β_1 -adrenoceptor stimulation during salbutamol infusion is more likely to have occurred than β_2 -adrenoceptor blockade during simultaneous atenolol infusion. The significant decreases in plasma glycerol and NEFA concentrations after the addition of atenolol might therefore be due to the blockade of the β_1 -adrenoceptor-mediated effects of salbutamol. Another explanation might be that atenolol blocked the basal β_1 -adrenoceptor-mediated effects of the endogenous catecholamines on lipolysis.

It is still uncertain which processes are responsible for sympathetically mediated thermogenesis and in which tissues these processes are localized. Several authors (9, 18) have suggested that the catecholamine-induced increase in whole body energy expenditure may partly be explained by the increase in myocardial energy expenditure caused by an increase in cardiac output. Myocardial energy expenditure can be estimated by the rate-pressure product (heart rate \times systolic blood pressure) (20). In our study, the estimated increase in myocardial energy expenditure would result in an overall increase in energy expenditure of 14% during the dobutamine test and of 2% during the dobutamine plus atenolol test. Whole body energy expenditure, however, increased 33% during the dobutamine test and 5% during the dobutamine plus atenolol infusion. The majority of the increase in energy expenditure, therefore, appeared to result from substrate oxidation in other tissues.

In summary, the results of this study indicate that, at dosages of 2.5, 5, and 10 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, the predominantly β_1 -adrenoceptor agonist dobutamine caused significant increases in energy expenditure, lipid oxidation, and lipolysis. The β_1 -adrenoceptor-antagonist atenolol blocked all dobutamine-induced increases in thermogenesis and lipid utilization. All parameters remained at levels comparable with those during saline infusion. The dose of atenolol used was specific for β_1 -adrenergic blockade and therefore did not camouflage concomitant β_2 -adrenoceptor stimulation by dobutamine. Therefore, we conclude that dobutamine can be used as selective β_1 -adrenoceptor agonist at dosages $\leq 10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in in vivo studies on human thermogenesis and lipid utilization.

The authors thank Jos Stegen and Joan Senden for technical support during analysis of the blood samples and Dr. Arnold Kester for statistical advice.

This study was supported by Netherlands Organization for Scientific Research, Grant 903-39-138.

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Received 16 September 1998; accepted in final form 26 May 1999.

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