Temporal aspects of cyclic messenger signaling in object recognition memory: a pharmalogical approach

Summary

Phosphodiesterases (PDE's) are regulatory enzymes that hydrolyze the cyclic messenger molecules cGMP and cAMP which are involved in early and late consolidation processes, respectively. A large body of pre-clinical research has shown that phosphodiesterase inhibitors (PDE-I's) have the potential to enhance memory in animals. However, human studies into the effects of PDE-I's have not yet been very succesfull at replicating these findings. The aim of this thesis was to extend our knowledge about the pharmacology of PDE4-I rolipram and the PDE5-I vardenafil and further our understanding about the temporal characteristics of cGMP and cAMP signaling in memory formation. Combining behavioral data from the object recognition task (ORT) with the pharmacokinetic (PK) profiles of our test drugs we were able to accurately determine the active windows of cAMP and cGMP in early- and late consolidation processes. Furthermore, we investigated the expression of memory improvement over time and the effects of environmetal conditions on drug efficacy in an attempt to find explanations for the discrepancies between pre-clinical PDE-I work and human studies.

Chapter 1

In this chapter we provide a general introduction into declarative memory and the the impact of cognitive empairment on our society. Furthermore we discribe some of the biological processess that are believed to be underlying memory, and the way in which PDE's are involved in these processes. Next, we adress how PDE-I's can be applied to manipulate these memory processes and enhance cognition and finally, we explain how the ORT can be used to separately investigate different stages of memory formation.

Chapter 2 and 3

In this thesis we exclusively used the ORT for the assessment of memory performance. The ORT is a widely used method to assess memory performance in animals. However, there are many different variants of the ORT and there is debate about the exact memory type that is measured and the relationship between exploration and

discrimination measures. To obtain a proper understanding about our ORT setup, we performed a meta-analysis of over 50 studies with Wistar rats that were performed in our lab. We found that the ORT is a sensitive task, which only requires a short learning trial for the reliable assessment of memory performance. In terms of analysis the actual occurrence of memory is assessed by comparing the discrimination measure to zero, preferably including realistic level of variation. Additionally, effects on memory performance should be assessed by comparison with a vehicle condition. We found that the relative discrimination measures d2 and d3 are very robust against exploration differences, in contrast to the absolute measure (d1) which has a strong positive correlation with the level of exploration. Although naïve animals are instinctively able to successfully discriminate between objects, it is preferable to familiarize the animals to the ORT prior to the actual experiment. Differences in object preference between condition including truly novel and relatively novel objects suggest that rats retain information about prior trials over long periods of time.

CHAPTER 4

There are not many publications about the PK properties of the PDE5-I vardenafil in rats, especially with regards to oral administration. Hence we performed a pharmacokinetic analysis using our preferred administration route for vardenafil (PO). We found that the drug was detectable in the brain within 2 min and unbound brain levels of vardenafil were above IC50 between 10-60 min after administration. The highest central concentration of vardenafil was measured 20 min after administration. Comparing behavioral data from intracerebroventricular (ICV) and oral vardenafil injections in a cholinergic deficit model and interpreting the PK data against a 1h and a 10 min retention intervalr, our data suggest that PDE5 is involved in acquisition processes, which may linger for at least 4-6 min after learning. In addition, the effectiveness of very small amounts of ICV-administered vardenafil provides further evidence for the notion that PDE5-Is improve memory via a central mechanism.

CHAPTER 5

In addition to the PDE5-I vardenafil, we investigated the pharmacokinetic profiles of the PDE4-I rolipram and AChE-I donepezil, in order to further elaborate the signaling windows of acetylcholine (Ach), cGMP and cAMP during memory consolidation in vivo. PK measurements revealed that PO andministered donepezil was detectable in the brain within 4 min after injection. Free brain concentrations above IC50 were reached between 10-60 min after administration and the highest free brain concentration was measured 20 min after the oral injection. In addition, IP administered rolipram was detected in the brain 2 min after injection and reached unbound brain concentrations above IC50 between 4-60 min after injection. The highest central concentration of rolipram was detected at 6 min after injection. Combining the data from the PK measurements to the behavioral data obtained in the ORT using a 1 h interval (with a scopolamine-induced short-term memory deficit) or a 24 h interval (natural forgetting of long-term memory) leads to the suggestion that acquisition processes continue for 6-12 min after the learning trial where they share a short common time frame of with early consolidation process. These acquisition processes also seemed to be affected by vardenafil and rolipram, possibly via an increase in cGMP/cAMP mediated ACh release. Finally, our data suggests that there is a critical window in memory consolidation for cGMP signaling within 47-55 min, and cAMP signaling between 3 to 5.5 h after the learning trial.

CHAPTER 6

It is known that long term memories (LTM) have to be physically incorporated in the synaptic architecture of the brain and that memories are not constant over time. In this chapter we investigated natural forgetting in the ORT in the first 24 h hours after learning and consequently, within these initial 24 h, studied the expression profiles of object memory enhanced by PDE5-I vardenafil and PDE4-I rolipram. Rats were tested at different retention intervals ranging from 1 h to 24 h, using a fixed time point for administration. Object discrimination performance of all groups time-dependently decreased up to an interval of 8 h. We discovered that only after a 10 h retention

interval the memory improving effects of vardenafil and rolipram started to emerge. This delayed manifestation of drug-enhanced memory suggests that two separate memory mechanisms are at play, a quick transient form of memory and a more stable memory form that requires several hours to develop. Furthermore, as both vardenafil and rolipram displayed identical memory expression profiles it appears that the cGMP and cAMP signaling pathways converge on the same downstream target.

CHAPTER 7

Many pre-clinical studies that have shown the potential of PDE-Is a cognition enhancing drugs. However, there convincing evidence about their effectiveness in humans has yet to be found. To explore whether environmental factors can partly explain the discrepancy between human- and animal studies we investigated the effects of environmental enrichment (EE) on the efficacy of PDE5-I vardenafil. Rats were either housed solitarily (SOL) or socially (SOC) under standard conditions, or socially in an EE. We found that EE animals were able remembered object information over longer periods of time in the vehicle conditions compared to SOL and SOC animals. However, vardenafil only improved object memory in SOL and SOC animals. Furthermore, the most effective dose in was lower and the effective dose-range was smaller in SOC animals compared to SOL animals. Our findings indicate that housing conditions have a marked effect on the effectiveness of vardenafil as a cognition enhancer. As PDE5 mRNA and protein expression were not altered between housing conditions it is conceivable that environmentally induced alterations in efficacy may also apply to other classes of cognition enhancers.

CHAPTER 8

In the final chapter of this thesis we discussed our main findings starting with the methodology of the ORT. We discuss how memory stages can be separately assessed using the ORT and the importance of the timing or pharmacological manipulation in relation to the learning- and test-trial. In the following section our findings about the pharmacology of donepezil, vardenafil and rolipram are discussed after which the PK

data are combined with our behavioral findings to determine the active windows of ACh, cGMP and cAMP. Finally, we discuss the implications of the time-dependency of the expression of pharmacologically enhanced memory and environmental influences on drug efficacy. In the final part of our discussion shed light on possible future directions and the potential benefits of multi-drug treatment for cognitive impairments.