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Object memory enhancement by combining sub-efficacious doses of specific phosphodiesterase inhibitors

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ABSTRACT

The second messengers cGMP and cAMP have a vital role in synaptic plasticity and memory processes. As such, phosphodiesterases inhibitors (PDE-Is), which prevent the breakdown of these cyclic nucleotides, represent a potential treatment strategy in memory decline. Recently it has been demonstrated that cGMP and cAMP signaling act in sequence during memory consolidation, with early cGMP signaling requiring subsequent cAMP signaling. Here, we sought to confirm this relationship, and to evaluate its therapeutic implications. Combining sub-efficacious doses of the cGMP-specific PDE type 5 inhibitor vardenafil (0.1 mg/kg) and cAMP-specific PDE type 4 inhibitor rolipram (0.01 mg/kg) during the early and late memory consolidation phase, respectively, led to improved memory performance in a 24 h interval object recognition task. Similarly, such a sub-efficacious combination treatment enhanced the transition of early-phase long-term potentiation (LTP) to late-phase LTP in hippocampal slices. In addition, both object memory and LTP were improved after administration of two sub-efficacious doses of the dual substrate PDE type 2 inhibitor BAY60 7550 (0.3 mg/kg) at the early and late consolidation phase, respectively. Taken together, combinations of sub-efficacious doses of cAMP- and cGMP-specific PDE-Is have an additive effect on long-term synaptic plasticity and memory formation and might prove a superior alternative to single PDE-I treatment.

1. Introduction

Cognitive decline is a hallmark symptom in a wide range of disorders including Alzheimer's disease, Parkinson's disease, and schizophrenia. However, as yet, no satisfying treatment has been found for alleviating this symptom in patients. Therefore, the search for more efficacious nootropic drugs is ever increasing. Over the last years, phopshodiesterase inhibitors (PDE-Is) have been repeatedly reported to demonstrate cognition enhancing effects in preclinical studies (Blokland et al., 2012; Reneerkens et al., 2009). Positive effects of PDE inhibition were reported on memory formation, executive functioning, information processing and attention. Phosphodiesterases (PDE) are enzymes that are responsible for the breakdown of cyclic nucleotides cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) into their respective inactive forms. These cyclic nucleotides are ubiquitous second messenger molecules. Among other functions, they have a key role in relaying incoming signals at the neurons to downstream effectors which enhance synaptic plasticity (Bach et al., 1999; Son et al., 1998; Frey et al., 1993; Lu et al., 1999; Bernabeu et al., 1996; Prickaerts et al., 2002a; Bourtchouladze et al., 1998). It has been demonstrated that enhancing cAMP or cGMP levels enhances hippocampal long-term potentiation (LTP) (Bollen et al., 2014; Puzzo et al., 2009; Vitolo et al., 2002; Palmeri et al., 2013), a physiological phenomenon which is generally considered to be the neuronal correlate of memory (Bliss and Lomo, 1973; Lynch, 2004). The importance of cyclic nucleotides in

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neuronal signaling has consequently led to an increasing amount of studies evaluating the cognition enhancing effects of inhibiting different PDEs.

An interesting feature of the PDE family is that 11 different subfamilies (PDE1–PDE11) have been identified, each with their specific function, regulation and localization pattern (Bender and Beavo, 2006). An important distinction can be made based on the target cyclic nucleotide. PDEs are cAMP-specific (PDEs 4,7,8), cGMP-specific (PDEs 5,6,9) or have dual substrate properties (i.e. cAMP and cGMP-targeting; PDEs 1,2,3,10,11). Memory enhancing effects have been reported for all three types of PDE-Is (Boess et al., 2004; Prickaerts et al., 2004; Rutten et al., 2007). Furthermore, our group has recently shown that, although cGMP and cAMP signaling are both important for consolidation of information into long-term memory, they are involved in different phases of the consolidation process. cGMP signaling is important immediately after acquisition, while cAMP signaling is critically involved at a late consolidation phase which is associated with the implementation of de novo transcribed proteins (Bollen et al., 2014; Rutten et al., 2007). The relationship of cGMP signaling and cAMP signaling has shown to be sequential in both LTP and memory, with activation of the cAMP signaling pathway during the late LTP and consolidation phases being a prerequisite for cGMP-mediated plasticity and cognition enhancement (Bollen et al., 2014).

Cyclic nucleotides and most PDEs are abundantly present throughout the body and the brain (Lakics et al., 2010). Therefore, PDE-Is are likely to instigate adverse side-effects through elevations of cyclic nucleotide levels in non-targeted areas. A well-known example is the prototypical cAMP-specific PDE type 4 (PDE4) inhibitor rolipram, which showed promising antidepressant effects in clinical trials, but the development was eventually stopped because of the severe emetic effects (Hebenstreit et al., 1989).

Given these findings, the aim of the present study is to evaluate whether our knowledge regarding the sequential relationship of cGMP and cAMP signaling can be translated into a superior treatment option by combining different types of PDE-Is. Specifically, we hypothesize that a sub-efficacious dose of a cGMP-targeting PDE-I can facilitate the effects of a sub-efficacious dose of cAMP-targeting PDE-I. This could have a substantial advantage over normal singular PDE treatment as it will lead to less unwanted side-effects. In this study we will combine a sub-efficacious dose of rolipram (late consolidation phase) with the cGMP-specific PDE type 5 (PDE5) inhibitor vardenafil (early consolidation phase) to increase memory performance as measured in the object recognition task (ORT). Additionally, we assessed the effects of administration of two likewise temporally separated sub-efficacious doses of the dual substrate PDE type 2 (PDE2) inhibitor BAY60 7550 on object recognition. Finally, we verified if the effects of our sub-efficacious treatments on memory performance can be attributed to changes in synaptic plasticity by measuring LTP in response to the combined PDE-Is treatment at sub-efficacious concentrations.

2. Methods

2.1. Subjects

The experimental procedures described in this study were approved by the local ethical committee for animal experiments of Maastricht University or of the University of Catania and were in agreement with the respective governmental guidelines.

For behavioral experiments, 3–4-months old male Wistar rats (Harlan, Horst, the Netherlands) were used. Rats were individually housed in standard type 3 Makrolon cages on sawdust bedding. The animals were held in an air-conditioned room (approximately 21 °C) and had free access to food and water. A soft-playing radio provided background noise. A reversed light–dark cycle was applied in the room (lights on between 7.00 PM and 7.00 AM) in order to test the animals during their naturally active period.

For electrophysiological studies C57BL/6J 3-months old male mice were obtained from a breeding colony housed in the animal facility of the University of Catania. Housing conditions of the mice were the same as for rats, except that they were housed socially with 5 animals per cage.

2.2. Object recognition

2.2.1. Apparatus

Animals were subjected to the object recognition task (ORT) (Bollen et al., 2014; Akkerman et al., 2012a). This task was performed in a circular arena with a diameter of 83 cm and walls of 40 cm high. The backside half of the arena wall was made of gray polyvinyl chloride, and the front half of transparent polyvinyl chloride. The objects consisted of four sets including 1) a cone made of brass, 2) a transparent glass bottle, 3) a solid metal beam with two holes and 4) a massive aluminum cube with a tapered top. The animals were unable to displace the objects. All objects were present in three-fold and were cleaned thoroughly after each trial to remove all olfactory traces.

2.2.2. Procedure

ORT procedures were adapted from previous literature (Ennaceur and Delacour, 1988), with modifications as stated elsewhere (Bollen et al., 2014; Prickaerts et al., 1997). During a first trial, rats were put in a circular apparatus, in which two identical objects were placed. 24 h later, the procedure was repeated with one of the objects from the initial trial replaced by another object. During both trials, exploration times were manually scored using a personal computer by the experimenter, who was unaware of the treatment condition tested. Exploration was defined as directing the nose to the object, with a maximal distance between nose and object of 2 cm. Leaning or sitting on the object was not considered exploratory behavior. A relative measure of discrimination was calculated, which was corrected for total exploration time. The resulting discrimination index (exploration time new object − exploration time old object)/(exploration time new object + exploration time old object) reflects recognition memory independent of normal exploratory behavior (Akkerman et al., 2012a). In addition, total time spent exploring objects during trial 1 and 2 (e1 and e2 respectively) was calculated to ascertain that treatment did not affect exploration in general. Animals that did not show normal exploration (T1 < 6 s or T2 < 9 s) were excluded from analysis, as a recent study of our group revealed that in animals with lower exploration times than the given limits, the reliability of discrimination measures weakens considerably (Akkerman et al., 2012a). Testing sessions were between 9.00 AM and 17.00 PM, and were performed under red light conditions while the test room was dimly lit by a lamp (25 W), located in the corner of the room.

2.2.3. Treatment

PDE2-I BAY60 7550 (kindly donated by BAYER AG, Wuppertal, Germany), the PDE4-I rolipram (Sigma Aldrich, Zwijndrecht, Netherlands) and the PDE5-I vardenafil (kindly donated by BAYER AG) were freshly dissolved on the day of testing. Drug administration of the PDE-Is was done either orally (BAY60 7550 0.3 mg/kg and vardenafil 0.1 mg/kg) or intraperitoneally (rolipram 0.01 mg/kg). All PDE-Is were dissolved in the same vehicle (98% methyl cellulose [tylose] solution (0.5%) and 2% tween80) and administered in a volume of 2 ml/kg. To target early and late phases of memory consolidation, the drugs were administrated immediately (T1 + 0h) or 3 h after the learning trial (T1 + 3h). Of note, all treatments were based on previously established sub-efficacious doses and concentrations (Bollen et al., 2014; Rutten et al., 2007, 2006).

2.3. Electrophysiology

Electrophysiological recordings were performed as previously described (Puzzo et al., 2009). Briefly, transverse hippocampal slices (400 μm) were cut and transferred to a recording chamber where they were maintained at 29 °C and perfused with ACSF continuously bubbled with 95% O_2 and 5% CO_2. The ACSF composition was composed of the following (in mM): 124.0 NaCl, 4.4 KCl, 1.0 Na_2HPO_4, 25.0 $NaHCO_3$, 2.0 $CaCl_2$, 2.0 $MgCl_2$, and 10.0 glucose. Field extracellular recordings were performed by stimulating the Schaeffer collateral fibers through a bipolar tungsten electrode and recording in CA1 stratum radiatum with a glass electrode filled with ACSF. A 15 min baseline was recorded with recordings every minute at an intensity that evoked a response approximately 35% of the maximum evoked response. LTP was induced using one 10-burst train (weak tetanus). Responses were recorded for 3 h after tetanization and measured as field excitatory post-synaptic potentials (f-EPSP) slope expressed as percentage of baseline. For electrophysiological experiments, vardenafil (0.3 nM), rolipram (1 nM) or BAY60 7550 (1 nM) were diluted in artificial CSF (ACSF) immediately before use, and applied in the bath solution at different time points before or after the induction of LTP.

2.4. Statistical analysis

According to statistical guidelines for ORT analysis (Akkerman et al., 2012b), we compared all experimental conditions with a fictive group (discrimination index = 0 ± 0.65) using two-sided student t-tests to evaluate whether animals