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Short communication

cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation

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Abstract

The present study investigates the role of cGMP and cAMP on the memory performance in the object recognition task in rats. The analogue 8-Br-GMP or 8-Br-cAMP was administered bilaterally into the hippocampus (0, 1, 3 and 10 μg in 0.5 μl saline/site) immediately after the exposure to two identical objects. After 24 h, saline-treated animals spent equal times exploring a new and the familiar object demonstrating that they did not recognize the familiar one. However, a dose-dependent improvement in object recognition was found after injection of 8-Br-cGMP. In contrast, 8-Br-cAMP did not improve the memory performance at the doses tested. These results indicate that hippocampal cGMP but not cAMP is involved in early stages of consolidation of object memory.

Keywords: Cyclic nucleotide; Phosphodiesterase; Cognition; Hippocampus

1. Introduction

The role of cAMP in memory processes is well established (e.g., Impey et al., 1998). Recently, the cGMP-signaling pathway has been found to play a role in memory processes as well. However, the cGMP-signaling pathway is different from that of cAMP. In addition, it has been suggested that cGMP is involved in early stages of memory formation, whereas cAMP plays a role in later stages of consolidation of memory (Bernabeu et al., 1996, 1997a,b). It should be noted that all these findings are based on one behavioral model, viz. the passive avoidance task. The present study investigated whether cGMP and/or cAMP play a role in early stages of consolidation in another behavioral task, viz. the object recognition task. Recently, we have found that administration of zaprinast, an inhibitor of phosphodiesterase enzymes that selectively break down cGMP, improved the performance in the object recognition task (Prickaerts et al., 1997). The latter already points to an involvement of cGMP in object memory processes. Therefore, the present study evaluated the effects of injection of analogues of cGMP as well as cAMP into the dorsal hippocampus of rats on the memory performance in the object recognition task.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the local ethical committee of the Maastricht University for animal experiments according to governmental guidelines. Twelve 4-month-old male Wistar rats (Charles River, Someren, The Netherlands) were used. The animals were housed individually in standard Makrolon cages on sawdust bedding in an air-conditioned room (about 20 °C). They were kept under a reversed 12/12-h light/dark cycle (lights on from 18:00 to 6:00 h) and had free access to food and water.

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2.2. Treatment

Two weeks before habituation of the object recognition task, the rats were anesthetized with an intraperitoneal (i.p.) injection of a mixture of 0.8 ml xylazine (2%), 1.2 ml ketamine (100 mg/ml) and 8 ml saline (0.9% NaCl). Injection volume was 1.5 ml/250 g body weight. After placement in a stereotaxic frame, bilateral steel cannulas (outer diameter 0.5 mm) were placed above the dorsal part of the hippocampus. The cannulas were fixed to the skull with acrylic dental cement (Paladur) and the tip of the cannulas were at the following coordinates: −3.6 mm anterior, 3.0 mm lateral and 3.0 mm ventral from the bregma (Paxinos and Watson, 1996). The injection cannula was 0.5 mm longer than the guide cannula, so the depth of the injection site was 3.5 mm and aimed at the dorsal hippocampus. After behavioral testing, the brains of the rats were dissected and stored in 4% formaldehyde. The brains were sliced at the level of the cannulas and the infusion site was verified at a macroscopic level.

8-Br-cGMP and 8-Br-cAMP (both Sigma) were freshly dissolved in saline (0.9% NaCl) on every experimental day. 8-Br-cGMP or 8-Br-cAMP was injected bilaterally in a volume of 0.5 μl over a 1-min period. After the injection, the injection needles were left in place for another minute to allow diffusion of the drugs. The different doses of 0, 1, 3 and 10 μg for each drug were given immediately after the first trial in the object recognition task.

2.3. Object recognition task

The object recognition test was performed as described elsewhere (Ennaceur and Delacour, 1988). The apparatus consisted of a circular arena, 83 cm in diameter. Half of the 40-cm high wall was made of grey polyvinyl chloride, the other half of transparent polyvinyl chloride. The light intensity (20 lx) was equal in the different parts of the apparatus. Two objects were placed in a symmetrical position about 10 cm away from the grey wall. We used three different sets of objects. The different objects were: (1) a cone consisted of a grey polyvinyl chloride base (maximal diameter 18 cm) with a collar on top made of brass (total height 16 cm), (2) a standard 1-l brown glass bottle (diameter 10 cm, height 22 cm) filled with water, and (3) a massive metal cube ($10.0 \times 5.0 \times 7.5$ cm) with two holes (diameter 1.9 cm). The objects could not be displaced by a rat.

In the first week, the animals were handled daily and were adapted to the procedure in 2 days, i.e., they were allowed to explore the apparatus (without any objects) twice for 3 min each day. In the two following weeks, the rats were adapted to the testing and infusion procedures after the first trial until they showed a stable discrimination performance, i.e., a good object discrimination at a 1-h interval. Subsequently, testing of the drugs began. A testing session comprised two trials. The duration of each trial was 3 min. During the first trial, the apparatus contained two identical objects. A rat was always placed in the apparatus facing the wall at the middle of the front (transparent) segment. After the first exploration period, the rat was put back in its home cage. Subsequently, after a delay interval, the rat was put back in the apparatus for the second trial, but now with two dissimilar objects, a familiar one and a new one. The times spent in exploring each object during the first and second trial were recorded manually with a personal computer. Exploration was defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. In order to avoid the presence of olfactory trails, the objects were always thoroughly cleaned. Moreover, each object was available in triplicate so none of the two objects from the first trial had to be used as the familiar object in the second trial. In addition, all combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects.

Since we expected the drug treatments to improve memory performance, we needed a delay interval at which no more discrimination between the objects occurs. Therefore, it was chosen to use a delay interval of 24 h, since there is no discrimination between the two objects after this interval. Each week, two testing sessions were given, one session comprised Monday and Tuesday and the other one comprised Thursday and Friday. The four doses of each drug were tested in a random order. First, all doses of 8-Br-cGMP were tested and thereafter, the rats were treated with all doses of 8-Br-cAMP.

2.4. Statistical analysis

The basic measures were the times spent by rats in exploring an object during the first and second trial. On the basis of these exploration measures, habituation and discrimination measures were calculated. Here, we report

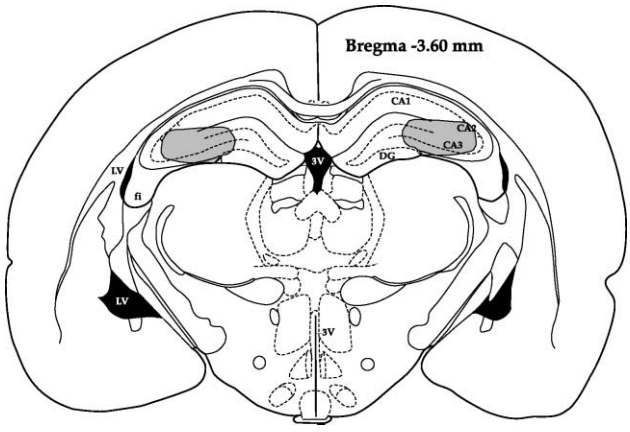

Fig. 1. Schematic outline of the location (shaded area) of the tip of the cannulas in the dorsal hippocampus.

the habituation index *h1*: *h1* = total exploration time first trial – total exploration second trial. We also used the discrimination index *d2*: *d2*=(exploration new object – exploexploration familiar object)/total exploration time in second trial. Testing of each dose of a drug comprised two sessions. The results of similar sessions were averaged into one pooled session. Since each rat served as its own control, a within-subject design was used for overall comparisons (repeated factor Dose). In case of a statistically reliable effect, comparisons between means of the different dose sessions were analyzed in more detail using post hoc Sidak's *t*-tests ($P < 0.05$).

3. Results

Verification of the location of the cannulas revealed that the infusion sites were located in the central part of the dorsal hippocampus (Fig. 1). The index measure of habituation of exploratory behavior *h1* was not affected in both the cGMP and cAMP sessions (Dose effect: F's < 2.28, n.s.). Thus, treatment with the nucleotides did not affect exploratory behavior, which could indirectly affect the measure of discrimination performance of the rats.

After a retention interval of 24 h, rats spent equal times exploring the new and familiar object when treated with saline, which demonstrates that they did not recognize the familiar one (*d2* value not different from zero; $t(11) = 1.14$, n.s.). A dose-dependent improvement in object recognition was found after injection with 8-Br-cGMP (Dose effect: $F(3,33) = 7.37$, $P < 0.01$). Post hoc analysis showed that the *d2* value of the high dose session was higher when compared with the other three doses. No other differences between dose sessions were observed (Fig. 2A).

A within-subjects analysis on *d2* showed again that 24 h after the first trial, the rats did not discriminate between the objects when treated with saline ($t(11) = 1.47$, n.s.). Treatment with 8-Br-cAMP did not improve the object recognition memory since the *d2* indices of all sessions were not different between each other (Dose effect: $F(3,33) = 0.58$, n.s.; Fig. 2B).

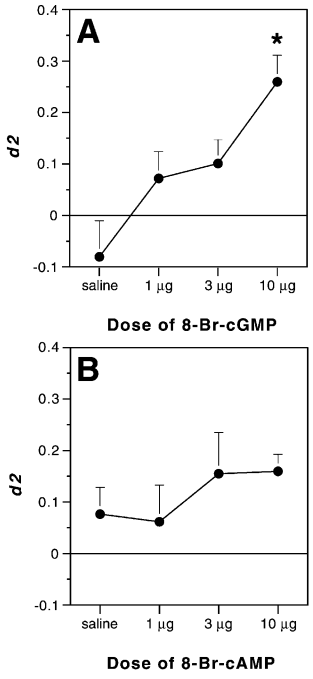

Fig. 2. Effects of 8-Br-cGMP (A) and 8-Br-cAMP (B) on the index of discrimination *d2* in the object recognition task (mean values + S.E.M.). In the nucleotide sessions, rats were treated with doses of 1, 3 and 10 µg (in 0.5 µl saline), bilaterally injected into the hippocampus. * Different from saline (Sidak's *t*-test, $P < 0.05$).

4. Discussion

Our findings showed that administration of 8-Br-cGMP, but not 8-Br-cAMP, into the dorsal hippocampus immediately after the first trial (training) improved the object memory performance. These findings are in agreement with a previous study using the passive avoidance task with rats (Bernabeu et al., 1996). It was shown that 8-Br-cGMP, but not 8-Br-cAMP, injections into the dorsal hippocampus, directly after the training trial, improved the memory performance. The testing paradigm of this task is basically the same as in our task since there is a training trial followed by a test trial after a predetermined interval. However, in this task, rats are shocked and have to remember an aversive stimulus that is in obvious contrast to the task we used in which object information has to be remembered. In untreated rats, the hippocampal cGMP levels peaked immediately after the training trial in the passive avoidance task, whereas a peak in hippocampal cAMP levels was observed at 3 h after training (Bernabeu et al., 1996). Interestingly, when 8-Br-cAMP was injected 3 h after the test trial in the avoidance task, the memory performance was improved. Accordingly, it was concluded that cGMP is important for early stages of memory consolidation, whereas cAMP is necessary for later stages of consolidation processes (Bernabeu et al., 1996). Our findings suggest that this also applies to object memory, i.e., cGMP is also involved in early stages of consolidation of object information, whereas cAMP is not.

Several mechanisms of action of cGMP have been suggested to explain how cGMP exerts its action in processes of memory formation. For example, cGMP is thought to act through regulation of cGMP-gated ion channels, regulation of cAMP-selective phosphodiesterase enzymes or activation of cGMP-dependent protein kinases (Schmidt et al., 1993; Wei et al., 1998). The latter was confirmed by the finding that cGMP levels and cGMP-dependent protein kinase activity were increased in the hippocampus immediately after passive avoidance training

(Bernabeu et al., 1997b). As already mentioned, cAMP was found to play a role in the passive avoidance task at later stages of consolidation processes (Bernabeu et al., 1996). cAMP is thought to act through cAMP-gated ion channels and cAMP-dependent protein kinases (Schmidt et al., 1993; Wei et al., 1998). Especially the latter is interesting since passive avoidance learning resulted in a peak of hippocampal cAMP-dependent protein kinase activity at the same time when cAMP administration was effective, i.e., 3 h after training (Bernabeu et al., 1997a; Vianna et al., 2000). Furthermore, an increase in immunoreactivity of the phosphorylated form of the cAMP responsive element binding protein (CREB) was also found 3 h after training (Bernabeu et al., 1997a).

Despite these findings regarding the possible mechanisms of cGMP and/or cAMP action, it remains unclear how cGMP, or in a later stage cAMP, could result in a changed signal transduction, thereby improving memory performance. In addition, it has to be noted that, although assumptions can be made based on interesting findings (e.g., Impey et al., 1998; Zhuo et al., 1998; Lu et al., 1999), it is in fact unclear where the changes in the signal transduction take place, i.e., in pre- and/or post-synaptic terminals. Besides a change in signal transduction as an explanation for the memory improving effects of cGMP, there is also an explanation based on an increased blood flow since cGMP (and cAMP) is known to result in vasodilatation (e.g., Dundore et al., 1993). Accordingly, it has been shown that topical application of both cGMP and cAMP analogues induced dilatation of cerebral arterioles in the parietal cortex of rats (Paterno et al., 1996). However, cAMP was found to be more effective than cGMP, while we found that the memory performance was only improved after cGMP treatment. This does not support a simple explanation in terms of a vascular mechanism.

Our findings argue for a role of cGMP in the (dorsal) hippocampus in object recognition. However, various rat studies, in which lesions were made of hippocampal pathways (e.g., fornix) and/or rhinal cortex, reported that object recognition memory does not depend on the hippocampus but is dependent on the rhinal (both perirhinal and postrhinal) cortex instead (e.g., Ennaceur and Aggleton, 1997; Bussey et al., 1999). The hippocampus was assumed to be only necessary for spatial information processing (e.g., Ennaceur and Aggleton, 1997). However, recently, there is evidence for a role of the hippocampus in object memory processes since selective lesions of the complete (i.e., dorsal and ventral) hippocampus proper resulted in an object memory deficit (Clark et al., 2000). Nevertheless, the role of the hippocampus in object recognition is still a matter of recent debate (for a review, see Mumby, 2001).

In summary, since the cGMP analogues were administered immediately after training, it is argued that cGMP in the dorsal hippocampus is involved in early processes of object memory consolidation. In contrast, cAMP is probably not involved in early memory consolidation. However, the site and mechanism of action of cGMP in memory processes have to be further elucidated.

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