Research report

Effects of activation and inhibition of cAMP-dependent protein kinase on long-term habituation in the crab *Chasmagnathus*

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Abstract

On sudden presentation of a danger stimulus, the crab *Chasmagnathus* elicits an escape response that habituates promptly and for a long period. We have previously reported that administration of a cAMP-permeable analog (CPT-cAMP) along with a phosphodiesterase inhibitor (IBMX) improves long-term habituation (LTH). In present experiments we studied the effect of systemic administration of the protein kinase A (PKA) activator Sp-5,6-DCI-cBIMPS and that of the PKA inhibitor Rp-8-Cl-cAMPS on LTH tested 24 h after a weak training protocol (5 trials of danger stimulus presentation) or a strong training protocol (15–30 trials), respectively. A 50 µl pre-training injection of 75 µM Sp-5,6-DCI-cBIMPS, and to a lesser degree of 25 µM, improved retention of the habituated response but not affect short-term habituation (STH). Like pre-training injection, post-training administration of Sp-5,6-DCI-cBIMPS proved to exert a facilitatory action on retention though with 75 µM dose only. Conversely, both pre- and post-training injection of 25 µM Rp-8-Cl-cAMPS impaired LTH without affecting STH. Thus, the PKA activator Sp-5,6-DCI-cBIMPS enables a weak training to produce LTH while the PKA inhibitor Rp-8-Cl-cAMPS impairs LTH when a strong training is given. Activation of crab PKA by Sp-5,6-DCI-cBIMPS and its inhibition by Rp-8-Cl-cAMPS were assessed using an in vitro PKA activity assay. These results provide independent evidences supporting the view that PKA plays a key role in long-term memory storage in this learning paradigm.

Keywords: Long-term memory; Learning; Protein kinase A; cAMP analog; Habituation; Crustacea

1. Introduction

Activation of the cyclic AMP (cAMP) pathway was found to be implicated in models of memory and neural plasticity, such as sensitization and synaptic facilitation in *Aplysia* [20], *Drosophila* learning mutants [6,21], and in two types of long-term potentiation in rat hippocampus [13,18,35]. The dependence on the protein synthesis of the long-term stage of memory storage has been a recurrent finding in these and many other models [11].

In the crab *Chasmagnathus* the presentation of a danger stimulus, an opaque rectangular figure passing overhead, elicits an escape response that declines promptly and for a long period (5 days at least) [22,24]. Although this instance of long-term memory meets most of the parametrical conditions of habituation, several results from our laboratory suggest that it is mediated by a conditioned association between contextual cues and the eliciting stimulus [34]. A robust retention of the habituated response is found 24 h after a training session consisting of 15 iterated stimulus presentation or more, but not when only five trials are given [26]. The crab’s long-term habituation (LTH) proved dependent on protein synthesis since drugs interfering with transcription or translation processes induce amnesia without affecting short-term habituation (STH) [23,24]. We have previously reported that systemic administration of the cAMP membrane permeable analog 8-(4-chlorophenylthio)-cAMP (CPT-cAMP), plus the phosphodiesterase inhibitor isobutyl methylxanthine (IBMX) can induce long-term memory of the habituated response when injected before or immediately after a 5-trial training session [25].

Although such a result supports the view that an augment in cAMP level is one of the key steps in the consolidation of crab’s LTH, it is not possible to conclude...
straightforwardly that cAMP-dependent protein kinase A (PKA) is mediating the mnemonic process. In fact, it is known that CPT-cAMP also activates cGMP-dependent protein kinase [29] and that either IBMX or CPT-cAMP increases cGMP levels [9], so that an explanation in terms other than activation of PKA could account for the facilitatory effect of CPT-cAMP + IBMX.

Present experiments are aimed at studying the effect of the PKA activator Sp-5,6-DCI-cBIMPS and the inhibitor Rp-8-CI-cAMPS on retention level of the habituated response, tested 24 h after a weak training protocol (5 trials) or a strong training protocol (15 or 30 trials), respectively. If PKA activation is a necessary step for memory storage in this learning paradigm, it should be expected a facilitatory effect of the activator on retention after the weak protocol and an amnesic effect of the inhibitor after the strong protocol.

Sp-5,6-DCI-cBIMPS and Rp-8-CI-cAMPS are membrane permeant, metabolically stable, analogs of cAMP whose action are highly specific to PKA, both interacting with the regulatory subunit of this kinase [29,37].

2. Materials and methods

2.1. Animals

Animals were adults male Chasmagnathus crabs 2.6-2.9 cm across the carapace, weighing 17 ± 0.2 g (n = 60), collected from water less than 1 m deep in the rias (narrow coastal inlets) of San Clemente del Tuyú, Argentina, and transported to the laboratory, where they were lodged in plastic tanks (35 × 48 × 27 cm) filled to 2 cm depth with diluted marine water, to a density of 35 crabs per tank. Water used in tanks and other containers during experiments was prepared using hw-Marinex (Winex, Germany) every 3 days and after feeding the water was maintained within a range of 22-24°C as well as the alley between them.

Experiments were carried out within the first week after the animal’s arrival. Each crab was used only in one experiment.

2.2. Apparatus

The apparatus is described in detail elsewhere [27]. Briefly, the experimental unit was the actometer: a bowl-shaped plastic container with a steep concave wall and a circular central flat floor 10 cm diameter, covered to a depth of 0.5 cm with marine water. The crab was lodged in the container which was suspended by three strings from an upper wooden framework (23 × 23 × 30 cm) and illuminated by a 10 W lamp placed 30 cm above the animal.

An opaque rectangular screen (a strip of 25 × 7.5 cm) could be moved horizontally over the animal and across the upper surface of the framework in 2.3 s by means of a motor. Screen displacements provoked a crab’s running response and consequent container oscillations. A stylus was centrally cemented to the bottom of the container and connected to a piezoelectric transducer. Container oscillations induced electrical signals proportional to the velocity of the oscillations through the transducer. Such signals were amplified, integrated during the recording time (9 s) and translated into numerical units ranging from zero to 1530, before being processed by computer. Thus, the scores were proportionally correlated to the velocity and number of oscillations recorded during 9 s. The experimental room had 40 actometers, isolated from each other by partitions.

A computer was employed to program trial sequences, trial duration and intertrial intervals, as well as to monitor experimental events.

2.3. Drugs and injection procedure

Crustacean saline solution [17] was used as a vehicle. Drugs were diluted in vehicle from a distilled water concentrated stock solution. Fifty µl of saline or drug solution were given through the right side of the dorsal cephalothoracic-abdominal membrane by means of a syringe fitted with a sleeve to control depth of penetration to 4 mm, thus ensuring that the injected solution was released roughly at the center of the pericardial sac. 5,6-Dichloro-1-β-D-ribofuranosyl-benzimidazole-3',5'-cyclic monophosphorothioate, Sp-isomer (Sp-5,6-DCI-cBIMPS) and 8-chloro-adenosine-3',5'-monophosphorothioate, Rp-isomer (Rp-8-CI-cAMPS) were purchased from Biolog, Germany.

2.4. Experimental procedure

A stimulation session had a fixed number of trials given with 180- or 81-s intertrial intervals and preceded by a 15 min of adaptation time in the actometer. Each trial lasted 9 s and consisted of passing the screen four times over the actometer, recording crab’s activity during the entire trial time. Two sessions per experiment were run, i.e. the training session (5 or 15 trials, 180-s intertrial interval; or 30 trials, 81-s intertrial interval) and the testing session (6 trials, 180-s intertrial interval; or 6 trials, 81-s intertrial interval), separated by a 24-h intersession interval. Crabs were individually housed during the entire intersession interval in plastic containers, covered to a depth of 0.5 cm with water and kept inside dimly lighted drawers.

One group of crabs was injected saline solution (SAL-group) and another one was given either the PKA activator Sp-5,6-DCI-cBIMPS (ACT-group), or the PKA inhibitor Rp-8-CI-cAMPS (INH-group). Half of the animals in each one of these groups were trained (TR-group); and the other half were placed in the actometers but remained untrained (control group, CT-group). Thus, each experi-
ment consisted of four groups \( (n = 20) \) termed SAL-CT, SAL-TR, ACT-CT (or INH-CT) and ACT-TR (or INH-TR). Saline or drug solutions were injected 15 min before the first training trial or immediately after the last training trial.

Before animals were placed in the actometers to start an experiment, they underwent a selection test: each crab was turned on its back and only animals that immediately returned to their normal position were used. The rationale behind this selection is that crabs with a slow righting reaction show a low responsiveness to a large diversity of stimuli and, at a later time, they usually present unhealthy symptoms. No more than 10% of tested crabs were eliminated.

Crab’s baseline responsiveness to the passing screen proved remarkably consistent up to 10 days after arrival, but on occasion animals coming from different capture efforts presented differences in level of responsivity. Therefore, only crabs belonging to the same capture were used in each experiment. In addition, an acceptance criterion aimed at ensuring a minimum level of responsiveness was employed. Every batch of 40 crabs (10 crabs per group) underwent a reactivity test consisting of two pre-training trials, separated by 9 s, given immediately after they had been placed in the actometers. If the mean response values corresponding to these two pre-training trials added scores was lower than 1000, such a batch of crabs was not included in the experiment.

2.5. Data analysis and evaluation of the facilitatory or amnesic effect

Long-term memory was assessed by focusing the data analysis on testing scores. Rescorla [25] convincingly argued in favor of using this sort of analysis at a paired training-testing comparison, stressing the need to clearly distinguish between time of input (training session) and time of assessment (testing session).

In all previous experiments at our laboratory, without exception, a significant difference \( (t\text{-test, } \alpha = 0.05) \) between mean testing scores of SAL-CT and SAL-TR was disclosed 24 h after training, provided that groups consisting of 20 or more crabs each were used, and that they were given a proper amount of training (i.e. 15 training trials with a 180-s intertrial interval for pre-training injected crabs or 30 trials separated by a 81-s interval for those injected immediately after training). By contrast, no significant difference at testing between SAL-groups was found in any case when only five training trials were given. Therefore, to test the amnesic effect of the PKA inhibitor Rp-8-C1-cAMPS, a strong training protocol was used while to test the facilitatory effect of the activator, Sp-5,6-DCI-cBIMPS, a weak training session of five trials was performed.

Throughout this paper, the statistical analysis of testing data included a set of three planned comparisons, namely SAL-CT vs. SAL-TR, ACT-CT vs. ACT-TR (or INH-CT vs. INH-TR) and SAL-CT vs. ACT-CT (or vs. INH-CT), using a weighted means ANOVA with \( \alpha \) (per comparison error rate) = 0.05.

In previous works [26,31] data analysis was confined to the first testing trial. Rationale for this choice was that the escape response level appears to be much more sensitive to changes in amount of training, as well as to amnesic or hyperamnesic agents, at the first testing trial than at the following ones. Thus, when animals undergo a strong training protocol the largest difference between CT and TR groups generally takes place at the first testing trial. When animals are given a weak training or are submitted to an amnesic agent, any CT–TR difference at first testing trial vanishes. On the other hand, the difference at this first trial is restored if a weak training protocol is used together with a facilitatory agent [12,26]. However, re-testing through the first trial may act as a recordatory [8] so that a CT–TR difference could emerge at second and/or successive trials yielding additional information concerning the retention level. Therefore, results interpretation in the present paper is focused on planned comparisons performed on first trial testing scores extending it to data from the following five trials. Training performances are evaluated by repeated measures ANOVA.

2.6. In vitro assay of cAMP analogs effect on PKA activity

The brain PKA activity was measured by cAMP-Dependent Protein Kinase Assay System (Gibco BRL – Life Technologies), based on the phosphocellulose method [28], by using Kemptide as a substrate. Sp-5,6-DCI-cBIMPS (0.1, 0.5 and 1 \( \mu \)M) or Rp-8-C1-cAMPS (10, 100, and 500 \( \mu \)M) was added to the reaction to assess the cyclic nucleotide effect on crab’s PKA activity using 5 \( \mu \)g and 50 \( \mu \)g of protein content of brain extracts, respectively. Animals were anesthetized on 0°C water, sacrificed and central brain were immediately dissected and homogenized in 5 mM EDTA and 50 mM Tris, pH 7.5 and centrifuged 5 min at 1000 rpm. Protein content of extracts was estimated by Bradford method.

2.7. Definitions

The following expressions are used with the meaning here defined: short-term habituation (STH) refers to the response decrement within training session; long-term habituation (LTH) to a retention of the response decrement demonstrated in the testing session.

3. Results

3.1. \( \text{Sp-5,6-DCI-cBIMPS improves LTH} \)

Since the hypothesis of this section is that an increase in PKA activity contingent with training improves long-term
memory, a 5-trial training session was used, that is, experimental conditions insufficient to ensure a significant long-term retention of the habituated response. Each experiment in this section consisted of the groups SAL-CT, SAL-TR, ACT-CT and ACT-TR.

In a first experiment 75 μM Sp-5,6-DCI-cBIMPS was injected pre-training. Performances of both trained groups during the 5-trial training session are illustrated in Fig. 1a. A 2 × 5 repeated measures ANOVA applied on these data yielded neither a significant between-group difference nor a group × trial interaction (statistical values in legend). Mean scores from pre-training trials (reactivity test) were closely similar for all four groups; this result was repeatedly found along the remaining experiments in this paper (Fig. 1, inset histograms).

Results corresponding to testing are shown in Fig. 2a. As a first step in data analysis, planned comparisons were performed on scores from the first testing trial. As expected, no retention concerning SAL-groups was disclosed, that is, the difference between SAL-CT and SAL-TR failed to reach the significance level (Fig. 2a, left curves), however a significant difference was found for ACT-CT vs. ACT-TR ($F_{1,76} = 6.74$) (Fig. 2a, right curves). When the analysis was extended to the remaining testing trials, a significant difference for SAL-CT vs. SAL-TR appeared only at the second trial ($F = 4.51$) while for ACT-CT vs. ACT-TR significant differences were found at trials 2 ($F = 5.13$), 3 ($F = 8.42$) and 6 ($F = 4.29$). No difference between control groups (i.e. SAL-CT vs. ACT-CT) was found at any trial.

In a second experiment we tested a lower 25 μM pre-training injection dose. Results at training were as above (data not shown). Concerning first testing trial scores (Fig. 2b), no significant difference between SAL groups (left curves) nor for a comparison between control groups were found, but a significant difference for ACT-CT vs. ACT-TR ($F = 5.78$) (right curves) was disclose. That is, the facilitatory effect of a 25 μM dose seems to be similar to that obtained with 75 μM. However, when planned comparisons were performed on data from the remaining testing trials, a single significant differences was revealed at second trial, either between SAL groups ($F = 4.63$) or between ACT groups ($F = 4.0$), thus suggesting that the effect of 25 μM would be weaker than that of 75 μM Sp-5,6-DCI-cBIMPS.

Therefore, pre-training injection of 75 mM Sp-5,6-DCI-cBIMPS, and to a lesser degree 25 μM, appears to improve retention of the habituated response at 24 h. This outcome could hardly be explained by an unspecific depressant effect of the drug, since no significant differences appear.
between trained groups at training (SAL-TR vs. ACT-TR) nor between control groups at testing (SAL-CT vs. ACT-CT) were found.

A third experiment was conducted to explore the effect of 75 μM Sp-5,6-DCI-cBIMPS when injected immediately after the last training trial. The experimental design was as above. No between group differences were found at training (data not shown). Results corresponding to the testing session (Fig. 3a) paralleled those obtained with pre-training injection of 75 μM (Fig. 2a). Namely, analysis of data from the first testing trial revealed no retention for SAL-groups but a good retention for ACT-groups (F = 4.75); on the other hand, analysis of the remaining testing trials disclosed significant difference for SAL-CT vs. SAL-TR at trials 2 (F = 6.02) and 4 (F = 7.23), and also for ACT-CT vs. ACT-TR at trials 2 (F = 4.62), 3 (F = 6.22) and 4 (F = 6.04). No difference between control groups (i.e. SAL-CT vs. ACT-CT) was found at any trial.

To ascertain the lowest effective post-training Sp-5,6-DCI-cBIMPS dose, 50 and 25 μM were tested. Results from the testing session corresponding to the experiment with 50 μM are presented in Fig. 3b. Planned comparisons focused on the first testing trial yielded no significant difference either between SAL-CT and SAL-TR or between ACT-CT and ACT-TR. Analysis performed on data from the remaining trials showed a single significant dif-
ference between ACT-groups at second trial ($F = 4.85$). No significant difference was found for any comparison when a 25 $\mu$M dose was tested (data not shown).

Therefore, post-training injection of Sp-5,6-DCI-cBIMPS proves to exert, as well as pre-training injection, a facilitatory effect on retention of the habituated response. However, unlike pre-training Sp-5,6-DCI-cBIMPS, a dose as high as 75 $\mu$M is necessary to show a clear-cut effect on retention. Recent results from our laboratory suggest that animals injected immediately after training show a mild amnesic effect apparently induced by the injection itself. Such a finding would account for the need of higher Sp-5,6-DCI-cBIMPS post-training doses to obtain a consistent facilitatory effect.

### 3.2. Rp-8-Cl-cAMPS induces amnesia

The purpose of experiments in this section is to test the hypothesis that inhibition of PKA activity hinders LTH to a danger stimulus in *Chasmagnathus*. Each experiment in this section consist of the groups SAL-CT, SAL-TR, INH-CT and INH-TR.

During the first experiment, trained crabs underwent 15 training trials separated by 180 s and were tested 24 h later. A 25 $\mu$M Rp-8-Cl-cAMPS dose was pre-training injected. Training results are shown in Fig. 1b. A $2 \times 15$ repeated measures ANOVA applied on training data showed neither a significant difference for SAL-TR vs. INH-TR nor a group per trial interaction. Fig. 4a displays

![Graph](image-url)
Fig. 4. Effect of Rp-8-Cl-cAMPS pre- or post training injection on long-term habituation. Mean response ± S.E.M. of the six testing trials. a: 25 μM Rp-8-Cl-cAMPS or saline was injected 15 min before training, testing at 24 h. b: 25 μM Rp-8-Cl-cAMPS or saline was injected immediately after training, testing at 24 h. n = 20. Symbols as in Fig. 2.

results corresponding to testing. Planned comparisons focused on the first testing trial disclosed a significant difference for SAL-CT vs. SAL-TR (F = 3.97) but not for either INH-CT vs. INH-TR or SAL-CT vs. INH-CT. When the analysis was extended to the remaining trials, differences reached significance for SAL-CT vs. SAL-TR at every testing trial but the fourth trial (trial 2, F = 3.99; trial 3, F = 5.67; trial 5, F = 5.77; trial 6, F = 3.96). By contrast, no significant difference was disclosed either for INH-CT vs. INH-TR or between CT-groups at any trial.

A second experiment was conducted to test the effect of 25 μM Rp-8-Cl-cAMPS when injected immediately after training. To ensure a good retention, a 30-trial training session with 81-s intertrial interval was given. No be-

Table 1
Sp-5,6-DCI-cBMPS activation of PKA in crab brain extracts: phosphotransferase activity (% of maximal minus basal activity)

<table>
<thead>
<tr>
<th>PKI (Sp)</th>
<th>0.1 μM</th>
<th>0.5 μM</th>
<th>1 μM</th>
<th>cAMP 10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>plus PKI</td>
<td>plus PKI</td>
<td>plus PKI</td>
<td>plus PKI</td>
</tr>
<tr>
<td>0.1 μM</td>
<td>13.1</td>
<td>-4.4</td>
<td>50.5</td>
<td>88.3</td>
</tr>
<tr>
<td>0.5 μM</td>
<td>-2.1</td>
<td>-4.4</td>
<td>10.1</td>
<td>17.3</td>
</tr>
<tr>
<td>1 μM</td>
<td>-2.1</td>
<td>-4.4</td>
<td>-2.1</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Data represent means of closely agreeing triplicates. 5 μg of protein content in brain extracts. PKI concentration: 1 μM.
between-group differences in training performances were found (data not shown). Results from testing are depicted in Fig. 4b. Analysis of data corresponding to the first trial disclosed a significant difference between SAL-groups ($F = 6.36$) but neither between INH-groups nor between control groups. As regards the remaining testing trials, a significant difference for SAL-CT vs. SAL-TR was disclosed at every trial (trial 2, $F = 5.64$; trial 3, $F = 8.26$; trial 4, $F = 6.73$; trial 5, $F = 11.88$ and trial 6, $F = 4.02$), while the between INH-group differences failed to reach significance at any trial except the fourth trial ($F = 5.23$).

Thus, pre- or post-training injection of 25 $\mu$M Rp-8-Cl-cAMPS impairs retention of the habituated response at 24 h. This outcome could hardly be explained by an unspecific enhancing effect of Rp-8-Cl-cAMPS, since no significant difference was found either between trained groups at training (SAL-TR vs. INH-TR) with pre-training injected drug or between control groups at testing (SAL-CT vs. INH-CT) either with pre- or post-training drug injection.

3.3. Activation and inhibition of PKA activity by the respective cAMP analogs in crab brain extracts

Foregoing results demonstrated that the two cAMP analogues employed are able to alter long-term memory in the crab. However, in order to conclude that PKA is actually implicated in the formation of Chasmagnathus long-term memory, biochemical characterization of the effects of both Sp-5,6-DCI-cBIMPS and Rp-8-Cl-cAMPS on the crab cAMP-dependent protein kinase was required. With such a purpose, in vitro kinase assays using extracts of the Chasmagnathus central brain were performed.

Results summarized in Table 1 show a dose dependent increase of phosphotransferase activity by Sp-5,6-DCI-cBIMPS in brain extracts with 5 $\mu$g of protein content. Addition of the pseudosubstrate PKI, a potent an specific inhibitor of PKA catalytic subunit, clearly reduced the level of PKA activity, thus indicating that the effect of the cAMP analogue was essentially due to the crab PKA activation.

Antagonist effect of Rp-8-Cl-cAMPS (Table 2) was only found when a larger amount of substrate (50 $\mu$g of protein content) was employed and the analogue concentration was 100- or 500-times higher than that of cAMP. By contrast, memory was impaired by a 50 $\mu$L injection of 25 $\mu$M Rp-8-Cl-cAMPS which may be considered in tissues as a concentration of less than 250 nM seeing that the hemolymph volume is roughly 5 ml – 30% of the body weight [15] – and assuming that drug diffuses evenly throughout the crab’s body. Such a discrepancy would be attributable to the difference in PKA concentration between that the intact cell and in current in vitro assay [14]. An increase of phosphotransferase activity was induced by the analogue in the absence of exogenous cAMP when a lower concentration was used (10 $\mu$M; Table 2) thus suggesting that Rp-8-Cl-cAMPS may be a partial agonist [32].

4. Discussion

Results from the first five experiments show that activation of PKA by means of pre- or post-training administration of the c-AMP permeant analog Sp-5,6-DCI-cBIMPS improves LTH. These findings are in keeping with our previous report [26] about the effect of another cAMP analog, CPT-cAMP, injected along with the phosphodiesterase inhibitor IBMX. In both cases manipulation that elevates cAMP enables a weak training protocol to produce a robust retention, thus suggesting that activation of the cAMP-PKA pathway during training is involved in the installation of LTH to a danger stimulus in Chasmagnathus.

The former interpretation is confirmed by results from the last two experiments in this paper showing that pre- or post-training injection of Rp-8-Cl-cAMPS, a competitive PKA inhibitor, induces amnesia in spite of the fact that animals were given a strong habituation protocol. The amnesic action of Rp-8-Cl-cAMPS suggests that the blockade of the cAMP cascade can interrupt LTH process, providing independent evidence for a key role of PKA in long-term memory storage in this associative learning paradigm. Such an interpretation is supported by the biochemical characterization of the effects of both Sp-5,6-DCI-cBIMPS and Rp-8-Cl-cAMPS (Tables 1 and 2) indicating that the analogues act essentially on the crab cAMP-dependent protein kinase.

By contrast, when experiments with pre-training injection are considered and attention is focused on the training performance (Fig. 1a, b), no effect of either Sp-5,6-DCI-cBIMPS or Rp-8-Cl-cAMPS on STH is manifest. Thus, the purported role for PKA in habituation seems to be circumspect to a late phase in this process, after acquisi-
tion of the habituated response. It is unlikely that the lack of effect in an early phase can be attributed to a delay in drugs reaching their peak action at training. Sp-5,6-DCI-cBIMPS is highly lipophilic and the time to reach the maximal PKA activation in intact cells is roughly between 5 and 10 min while the time of action of Rp-8-Cl-cAMPS is estimated in 15 min [29]. These assessments as well as the fact that both drugs are non hydrolysable by phosphodiesterase suggest that they are already present into the cells during the training course. It is worth noticing that Sp-5,6-DCI-cBIMPS improves andRp-8-Cl-cAMPS impairs 24 h retention when administered immediately after training (Figs. 3 and 4b), suggesting that these drugs do not act on the acquisition process. Ongoing experiments are aimed at determining the time window for these drugs action after training. Accordingly with the present results, previous experiments at our laboratory demonstrated that LTH, but not STH, requires new mRNA and protein synthesis [23,24], thus hinting at the possibility that the persistent phase of habituation entails a cAMP-dependent transcriptional activity.

Previous results from our laboratory suggest that LTH in the crab Chasmagnathus is mediated by an association between contextual cues and the eliciting stimulus [34], so that present findings could be considered as an instance of in vivo regulation by cAMP analogues in an associative form of learning behavior. Similar results were obtained by using other memory and neuronal plasticity models. A pivotal role of cAMP cascade has been found in sensitization of the gills and siphon withdrawal reflex in Aplysia [19]. More recently, it has been reported that long-term synaptic facilitation in Aplysia sensory neurons requires cAMP-mediated regulation of gene expression [4,18]. Early and late phases of long-term potentiation (LTP) of the mossy fiber in the CA3 hippocampal region are blocked by Rp-8-Cl-cAMPS and can be induced by Sp-cAMPS, another PKA activator [18,35]. Persistent phase of LTP (P-LTP), but not transient phase (T-LTP), in the Schaffer collateral pathway in the CA1 neurons of rat hippocampus is induced by Sp-cAMPS and blocked by the inhibitor Rp-cAMPS and by a protein synthesis inhibitor [13]. Mice with targeted mutation of the cAMP-responsive element-binding protein (CREB) are deficient in long-term memory of fear conditioning and Morris water maze task [5]. Heat shock-inducible transgene that expresses a negative dominant CREB impaired long-term memory of olfactory learning in Drosophila [36].

Thus, the cAMP mechanism of signal transduction seems to be a molecular conserved process implicated in long lasting storage of memory present in diverse phylogenetic groups and in a variety of learning tasks.

To our knowledge, the present work is the first report in which cAMP analogues’ action is studied by means of drug administration in intact animals. This family of compounds have been used in neuron cultures [4,10], hippocampal slices [13,18,35] and exposed ganglion prepara-

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