Pharmacology, Biochemistry and Behavior 92 (2009) 224–230

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The type 4 phosphodiesterase inhibitors rolipram and YM976 facilitate recall of the weak version of the passive avoidance task in the day-old chick

Simon F. Crowe [a,*], Joanna Neath [a], Matthew W. Hale [a,b]

[a] School of Psychological Science, Faculty of Science, Technology and Engineering, La Trobe University, Bundoora, Victoria 3086, Australia
[b] Department of Integrative Physiology, University of Colorado, Boulder, CO, 80309-0354, USA

ARTICLE INFO

Article history:
Received 20 August 2008
Received in revised form 9 November 2008
Accepted 24 November 2008
Available online 7 December 2008

Keywords:
Chick
Phosphodiesterase-4
Rolipram
YM976
Memory
Passive avoidance

ABSTRACT

This series of studies examined the effect of the PDE4 inhibitors rolipram and YM976 on the recall of the passive avoidance learning (PAL) task in the day old chick, with a particular focus on the role of D1 receptor in activating this pathway. The results indicated that 2.0 mg/kg rolipram administered 5 min before or immediately, 5, 15 or 30 min following training and 0.025 mg/kg YM976 administered 15 min before or immediately, 5, or 15 min following training facilitated recall of the weak form of the task (i.e. using 20% v/v methyl anthranilate (MeA) as the aversant) at 180 min following training. In each case the effect emerged from 60 min following the training, and was still observable from 180 min to 24 h after training. In addition, whilst administration of 0.5 mg/kg SCH23390, a D1 receptor antagonist, 10 min prior to training disrupted recall for the strong form of the task, co-administration of 2.0 mg/kg rolipram but not 0.025 mg/kg YM976 5 min prior to training prevented this disruption occurring. The results suggest a role for D1 receptor activation in the processes underlying the facilitation of memory by rolipram.

1. Introduction

Phosphodiesterase-4 (PDE4) is an enzyme that catalyzes the hydrolysis of cyclic adenosine monophosphate (cAMP) and plays a critical role in controlling its intracellular concentration (Zhang et al., 2007). Rolipram, a dialkoxyphenyl pyrrolidinone, belongs to a group of neuroactive compounds that have selective PDE4 inhibiting properties, and acts to increase intracellular cAMP levels (Cherry and Davis, 1999; Zhang and O'Donnell, 2000). This agent has been found to facilitate memory performance in mice and rats (Barad et al., 1998; Egawa et al., 1997; Imanishi et al., 1997; Randt et al., 1982; Schneider, 1984; See Blokland et al., 2006 for a recent review), and is also reported to have antidepressant (Bobon et al., 1988; Eckmann et al., 1988; Hebenstreit et al., 1989; O'Donnell and Frith, 1999), antipsychotic (Kanes et al., 2007) and anxiogenic (Zhang et al., 2007) properties. Its role in treatment however has been undermined by its marked side effect profile including emesis and nausea (Bureau et al., 2006; Dyke and Montana, 2002).

Recently a novel PDE4 inhibitor, 4-(3-Chlorophenyl)-1,7-diethylpyrido [2,3-*d*]pyrimidin-2(1-H)-one (YM976), has been developed (Aoki et al., 2000b). YM976 is a pyrimidine derivative and has a different structure from that of the earlier PDE4 inhibitors such as rolipram in that it lacks the 3-cyclopentyloxy-4-methoxyphenyl group (Moriuchi et al., 2003). Despite this structural difference, Aoki et al. (2000a) using *in vitro* cell free experiments, have demonstrated that YM976 is a strong inhibitor of PDE4 with its inhibitory effects noted to be 500 times stronger than those of rolipram. In addition, Aoki et al. have demonstrated that YM976 shows no inhibition of the other PDE isozymes (i.e. PDE1, −2, −3 and −5) indicating its specificity to PDE4 (Aoki et al., 2001), and both rolipram and YM976 appear to equally affect the subtypes of the enzyme, each facilitating the activities of PDE4A, 4B and 4D more strongly than their effect on PDE4C (Aoki et al., 2001).

One of the attractive features of YM976 is that it is reportedly not associated with the common side effects of nausea and emesis observed with rolipram (Aoki et al., 2001), providing a possible alternative avenue for the development of pharmacological treatments for various memory and other forms of physical and mental disorder. YM976 however shows little inhibition of brain, as compared to peripheral, PDE4 activity at a dose of 10 mg/kg, suggesting that YM976 has poor brain penetration as compared with rolipram (Aoki et al., 2001), possibly underlying its lessened emetic action. This aspect of the action of the respective PDE4 inhibitors was of particular interest to this investigation, particularly as these parameters pertain to the respective memory facilitative effects of the two agents.

Whilst it is evident that the administration of PDE4 inhibitors can facilitate memory by increasing cAMP signaling, it has also been demonstrated that manipulation of the biochemical events believed to both precede and follow cAMP synthesis can also impact upon memory processing. Studies have shown that cAMP is necessary for the activation of cAMP dependent protein kinase A (PKA), which results in turn in activation of its target transcription factor, cAMP

* Corresponding author. School of Psychological Science, Faculty of Science, Technology and Engineering, La Trobe University, Bundoora, Victoria 3086, Australia. Tel.: +61 3 9479 1380; fax: +61 3 9479 1956.
E-mail address: s.crowe@latrobe.edu.au (S.F. Crowe).

0091-3057/$ – see front matter.
doi:10.1016/j.pbb.2008.11.014

response element binding protein (CREB) culminating in new protein synthesis (Zhang and O'Donnell, 2000). It is these end processes of the cAMP pathway, that is, protein synthesis and CREB activation, which are believed necessary for long-term memory formation (Gibbs and Ng, 1977; Guzowski and McGaugh, 1997; Rose, 2000, 2004). Disruption of D1 receptor activation, which is believed to trigger the events necessary for cAMP synthesis (Ardenghi et al., 1997; Guzowski and McGaugh, 1997; Zhang and O'Donnell, 2000), can also disrupt long-term memory formation, as can inhibition of the protein synthesis believed to follow on from cAMP synthesis (Blair et al., 2001; Davis and Squire, 1984; Gibbs and Ng, 1977; Okulski et al., 2002; Schafe and LeDoux, 2000).

Our group has previously noted that the timing of the disruption of memory noted with the D1 receptor antagonist, SCH23390 (Hale and Crowe, 2003) corresponds with the increase in whole forebrain levels of cAMP noted between 30 and 60 min after passive avoidance learning (PAL) in the chick (Brown, 1987), as well as with the up-regulation of dopamine D1 receptors observed by Stewart et al. (1996). The onset of amnesia noted with SCH23390, also corresponds with the onset of amnesia reported both by Serrano et al. (1994) and by Zhao et al. (1995) following the application of protein kinase inhibitors. These findings support the notion that D1 receptor activation, cAMP levels and protein synthesis may each play a role in long term memory formation through the cAMP-dependent protein synthesis pathway.

To date, there have been a number of studies which have demonstrated that administration of PDE4 inhibitors, particularly rolipram, can overcome experimentally-induced amnesia following administration of protein synthesis inhibitors (e.g. Randt et al., 1982). Similar findings investigating the possibility of PDE4 inhibitors overcoming receptor antagonist-induced amnesia have also been noted (Imanishi et al., 1997; Rutten et al., 2007; Zhang and O'Donnell, 2000).

It has been argued that the amnesic effect of D1 receptor antagonists occurs by decreasing the synthesis of cAMP necessary to activate protein synthesis (Hale and Crowe, 2003). It is thus possible that concurrent administration of a PDE inhibitor may help to facilitate memory by overcoming the effects of the D1 antagonist. If PDE4 inhibitors were found to facilitate memory formation, an interesting follow up question would be to determine whether the agent can reverse the effects of D1 receptor antagonist-induced memory impairment, therefore facilitating memory by increasing cAMP levels and activating protein synthesis via protein kinase A.

In order to test these hypotheses, an appropriate measure of memory processing which provides precise temporal parameters in defining the stages of memory is required. The PAL task in the day old chick is such a measure (Crowe and Hale, 2002; Rose, 2000, 2004).

The aim of this set of three experimental series was to: 1) examine the ability of the PDE4 inhibitors rolipram and YM976 to facilitate memory consolidation for the weak version of the PAL in the day old chick; and 2) to determine whether D1 receptor activation plays a role in the processes underlying this facilitative effect.

2. Methods

2.1. Animals and housing

Day-old white-Leghorn×New Hampshire cockerels were obtained from a local hatchery on the morning of each experiment. On arrival, chicks were randomly placed in pairs into open-topped wooden boxes (20×25×20 cm). The boxes were maintained at a constant temperature of 20–25 °C using a white incandescent light bulb suspended above each box. Each chick had *ad lib* access to food for the duration of the experiment, but did not have access to water, as thirst acted to increase motivation on the learning task. The chicks were housed in pairs to reduce stress behaviours such as distress calling and attempts to escape, which may have independent effects on memory processing (De Vaus et al., 1980; Johnston and Rose, 1998). One chick in each box was marked for identification during data recording. A group of 20 chicks constituted one experimental group. The La Trobe University Animal Ethics Committee approved the experimental protocol. Cockerels are always employed in these experiments as they are excess to food production of this egg laying strain.

2.2. Drug preparation and administration

All drugs used during the series of experiments were administered via subcutaneous injection into a skin-fold ventral to the rib cage using a 1 ml syringe fitted with a 27.5 gauge needle. In experimental series 1, chicks were injected with rolipram (4-[3-(Cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidinone; Sigma, Castle Hill, New South Wales; Cat #R6520) or DMSO (dimethyl sulphoxide; Sigma, Castle Hill, New South Wales; Cat #D5879). In experimental series 2, chicks were injected with either YM976 (4-(3-Chlorophenyl)-1,7-diethylpyrido[2,3-d]pyrimidin-2(1H)-one; Sigma, Castle Hill, New South Wales, Cat #Y4877) or saline. In experimental series 3, chicks were pretreated with either SCH23390 (R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; Sigma, Castle Hill, New South Wales, Cat #D054) or saline and then treated with either rolipram, DMSO, YM976 or saline. All drugs were prepared on the morning of the experiment and were allowed to adjust to room temperature before use. The drugs and control substances were injected in a total volume of 100 µl. All injections were blind and the codes were not broken until after the behavioural data had been collected. The chemical aversant methyl anthranilate (MeA) was used in the training trial. In the experiments in which a weak training experience was required, MeA was diluted to 20% (v/v) in ethanol (Crowe et al., 1989, 1990). In experimental series 3, 100% MeA was also used.

2.3. Single-trial passive avoidance task

Chicks were trained on a modified version of the single-trial passive avoidance task which has been described in detail elsewhere (Crowe and Hale, 2002; Hale and Crowe, 2003). Briefly, the task involves three components: pretraining, training and retention trials.

2.3.1. Pretraining

At the pretraining stage, chicks were presented with chrome bead, approximately 2 mm in diameter, and a red bead, approximately 5 mm in diameter. Both beads were attached to a straight wire, 250 mm in length. First, the front of the box was tapped gently to gain the chick's attention; the chrome bead was then immersed in water and presented to the chicks to encourage their natural pecking response, as reinforced by the water reward. The bead was presented to the chick by hand for approximately 10 s and this process was repeated after a period of about 20 min. Following the second pretraining trial with the chrome bead, the chicks were presented with the red bead. Again, the bead was dipped in water, and after gently tapping on the front of the box, the bead was presented for 10 s. The number of pecks at the red bead was recorded using a hand held data event recorder. For all trials, a peck was scored when the chick made full contact with the bead. The use of the pretraining red bead was necessary to establish a baseline level of pecking for each chick. These data were used in the subsequent statistical analysis.

2.3.2. Training

A red bead, visually identical to that used in the pretraining, was used in the training trial. The bead was dipped in either the chemical aversant methyl anthranilate (MeA) or water. The bead was presented to the chick for 10 s. In experimental series 1 and 2, MeA was diluted to 20% v/v in ethanol; in experimental series 3, both 20% and 100% MeA were used. Chicks typically show a disgust reaction after pecking at

the aversive bead including shaking their heads, closing their eyes and occasionally wiping their beaks on the floor of the box. Chicks that failed to peck at the red bead during the 10 second trial were excluded from the subsequent data analysis. In keeping with our previous experience with this task, no more than 10% of the 20 chicks initially employed in each group were excluded on this basis (Crowe and Hale, 2002). In all experiments water trained chicks were used to control for the non-specific drug effects on motor or arousal functions (Crowe and Hale, 2002).

2.3.3. Retention trial

The retention trial involved presenting the chicks with a visually identical dry red bead. The retention trial was conducted at various times following training according to the experimental protocol. The number of pecks at the bead was recorded. An avoidance ratio (AR) was calculated as the number of pecks at the red pretraining bead divided by the number of pecks at the red test bead plus the number of pecks at the red pretraining bead (i.e. AR = pecks pre/pecks pre + pecks test).

2.4. Experimental series

Experimental series 1 consisted of a dose response, time of injection and time course of retention for rolipram. For the dose response, rolipram was prepared in doses of 0.125, 0.25, 0.5, 1.0, 2.0 mg/kg and compared with vehicle (DMSO)-treated controls. Chicks were tested for retention at 180 min post training. For the time of injection study, rolipram (2.0 mg/kg) or vehicle were injected at −30, −15, −5, 0, +5, +15, +30 and +45 min relative to the training trial and chicks were tested for retention at 180 min post training. For the retention time course rolipram (2.0 mg/kg) or vehicle was injected 5 min prior to training and retention was tested at 10, 30, 60, 90, 180 and 1440 min post training.

Experimental series 2 consisted of a dose response, time of injection and time course of retention for YM976. For the dose response, YM976 was prepared in doses of 0.025, 0.05, 0.5, 1.0, 2.0 mg/kg and compared with vehicle (saline)-treated controls. Chicks were tested for retention at 180 min post training. For the time of injection study, YM976 (0.025 mg/kg) or vehicle were injected at −30, −15, −5, 0, +5, +15 and +30 min relative to the training trial and chicks were tested for retention at 180 min post training. For the retention time course YM976 (0.025 mg/kg) or vehicle was injected immediately after training and retention was tested at 10, 30, 60, 90, 180 and 1440 min post training.

Experimental series 3 examined the effects of rolipram and YM976 on SCH23390-induced amnesia on chicks trained with the weakly reinforced training stimulus (20% MeA), strongly reinforced training stimulus (100% MeA) or water. Ten minutes prior to the training trial chicks were injected with either SCH23390 (0.5 mg/kg) or saline followed 5 min later (i.e. 5 min before the training trial) by injection of rolipram, DMSO, YM976 or saline. Retention was tested at 180 min post training.

2.5. Statistical analysis

The analysis was performed on the mean avoidance ratios of each group using analysis of variance (ANOVA), with post-hoc tests employed where appropriate. The analyses were conducted using SPSS software (Version 11.5 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Experimental series 1: The effects of rolipram on memory formation

Rolipram facilitated memory formation for the weakly reinforced training stimulus (20% MeA) as compared with the vehicle-treated control group (Fig. 1A). Factorial ANOVA for training (20% MeA and water-trained controls) and dose (DMSO, 0.125, 0.25, 0.5, 1.0 and 2.0 mg/kg rolipram) revealed a training × dose interaction ($F(5,214) = 3.66$, $p = 0.003$) and main effects for training ($F(1,214) = 57.23$, $p < 0.001$) and dose ($F(5,214) = 4.18$, $p = 0.001$). Levene's test of homogeneity of variance was not met. Dunnett's T3 post hoc tests were therefore used which indicated increased mean avoidance ratios in the 0.25, 0.5 and 2.0 mg/kg rolipram groups as compared with the vehicle treated controls. The 2.0 mg/kg dose of rolipram yielded the highest avoidance ratio (0.94 ± 0.02) and was therefore used in subsequent experiments. Water trained control groups were used to address the possibility that the decreased pecking at test was caused by a generalized effect of the drug rather than an effect on memory *per se*. Post hoc tests revealed no difference in mean avoidance ratio between vehicle-treated and rolipram-treated chicks (all $ps > 0.05$).

Rolipram facilitated memory formation when injected at various times both before and after training (Fig. 1B). Factorial ANOVA for the

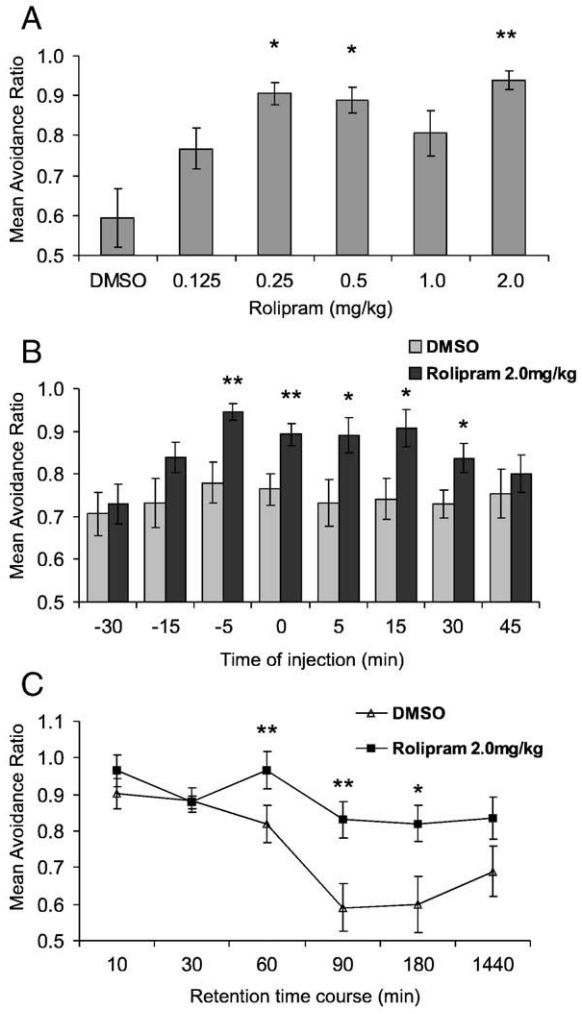

Fig. 1. Graphs illustrating the effects of the PDE4 inhibitor, rolipram, on memory formation following passive avoidance training (mean avoidance ratio ± SEM). Chicks were trained with a weakly reinforced (20% MeA) stimulus and tested for retention at various times after training. A) Dose response. Chicks were injected (s.c.) with vehicle (DMSO) or various doses of rolipram (0.125, 0.25, 0.5, 1.0 and 2.0 mg/kg) immediately after training with 20% MeA and tested for retention at 180 min post training. $^{*}p < 0.05$, $p^{*}0.01$ versus vehicle; post hoc Dunnett T3. B) Time of injection. Chicks were injected (s.c.) with vehicle or rolipram (2.0 mg/kg) at various times before and after training with 20% MeA and tested for retention at 180 min post training. $^{*}p < 0.05$, $^{**}p < 0.01$ versus time matched vehicle control; post hoc Fisher's LSD. C) Retention time course. Chicks were injected (s.c.) with vehicle or rolipram (2.0 mg/kg) 5 min prior to training and tested for retention at various times post training. $^{*}p < 0.05$, $^{**}p < 0.01$ versus time matched vehicle control; post hoc Fisher's Protected LSD tests.

time of injection study revealed main effects for both training ($F(7,628)=148.60$, $p<0.001$) and drug ($F(7,628)=28.43$, $p<0.001$). Post hoc pair wise comparisons revealed an increase in mean avoidance ratio in the rolipram- as compared to vehicle-treated chicks when the drug was injected between 5 min before training and 30 min after training. Among the water trained chicks, there were no differences between the rolipram- and vehicle-treated chicks at any time of injection (all $ps>0.05$).

When injected at the dose of 2.0 mg/kg 5 min prior to training, rolipram facilitated recall of memory as compared to DMSO (Fig. 1C). Factorial ANOVA for the time course of retention study revealed drug×training ($F(1,408)=6.15$, $p=0.014$) and time×training ($F(5,408)=7.41$, $p<0.001$) interactions and main effects for drug ($F(1,408)=14.96$, p, 0.001), time ($F(5,408)=4.01$, $p=0.001$) and training ($F(1,408)=88.53$, $p<0.001$). The drug×time and drug×time×training interactions were not significant (all $ps>0.05$). Post hoc tests revealed a decrease in mean avoidance ratio for the vehicle- as compared to rolipram-treated chicks at 60, 90 and 180 min post training. Unexpectedly there was no difference between DMSO and rolipram at 1440 min post training.

3.2. Experimental series 2: The effects of YM976 on memory formation

YM976 also facilitated memory formation for the weakly reinforced training stimulus (20% MeA) compared with the saline-treated control group when retention was tested at 180 min post training (Fig. 2A). Factorial ANOVA for training (20% MeA and water-trained controls) and dose (saline, 0.025, 0.05, 0.5, 1.0 and 2.0 mg/kg YM976) revealed main effects for training ($F(1,294)=114.81$, $p<0.001$) and dose ($F(5,294)=3.73$, $p=0.003$). Post hoc Dunnett's test indicated an increase in mean avoidance ratio between the 0.025, 0.05, 0.5 and 2.0 mg/kg doses of YM976 and saline. The 0.025 mg/kg dose of YM976 yielded the highest avoidance ratio (0.83±0.03) and was therefore used in subsequent experiments.

In the time of injection study, YM976 facilitated memory formation when injected at various times both before and after training (Fig. 2B). Factorial ANOVA revealed drug×training ($F(1,362)=8.61$, $p=0.003$) and time×training ($F(6,632)=2.45$, $p=0.024$) interactions and main effects for both training ($F(1,632)=432.58$, $p<0.001$) and drug ($F(1,632)=34.51$, $p<0.001$). Post hoc Fisher's LSD pair wise comparisons indicated an increase in mean avoidance ratio in the YM976- as compared to saline-treated chicks when the drug was injected at −15 min and also between 0 and 15 min after training. In the subsequent studies, YM976 was injected immediately after training. Among the water trained chicks, there were no differences between the rolipram- and vehicle-treated chicks at any time of injection (all $ps>0.05$).

For the time course of retention study for YM976, factorial ANOVA revealed a drug×training interaction ($F(5,466)=27.71$, $p<0.001$) and main effects for drug ($F(1,466)=18.40$, $p<0.001$), time ($F(5,466)=4.43$, $p=0.001$) and training ($F(1,466)=323.98$, $p<0.001$). The drug×time and drug×time×training interactions were not significant (all $ps>0.05$). Post hoc Fisher's LSD pair wise comparisons revealed a decrease in mean avoidance ratio for the saline-treated as compared to YM976-treated chicks at 60, 90, 180 and 1440 min post training. There were no differences between saline- and YM976-treated chicks in the water trained groups at any of the time points sampled (all $ps>0.05$).

3.3. Experimental series 3: The effects of rolipram and YM976 on SCH23390-induced amnesia

Rolipram (2.0 mg/kg) prevented SCH23390-induced amnesia in chicks trained with the strong aversant (100% MeA). Factorial ANOVA for pretreatment (SCH23390 or saline), treatment (rolipram or DMSO) and training (20% MeA, 100% MeA or water) revealed a pretreatment×treatment interaction ($F(2,204)=3.33$, $p=0.038$) and main effects for pretreatment ($F(2,204)=10.67$, $p=0.001$), treatment ($F(1,204)=19.46$, $p<0.001$) and training ($F(2,204)=22.54$, $p<0.001$) (Fig. 3A,B). Post hoc tests for the 20% MeA trained chicks revealed an increased AR in the rolipram-treated as compared to vehicle-treated chicks in both the SCH23390 and saline pretreatment groups (Fig. 3A).

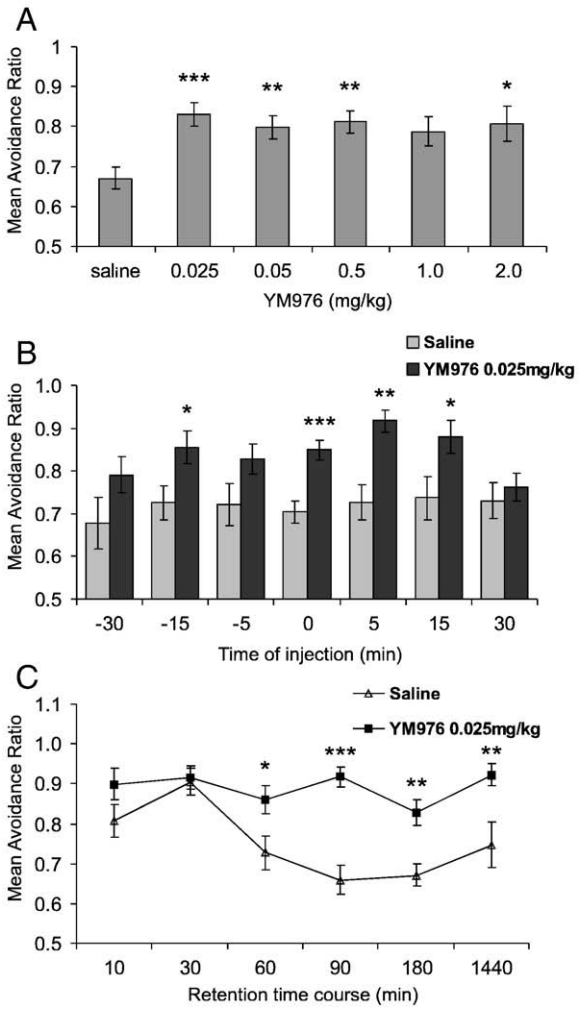

Fig. 2. Graphs illustrating the effects of the PDE4 inhibitor, YM976, on memory formation following passive avoidance training (mean avoidance ratio±SEM). Chicks were trained with a weakly reinforced (20% MeA) stimulus and tested for retention at various times after training. A) Dose response. Chicks were injected (s.c.) with vehicle (saline) or various doses of YM976 (0.025, 0.05, 0.5, 1.0 and 2.0 mg/kg) immediately after training with 20% MeA and tested for retention at 180 min post training. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ versus vehicle; post hoc Dunnett's test. B) Time of injection. Chicks were injected (s.c.) with vehicle or YM976 (0.025 mg/kg) at various times before and after training with 20% MeA and tested for retention at 180 min post training. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ versus time matched vehicle control; post hoc Fisher's LSD. C) Retention time course. Chicks were injected (s.c.) with vehicle or YM976 (0.025 mg/kg) immediately after training and tested for retention at various times post training. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ versus time matched vehicle control; post hoc Fisher's Protected LSD tests.

Consistent with previous research with the strongly reinforced (100% MeA) training stimulus (Hale and Crowe, 2003; Kabai et al., 2004), post hoc tests indicated a decrease in AR in chicks pretreated with SCH23390 as compared with saline-pretreated chicks (Fig. 3B). Post hoc tests indicated an increase AR of rolipram-treated as compared to vehicle-treated chicks in the SCH23390 pretreatment group. Unexpectedly, the post-hoc tests revealed a small but statistically significant difference between rolipram and DMSO-treated chicks in the saline pretreatment group (DMSO: 0.60±0.02; rolipram 0.69±0.03).

Unlike rolipram, YM976 (0.025 mg/kg) failed to prevent the SCH23390-induced amnesia. Factorial ANOVA for pretreatment

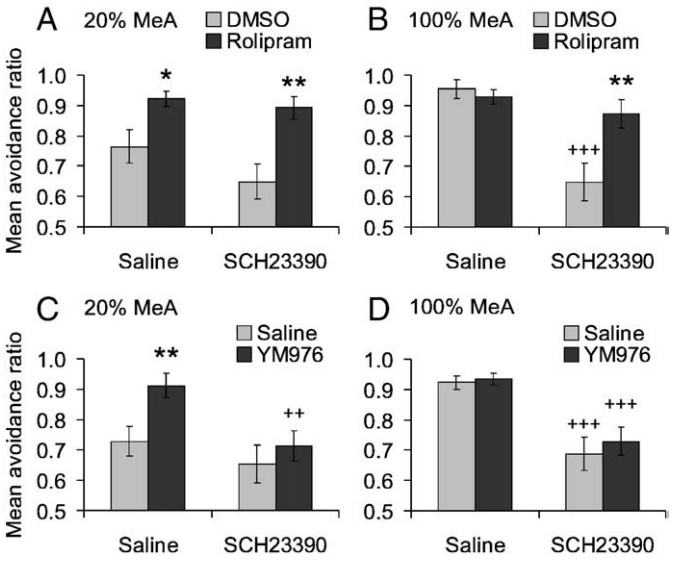

Fig. 3. Graphs illustrating the effects of rolipram and YM976 on dopamine D1 receptor antagonist, SCH23390 (0.5 mg/kg)-induced memory disruption for the passive avoidance task. In the pre-treatment phase, chicks were injected (s.c.) with either SCH23390 or saline. In the treatment phase chicks were injected with vehicle (DMSO), rolipram (2.0 mg/kg), vehicle (saline) or YM976 (0.025 mg/kg). A) Chicks were trained with 20% MeA and treated with either DMSO or rolipram. B) Chicks were trained with 100% MeA and treated with either DMSO or rolipram. C) Chicks were trained with 20% MeA and treated with either saline or YM976. D) Chicks were trained with 100% MeA and treated with either saline or YM976. $^{**}p<0.05$, $^{*}p<0.01$ versus treatment control (i.e. SCH23390/DMSO versus SCH23390/rolipram); Fisher's LSD. $+++p<0.001$, $++p<0.01$ versus pre-treatment control (i.e. SCH23390/DMSO versus saline/DMSO); post hoc Fisher's Protected LSD tests.

(SCH23390 or saline), treatment (YM976 or saline) and training (20% MeA, 100% MeA or water) revealed a pretreatment×treatment interaction ($F(2,213)=6.58$, $p=0.002$) and main effects for pretreatment ($F(1,213)=23.31$, $p<0.001$), treatment ($F(1,213)=4.51$, $p=0.035$) and training ($F(2,213)=54.89$, $p<0.001$) (Fig. 3C,D). For the weakly reinforced (20% MeA) training stimulus data, post hoc tests indicated an increase in mean AR in YM976-treated compared with saline-treated chicks when chicks were pretreated with saline. However, there was no difference in mean AR between YM976- and saline-treated chicks in the SCH23390-pretreated group (Fig. 3C). Among the YM976-treated chicks, SCH23390-pretreatment decreased mean AR when compared with saline-pretreated chicks for the strongly reinforced (100% MeA) training stimulus but there was no difference between YM976- and saline-treated chicks in either the SCH23390 or saline-pretreated groups (Fig. 3D). Post hoc tests did however reveal a decrease in mean AR in both the YM976- and saline-treated chicks in the SCH23390-pretreatment group as compared to the saline-pretreatment groups (Fig. 3D). Post hoc tests indicated no differences among the data for the water-trained chicks (all $ps>0.05$).

4. Discussion

The three experimental series reported in this study demonstrate that 2.0 mg/kg rolipram administered 5 min before or immediately, 5, 15 or 30 min following training and 0.025 mg/kg YM976 administered 15 min before or immediately, 5, or 15 min following training facilitated recall of the weak form of the task (i.e. using 20% v/v methyl anthranilate MeA) at 180 min following training. In each case the effect emerged from 60 min following the training, and was still observable from 180 min to 24 h after training. In addition, whilst administration of 0.5 mg/kg SCH23390, a D1 receptor antagonist, 10 min prior to training disrupted memory for the task, co-administration of 2.0 mg/kg rolipram 5 min prior to training prevented this memory disruption occurring using either the weak or the strong (100% MeA) version of the task. In contrast co-administration of 0.025 mg/kg YM976 failed to reverse the SCH23390-induced amnesia.

Specifically the results of experimental series 1 indicated: 1) that rolipram facilitated memory for the weak version of the PAL task at the doses of 0.25, 0.5 and 2.0 mg/kg with the latter dose producing the largest effect; 2) rolipram needed to be administered between 5 min before training and up to 30 min after training, and administration of rolipram 5 min following training proved to be the most effective time of administration, and 3) when rolipram was administered 5 min following training, the facilitative effect was evident from 60 min following training.

The timing of the facilitative effect of rolipram coincides with the later stages of long term memory processing described by Rose (2000, 2004). This timing also coincides with the timing of increased cAMP activity following PAL training previously noted by Brown (1987). It has been suggested that there is a complex cAMP dependent pathway which is involved in the formation of long-term memories (see Lynch, 2004 for a review). The literature on PDE4 inhibitors including rolipram, indicate that their main action is mediated by increased cAMP signaling by preventing its breakdown (Cherry and Davis, 1999; Zhang and O'Donnell, 2000).

The results of experimental series 2 explored the effects of the novel PDE4 inhibitor YM976 on memory functioning in the chick. These studies demonstrated that: 1) a dose of 0.025 mg/kg YM976, was most effective in facilitating memory performance for the weak version of the PAL task although doses of 0.05, 0.5 and 2 mg/kg were also found to be effective; 2) the facilitative effect of 0.025 mg/kg YM976 was dependent on the time of administration. YM976 facilitated memory performance when administered between 15 min before and up to 15 min following training, with the most effective time of administration being 5 min following training; and 3) administration of 0.025 mg/kg YM976, 5 min following training produced a facilitative effect on performance on the PAL task by 60 min following training with this effect still present at 1440 min following training.

YM976 has previously been demonstrated to be a specific inhibitor of PDE4, which is thought to explain the increase in cAMP activity following administration of this drug (Aoki et al., 2000b). It is most likely that in common with rolipram, YM976 facilitates memory performance by inhibiting the PDE4 enzyme responsible for breaking down cAMP and hence increasing signaling via the cAMP-dependent pathway involved in the formation of long term memory.

Hale and Crowe (2003) have suggested that the increase in cAMP involved in memory consolidation is triggered by activation of the D1 receptor subtype. In support of this suggestion, they have demonstrated that reduced memory performance for the PAL task occurs following administration of the D1 receptor antagonist SCH23390. The role of D1 receptor activation in triggering the processes through which rolipram, and YM976 are thought to facilitate memory was therefore examined in experimental series 3. The results demonstrated that: 1) consistent with the findings of Hale and Crowe (2003), SCH23390 was found to disrupt memory for the strong version of the PAL task; 2) co-administration of 2.0 mg/kg rolipram with SCH23390 produced a facilitative effect on the weak version of the task and prevented the amnesia induced by SCH23390 on the strong version of the task; and 3) co-administration of 0.025 mg/kg YM976 with SCH23390 was not found to prevent the memory disruption caused by SCH23390.

It was concluded from these findings that the facilitative role of rolipram was most likely related to D1 receptor activation. In contrast, the results from this study suggested that D1 receptor activation is possibly not involved in the memory facilitation following administration of YM976. This indicates that despite the similarities in their pharmacological action, that rolipram and YM976 differ in their ability to overcome the challenge to memory processing posed by SCH23390, indicating that there may be subtle differences in their mode of

activity. This difference may be attributable to the divergence of the two drugs with regard to their ability to penetrate the brain (Aoki et al., 2001) and/or their potential to cause emesis which may be mediated by a dopaminergic mechanism.

As noted by Hale and Crowe (2003) the significance of increased cAMP signaling and therefore PKA activation culminates in the phosphorylation of specific proteins including both CREB and DARP-32. When activated, DARP-32 inhibits protein phosphatase 1 (PP-1) (Greengard et al., 1999), which is responsible for dephosphorylating CREB (Liu and Graybiel, 1996). Hemmings et al. (1986) suggest that inhibition of PP-1 by DARP-32 acts to prolong and/or potentiate the physiological effects of CREB by preventing its breakdown. Phosphorylation of CREB is a cAMP/PKA dependent mechanism which has been implicated in memory (Ardenghi et al., 1997; see Lamprecht, 1999 for a review).

The results of this series of studies suggest a relationship between D1 receptor activation and cAMP activity in the processes underlying the facilitation of long term memory by PDE4 inhibitors, which culminates in activation of CREB and the protein synthesis necessary for long term memory consolidation. It is therefore suggested that the facilitative effect of both rolipram and YM976 on weak initial training is most likely related to cAMP dependent PKA molecular pathway, which results in CREB activation and protein synthesis necessary for long term memory consolidation. However neither their synaptic, nor their biochemical basis was directly investigated. The results of the current series of studies do suggest an interaction between rolipram but not YM976 on the D1 receptors in the regulation of learning and memory.

It is assumed that rolipram prevented the amnesia induced by SCH23390 via increasing cAMP intracellular signaling. It is thus possible that rolipram may act directly on the D1 receptor sites themselves as opposed to the processes triggered following their activation. Some support this contention arises from the work of Aoki et al. (2001), in their investigation of the lesser emetic effect of YM976 in comparison to rolipram. When they measured the brain penetration of both rolipram and YM976 by measuring cAMP content in peripheral tissues (peritoneal macrophages) and in brain, they noted that YM976 increased the level of cAMP in peritoneal macrophages, but did not cause significant increase in brain cAMP levels. Rolipram at the same dose on the other hand, elevated the cAMP content of both tissues. It thus seems possible that the differences in action noted with YM976 and rolipram may be attributable to the difference in their respective abilities to have a CNS action which only emerges in the context of the current study with the challenge to the D1 receptor.

It would be useful in further experimentation to determine whether administration of rolipram results in a direct up regulation of D1 receptors in the chick brain, and to determine to what degree this may be age related. Previous research (Harada et al., 2002) has indicated that cAMP activity as well as its functional response to dopamine D1 antagonists shows an age-related response in the brains of primates.

In addressing these issues simultaneous examination of the biochemical, physiological and behavioural effects of PDE4 in the day old chick would be worthwhile. The biochemical cascade that follows training on the PAL task has been noted to occur in two specific regions of the chick brain, the intermediate medial mesopallium and the medial striatum (Rose, 2000, 2004). Furthermore, Brown (1987) has demonstrated increased cAMP signaling in the medial striatum following training on this task. Thus it would be interesting to determine whether there are any changes in brain cAMP activation in these areas following administration of either rolipram or YM976. It would also be useful to determine whether there is direct up regulation of D1 receptors in these areas following administration of rolipram.

The importance of these results in combination with the minimal dose limiting side effects associated with the YM976, indicate that this drug type may potentially be worthwhile in the clinical treatment of age related memory disorders as well as having a possible role to play in the memory deficits associated with depression, anxiety and the psychotic disorders. However the specificity of this effect, particularly with regard to its action on dopaminergic processes, needs to be more clearly determined.

Acknowledgement

This work was supported by an Australian Research and Discovery grant awarded to Professor Simon Crowe [DP0663969].

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pbb.2008.11.014.

References

Aoki M, Fukunaga M, Kitagawa M, Hayashi K, Morokata T, Ishikawa G, et al. Effect of a novel anti-inflammatory compound, YM976, on antigen-induced eosinophil infiltration into the lungs in rats, mice and ferrets. J Pharmacol Exp Ther 2000a;295(3):1149–55.

Aoki M, Kobayashi M, Ishikawa G, Saita Y, Terai Y, Takayama K, et al. Novel phosphodiesterase type 4 inhibitor, YM976 (4-(3-chlorophenyl)-1,7 diethylpyrido [2,3-d]pyrimidin-2(1H)-one), with little emetogenic activity. J Pharmacol Exp Ther 2000b;297:165–73.

Aoki M, Fukunaga M, Kitagawa M, Sugimoto T, Hirano Y, Kobayashi M, et al. Studies on mechanism of low emetogenicity of YM976, a novel phosphodiesterase type 4 inhibitor. J Pharmacol Exp Ther 2001;298(3):1142–9.

Ardenghi P, Barros D, Izquierdo LA, Bevilaqua L, Schroder N, Quevedo J, et al. Late and prolonged post-training memory modulation in entorhinal and parietal cortex by drugs acting on the cAMP/protein kinase A signaling pathway. Behav Pharmacol 1997;8:745–54.

Barad M, Bourtchouladze R, Winder DG, Golan H, Kandel E. Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. P Natl Acad Sci USA 1998;95(25):15020–5.

Blair HT, Schafe GE, Bauer EP, Rodrigues SM, LeDoux JE. Synaptic plasticity in the lateral amygdala: A cellular hypothesis of fear conditioning. Learn Mem 2001;8:229–42.

Blokland A, Schreiber R, Prickaerts J. Improving memory: a role for phosphodiesterases. Curr Pharm Design 2006;12(20):2511–23.

Bobon D, Breulet M, Gerard-Vandenhove MA, Gurot-Goffioul F, Plomteux G, Sastre-y-Hernandez M, et al. Is phosphodiesterase inhibition a new mechanism of antidepressant action? Eur Arch Psy Clin N 1988;238:2–6.

Brown DF. 1987. *The Role of Adenosine 3':5'-cyclic Monophosphate (cAMP) in Memory Formation: A Behavioural, Pharmacological and Biochemical Study.* PhD Thesis, La Trobe University, Melbourne, Australia.

Bureau Y, Handa M, Zhu Y, Laliberte F, Moore CS, Liu S, et al. Neuroanatomical and pharmacological assessment of Fos expression induced in the rat brain by the phosphodiesterase-4 inhibitor 6-(4-pyridylmethyl)-8-(3-nitrophenyl) quinoline. Neuropharmacology 2006;51(5):974–85.

Cherry JA, Davis RL. Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement and affect. J Comp Neurol 1999;407:287–301.

Crowe SF, Hale MW. Carryover effects associated with the single trial passive avoidance learning task in the young chick. Neurobiol Learn Mem 2002;78:321–31.

Crowe SF, Ng KT, Gibbs ME. Memory formation processes in weakly reinforced learning. Pharmacol Biochem Beh 1989;33:881–7.

Crowe SF, Ng KT, Gibbs ME. Memory consolidation of weak training experiences by hormonal treatments. Pharmacol Biochem Beh 1990;37:729–34.

Davis HP, Squire LR. Protein synthesis and memory: a review. Psychol Bull 1984;96(3): 518–59.

De Vaus JE, Gibbs ME, Ng KT. Effects of social isolation on memory formation. Behav Neural Biol 1980;29(4):473–80.

Dyke HJ, Montana JG. Update on the therapeutic potential of PDE4 inhibitors. Expert Opin Inv Drug 2002;11(1):1-13.

Eckmann F, Fichte K, Meya U, Sastre-y-Hernandez M. Rolipram in major depression: result of a double blind comparative study with amitriptyline. Curr Ther Res Clin E 1988;43:291–5.

Egawa T, Mishima K, Matsumoto Y, Iwasaki K, Iwasaki K, Fujiwara M. Rolipram and its optimal isomers, phosphodiesterase 4 inhibitors, attenuated the scopolamine-induced impairments of learning and memory in rats. Jpn J Pharmacol 1997;75:275–81.

Gibbs ME, Ng KT. Psychobiology of memory: towards a model of memory formation. Biobehav Rev 1977;1:113–36.

Greengard P, Allen PB, Nairn AC. Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron 1999;23(3):435–47.

Guzowski J, McGaugh JL. Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. Proc Natl Acad Sci USA 1997;94:2693–8.

Hale MW, Crowe SF. Facilitation and disruption of memory for the passive avoidance task in the day-old chick using dopamine D1 receptor compounds. Behav Pharmacol 2003;14(5):525–32.

Harada N, Nishiyama S, Ohba H, Sato K, Kakiuchi T, Tsukuda H. Age differences in phosphodiesterase type-IV and its functional response to dopamine D1 receptor modulation in the living brain: a PET study in conscious monkeys. Synapse 2002;44:139–45.

Hebenstreit GF, Fellerer K, Fichte K, Fischer G, Geyer N, Meya U, et al. Rolipram in major depressive disorder: results of a double blind comparative study with imipramine. Pharmacopsychiat 1989;22:156–60.

Hemmings HC, Nairn A, Greengard P. Protein kinases and phosphoproteins in the nervous system. In: Martin JB, Barchas JD, editors. Neuropeptides in neurologic and psychiatric disease. New York: Raven Press; 1986. p. 47–69.

Imanishi T, Sawa A, Ichimaru Y, Miyashiro M, Kato S, Yamamoto T, et al. Ameliorating effects of rolipram on experimentally induced impairments of learning and memory in rodents. Eur J Pharmacol 1997;321:273–8.

Johnston ANB, Rose SPR. Isolation-stress induced facilitation of passive avoidance memory in the day-old chick. Behav Neurosci 1998;112:1–8.

Kabai P, Stewart MG, Tarcali J, Csillag A. Inhibiting effect of D1, but not D2 antagonist administered to the striatum on retention of passive avoidance in the chick. Neurobiol Learn Mem 2004;81:155–8.

Kanes SJ, Tokarczyk J, Siegel SJ, Bilker W, Abel T, Kelly MP. Rolipram: a specific phosphodiesterase 4 inhibitor with potential antipsychotic activity. Neurosci 2007;144(1):239–46.

Lamprecht R. CREB: a message to remember. Cell Molecular Life Sci 1999;55(4):554–63.

Liu F, Graybiel M. Spatiotemporal dynamics of CREB phosphorylation in the developing striatum. Neuron 1996;17:1133–44.

Lynch MA. Long-term potentiation and memory. Physiol Rev 2004;84:87-136.

Moriuchi H, Nakahara T, Maruko T, Sakamoto K, Ishii K. Relaxant effect of YM976, a novel phosphodiesterase 4 inhibitor, on bovine tracheal smooth muscles. Eur J Pharmacol 2003;470(1–2):57–64.

O'Donnell JM, Frith S. Behavioral effects of family-selective inhibitors of cyclic nucleotide phosphodiesterases. Pharmacol Biochem Beh 1999;63:85-192.

Okulski P, Hess G, Kaczmarek L. Anisomycin treatment paradigm affects duration of long-term potentiation in slices of the amygdala. Neurosci 2002;114(1):1–5.

Randt CT, Judge ME, Bonnet KA, Quartermain D. Brain cyclic AMP and memory in mice. Pharmacol Biochem Beh 1982;17:677–80.

Rose SPR. God's organism? The chick as a model system for memory studies. Learn Mem 2000;7(1):1-17.

Rose SPR. Memory beyond the synapse. Neuron Glia Biology 2004;1:211–7.

Rutten K, Lieben C, Smits L, Blokland A. The PDE4 inhibitor rolipram reverses object memory impairment induced by acute tryptophan depletion in the rat. Psychopharmacol (Berlin) 2007;192(2):275–82.

Schafe GE, LeDoux JE. Memory consolidation of auditory Pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. J Neurosci 2000;20:1–5.

Schneider HH. Brain cAMP response to phosphodiesterase inhibitors in rats killed by microwave irradiation or decapitation. Biochem Pharmacol 1984;33:1690–3.

Serrano PA, Beniston DC, Oxonian MG, Rodriguez WA, Rosenzweig MR, Bennett EL. Differential effects of protein kinase inhibitors and activators on memory formation in the 2 day-old chick. Behav Neural Biol 1994;61:60–72.

Stewart MG, Kabai P, Harrison E, Steele RJ, Kossut M, Gierdalsko M. The involvement of dopamine in the striatum in the passive avoidance training in the chick. Neurosci 1996;70:7-14.

Zhang HT, O'Donnell JM. Effects of rolipram on scopolamine-induced impairment of working and reference memory in the radial-arm maze tests in rats. Psychopharmacol 2000;150(3):311–6.

Zhang HT, Huang Y, Masood A, Stolinski LR, Li Y, Zhang L et al. 2007. Anxiogenic-like behavioral phenotype of mice deficient in phosphodiesterase 4B (PDE4B). Neuropharmacol 2008; 33(7):1611–162.

Zhao WQ, Polya GM, Wang BH, Gibbs ME, Sedman GL, Ng KT. Inhibitors of cAMP-dependent protein kinase impair long-term memory formation in day-old chicks. Neurobiol Learn Mem 1995;64:106–18.