Available online at www.sciencedirect.com
SCIENCE DIRECT®
Behavioural Brain Research 147 (2003) 41–48
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

Intra-accumbens protein kinase C inhibitor NPC 15437 blocks amphetamine-produced conditioned place preference in rats

Harinder Aujla, Richard J. Beninger*

Department of Psychology, Queen's University, Kingstone, Ont., Canada

Received 3 January 2003; received in revised form 1 April 2003; accepted 1 April 2003

Abstract

In a previous study, intracerebroventricular calcium-dependent protein kinase (PKC) inhibition attenuated cocaine place conditioning. This suggested the hypothesis that intra-nucleus accumbens (NAc) injections of the PKC inhibitor NPC 15437 may block place conditioning produced by NAc injections of amphetamine. An unbiased conditioned place preference paradigm was employed to evaluate the present hypothesis. Thus, during pre-conditioning rats had access to an apparatus consisting of two chambers connected by a tunnel for three 15-min sessions. During 8 conditioning days with the tunnel blocked, one chamber was paired with NAc injections of drug for four 30-min sessions alternating with pairing of the other chamber with NAc injections of saline. Time spent on the drug-paired side was assessed in a final drug-free test session and compared to the amount of time spent there in pre-conditioning; a significant increase was defined as a place preference. Intra-NAc amphetamine (20.0 μg/0.5 μl/side) produced a place preference. This effect was blocked dose dependently by NPC 15437 (0.1, 0.5 and 1.0 μg/0.5 μl/side). NPC 15437 (1.0 μg) alone did not produce a place preference or aversion. None of the doses of NPC 15437 affected the locomotor stimulant effect of intra-NAc amphetamine during conditioning revealing a dissociation between the locomotor stimulating and rewarding effects of intra-NAc amphetamine. Results implicate PKC in the NAc in reward-related learning. More work is needed to elucidate the signaling pathways involved in this type of learning.

Keywords: Amphetamine; Conditioned place preference; Dopamine; Learning; Locomotion; Nucleus accumbens; NPC 15437; PKC; Place conditioning; Reward

1. Introduction

The signaling molecule calcium-dependent protein kinase (PKC) has been implicated in learning and memory in a number of central nervous system regions in a variety of paradigms. These include the hippocampus in spatial memory [6,12], or contextual fear conditioning in mice [6], the cerebellum in eye-blink conditioning in rabbits [20], the robust nucleus of the archistriatum in song learning in zebra finches [16], and the mushroom bodies in associative conditioning of courtship in *Drosophila* [8]. The role of PKC in synaptic plasticity has been reviewed by Nogues [13]. At present little is known about the role of PKC in reward-related learning.

The conditioned place preference (CPP) paradigm has been used extensively to assess reward-related learning in rats. In one version of the CPP paradigm, drug treatments are differentially paired with one of two distinct compartments in a two-chambered apparatus. On a drug-free test day, the compartments are connected by a tunnel and reward-related learning is defined as a significantly greater amount of time spent on the drug-paired side. Previous studies have shown that systemic or intra-nucleus accumbens (NAc) injections of pharmacological agents that augment dopaminergic transmission such as amphetamine or cocaine produce a place preference (reviewed in [19]). Results implicate dopamine in CPP learning.

One previous study has evaluated the role of PKC in CPP produced by cocaine. Thus, Cervo et al. [4] reported that the PKC inhibitors H7 or chelerythrine injected intracerebroventricularly (icv) immediately following conditioning sessions with systemic cocaine blocked the CPP effect. They also evaluated the effects of H7, that inhibits both PKC and cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA), injected prior to cocaine conditioning sessions but found no effect. Further studies are needed to identify the specific roles of PKA and PKC in specific brain regions in reward-related learning. Thus, the present study tested the hypothesis that NAc co-injection of amphetamine plus the PKC inhibitor

* Corresponding author.
E-mail addresses: harinder@psyc.queensu.ca (H. Aujla), beninger@psyc.queensu.ca (R.J. Beninger).

0166-4328/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0166-4328(03)00136-0

S-2,6-diamino-*N*-[[1-(1-oxotridecyl)-2-piperidinyl] methyl]-hexanimide dihydrochloride (NPC 15437) will dose dependently block the place preference produced by amphetamine alone.

2. Methods

2.1. Subjects

Treatment of rats was in accordance with the guidelines of the Animals for Research Act, the Canadian Council on Animal Care, and relevant University policies and was approved by the Queen's University Animal Care Committee. Experimentally naive male Wistar rats ($N = 72$) (Charles River, St.-Constant, Quebec) weighing between 225 and 250 g were housed in pairs in clear plastic shoebox-style cages with sterilized wood-chip bedding material. Animals were maintained in a temperature-controlled ($21 \pm 1\,^{\circ}C$) colony room on a reversed 12-h light–dark cycle (lights off at 07:00 h). Food (Purina rodent laboratory chow #5001) and water were freely available in the home cages throughout the experiment. All rats were handled daily for at least 5 days prior to surgery.

2.2. Surgery

Rats were anesthetized with an oxygen flow containing 4% halothane (Halocarbon Laboratories, River Edge, New Jersey). During surgery the concentration of halothane was adjusted to maintain a deep level of anesthesia. Rats then were placed into a stereotaxic device. An incision was made along the midline, the scalp retracted, and the area surrounding bregma was cleaned and dried. The skull was leveled using the line connecting bregma and lambda. Two stainless steel guide cannulae (0.64 mm diameter, 13 mm long) were lowered through two small holes drilled through the skull at the following coordinates: 1.2 mm anterior to bregma, 1.5 mm lateral to the midline, and 6.7 mm ventral to the surface of the skull [15]. The cannulae were anchored to the skull with four stainless steel screws and dental cement. Stainless steel pins (0.31 mm diameter) were inserted into the guide cannulae to ensure that they remained unoccluded. Each rat received a 0.2-ml subcutaneous injections of the analgesic buprenorphine (0.03 mg/kg) (10% in saline solution) at the onset of surgery and 8–12 h after surgery for pain relief. In addition, lidocaine with epinephrine was injected (0.3 ml) in several locations around the incision for local analgesia. Animals were allowed to recover for 1 week prior to testing and during this time the pins were replaced daily.

2.3. Drugs

D-Amphetamine sulphate (amph) (USP, Rockville, MD) (40.0 mg/ml) was dissolved in sterile saline, and this preparation was made fresh daily, a maximum of 30 min prior to the onset of the experiment.

NPC 15437 (Research Biochemicals International, Oakville, Ontario, Canada) (0.2, 1.0, 2.0 mg/ml) was dissolved in distilled water, alliquotted into equal portions and frozen until needed. Equal volumes of NPC 15437 and amphetamine were mixed together to achieve the final concentrations.

2.4. Intra-cranial drug injection

Two 10.0 μl microsyringes (Hamilton Co., Reno, NV) mounted on an infusion pump (Sage Instrument Pump Model 355) were used to infuse the drug at a constant rate of 1.0 μl/min. The injection cannulae, cut 1.0 mm longer than the implanted guide cannulae, were made from stainless steel tubing (0.31 mm diameter). Polyethylene tubing was used to attach injection cannulae to the microsyringe. Bilateral drug injections (0.5 μl/side) were delivered over a 30-s interval and the injection cannulae remained in place for an additional 30 s to promote diffusion.

2.5. Apparatus

Four rectangular wooden boxes consisted of two compartments (38 cm × 27 cm × 36 cm) with removable Plexiglas covers, connected by a tunnel (8 cm × 8 cm × 8 cm). The tunnel could be closed by inserting removable plastic guillotine-style doors. The two initially neutral compartments were visually distinct: each compartment wall consisted of either urethane-sealed wood or painted black and white vertical stripes (1 cm wide). The compartment floors were also distinct: one type was made of galvanized steel mesh, and the other was constructed of parallel stainless steel bars (1 cm apart). Tunnel floors were constructed of galvanized sheet metal. The arrangement of walls and floor types was such that each box had a unique configuration. Six photo-cell emitters and detectors were located in each box: two in each compartment (height 5 cm) and one at each end of the tunnel (height 3 cm). A 6809 micro-controller using custom-made software, controlled by a Macintosh computer, was used to record the time spent in each compartment. For further details of the apparatus, see [2].

2.6. Procedure

Using an unbiased conditioned place preference paradigm, the procedure consisted of three phases: pre-conditioning, conditioning and test. For all phases, animals were tested during the same time period (09:00–1300 h) each day and received one session per day.

2.6.1. Pre-conditioning

During three 15-min sessions, rats were exposed to the entire box. There were no drugs administered during this phase. Rats were placed in a particular compartment designated as the start compartment. The start compartment was

counterbalanced across rats: half of the rats started on the left side, and the other half started on the right side of the conditioning chamber. The amount of time spent in each compartment was recorded.

2.6.2. Conditioning

During eight 30-min sessions, rats were confined to one compartment only, different compartments on alternate days. Drug injections preceded placement into one compartment on days 1, 3, 5, and 7, while saline injections preceded placement into the other compartment on days 2, 4, 6, and 8. NPC 15437 in doses of 0, 0.1, 0.5, and 1.0 μg/0.5 μl/side was co-administered to different groups in a mixture with 20.0 μg/0.5 μl/side of amphetamine; the groups were designated amph, NPC (0.1 μg) + amph, NPC (0.5 μg) + amph and NPC (1.0 μg) + amph, respectively. An additional group received NPC 15437(1.0 μg/0.5 μl/side) alone paired with one side of the box during conditioning; this group was designated NPC (1.0 μg) alone. The designation of the drug-paired compartment was counterbalanced, so that for half of the rats the start compartment (designated in pre-conditioning) was paired with drug, whereas for the other half it was paired with saline. The counterbalancing procedure was determined prior to the start of the current experiment; thus, the distribution of pre-conditioning durations did not influence assignment of conditioning chamber. On all conditioning days, the number of beam breaks was recorded as a measure of activity.

2.6.3. Testing

During one 15-min test session, rats were exposed again to the entire apparatus with the guillotine doors removed. The amount of time spent in each compartment and the number of beam breaks was recorded.

2.7. Histological analysis

Following behavioral testing, all animals were sacrificed by carbon dioxide gas exposure and decapitated. Brains were extracted immediately and placed in a 10% formalin/sucrose solution. At least 2 days following extraction, brains were frozen and sliced in 70 μm coronal sections using a microtome. Brain slices were mounted on glass slides and stained with Nissl cell body stain. Verification of injection sites was performed by an observer who was blind to the behavioral results. Animals were considered to be cannulated properly if the entire tip of both cannulae was located in the region of core or shell of the NAc.

2.8. Statistical analyses

Planned *t*-tests comparing time spent in each compartment during pre-conditioning assessed possible compartment bias for each group. Planned *t*-tests comparing time spent in the tunnel during pre-conditioning versus test for each group were run in order to ensure that any place preference effect was not an artifact of a general increase in compartment to tunnel time ratio.

Planned *t*-tests comparing time spent on the drug-paired side during pre-conditioning to time spent on the drug-paired side during test were used to assess place preference. Conditioned place preference occurs if animals spend significantly more time in the drug-paired compartment during the test session as compared to the average from the three pre-conditioning sessions. A mixed design two-way analysis of variance (ANOVA) followed by Tukey Honestly Significant Difference (HSD) post-hoc analysis comparing time spent on the drug-paired side across treatment conditions supplemented the initial analysis. Two two-way ANOVA followed by simple effects analysis were used to examine activity during conditioning sessions. An alpha level of 0.05 was used for all analyses.

3. Results

3.1. Histology

Histological examination revealed that of the 72 rats that underwent surgery, 53 placements were considered to be in the target region of the NAc (Fig. 1). Final numbers were amph ($N = 21$), NPC (0.1 μg) + amph ($N = 7$), NPC (0.5 μg) + amph ($N = 8$), NPC (1.0 μg) + amph ($N = 7$) and NPC (1.0 μg) alone ($N = 10$).

3.2. Place conditioning

There was no significant difference in time spent on the drug-paired side versus the vehicle-paired side during

Table 1
Time (s) spent in the to-be-drug- and vehicle-paired sides during pre-conditioning

Treatment (dose)	Time (s)				
	N	Vehicle (S.E.M.)	Drug (S.E.M.)	*t*	*P*
Amph (20.0)	21	428.7 (15.3)	401.4 (14.7)	0.93	n.s.
NPC (0.1) + amph	7	435.1 (35.6)	394.0 (34.9)	0.58	n.s.
NPC (0.5) + amph	7	455.7 (49.2)	385.6 (46.0)	0.74	n.s.
NPC (1.0) + amph	8	427.0 (40.4)	403.8 (36.0)	0.31	n.s.
NPC (1.0) alone	10	440.8 (24.4)	388.8 (26.6)	1.02	n.s.

Abbreviations: amph: amphetamine, NPC: NPC 15437, n.s.: not significant; S.E.M.: standard error of the mean, dose in μg/0.5 ml/side.

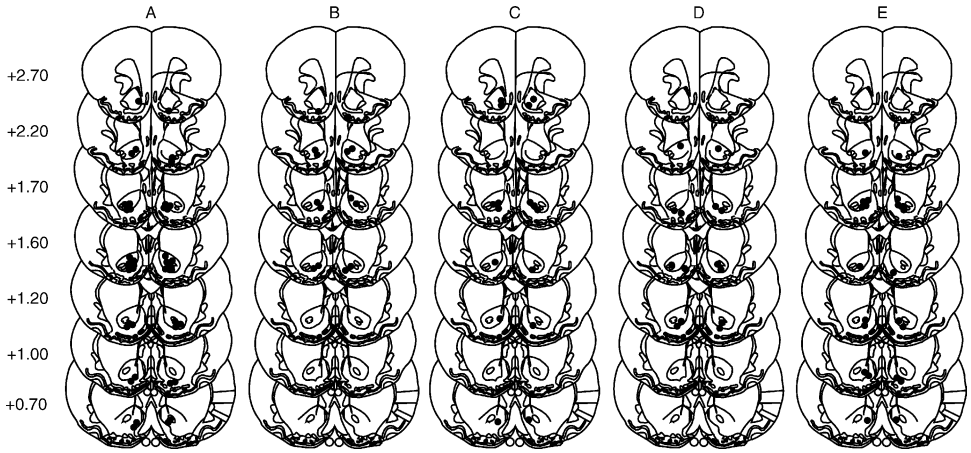

Fig. 1. Location of cannulae tips in the (A) amphetamine ($N = 21$), (B) NPC 15437 (0.1 μg) + amphetamine ($N = 7$), (C) NPC 15437 (0.5 μg) + amphetamine ($N = 8$), (D) NPC 15437 (1.0 μg) + amphetamine ($N = 7$) and (E) NPC 15437 (1.0 μg) alone ($N = 10$) groups.

Table 2
Tunnel time (s): pre-conditioning vs. test

Treatment (dose)	Time (s)			
	Pre-cond (S.E.M.)	Test (S.E.M.)	*t*	*P*
Amph (20.0)	69.9 (2.3)	76.9 (3.0)	1.91	n.s.
NPC (0.1) + amph	70.9 (5.0)	72.9 (4.6)	0.32	n.s.
NPC (0.5) + amph	58.7 (5.2)	70.6 (3.0)	2.38	0.05
NPC (0.1) + amph	69.3 (6.5)	66.0 (2.5)	0.62	n.s.
NPC (1.0) alone	70.4 (3.4)	70.2 (3.1)	0.04	n.s.

Abbreviations: amph: amphetamine, NPC: NPC 15437, n.s.: not significant; pre-cond: pre-conditioning, S.E.M.: standard error of the mean, dose in μg/0.5 ml/side.

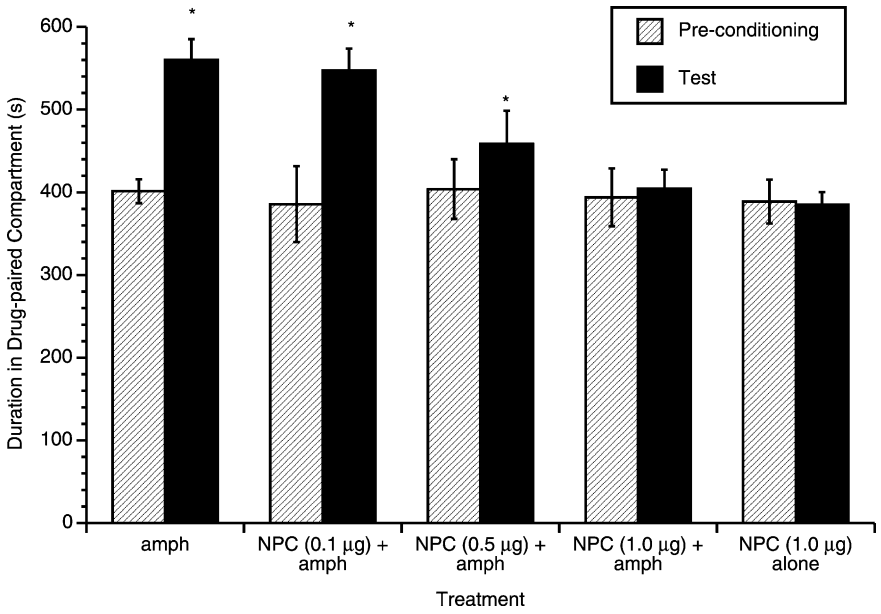

Fig. 2. Mean time (s) spent on drug-paired side during pre-conditioning and test phases across treatment groups. The symbol (∗) indicates a significant difference from pre-conditioning. Abbreviations: amph: amphetamine; NPC: NPC 15437.

preconditioning in any of the groups (see Table 1). There was no significant change in tunnel time from pre-conditioning to test in any of the treatment conditions (see Table 2); for the NPC (0.5 μg) + amph group, the *P*-value for the *t*-test equaled 0.05.

The change in time spent in the drug-paired side from pre-conditioning to test was used to evaluate place conditioning for each group. The mean of the three pre-conditioning days for each group was used for the purpose of this analysis (see Fig. 2). There was a significant increase in time spent on the drug-paired side from pre-conditioning to test for the amph ($t(20) = 9.69$, $P < 0.001$) and the NPC (0.1 μg) + amph group ($t(6) = 5.22$, $P < 0.01$). There was no significant change in time spent on the drug-paired side for the NPC (0.5 μg) + amph ($t(7) = 2.37$, $P = 0.06$), the NPC (1.0 μg) + amph ($t(6) = 0.56$, $P > 0.05$), and the NPC (1.0 μg) alone groups ($t(9) = 0.15$, $P > 0.05$).

Mean pre-conditioning and test day results were subjected to a two-way mixed design ANOVA followed by simple effects analysis and Tukey post-hoc tests. Results yielded a significant group by phase interaction ($F(4, 48) = 13.31$, $P < 0.01$) as well as a significant main effect of group ($F(4, 48) = 3.65$, $P < 0.01$) and phase ($F(1, 48) = 50.4$, $P < 0.01$). Simple effects analysis of group at each phase revealed a significant effect of group during the test day ($F(4, 70) = 8.528$, $P < 0.01$) but not on the pre-conditioning days ($F(4, 70) = 0.08$, $P > 0.05$). Tukey HSD post-hoc tests revealed that the NPC (0.5 μg) + amph, NPC (1.0 μg) + amph and NPC (1.0 μg) alone groups spent less time on the drug-paired side than the amph and the NPC (0.1 μg) + amph groups. Furthermore, the NPC (0.5 μg) + amph group spent more time in the drug-paired side on test than the NPC (1.0 μg) + amph group.

3.3. Activity

Amphetamine enhanced activity in all groups that received amphetamine (see Table 3). A two-way group × day ANOVA revealed a main effect of treatment ($F(4, 48) = 115.79$, $P < 0.01$) on activity during conditioning on the drug-paired side. Tukey HSD post-hoc tests revealed that the NPC (1.0 μg) alone group exhibited lower activity counts than the other groups at each day. None of the other treatment groups differed from one another. A similar ANOVA carried out on vehicle days yielded no significant effects.

4. Discussion

Results can be summarized as follows: (1) Intra-NAc amphetamine produced a place preference. (2) NAc injections of the PKC inhibitor NPC 15437dose dependently blocked the place preference produced by NAc amphetamine. (3) NAc injections of NPC 15437 alone did not affect place preference. (4) NAc NPC 15437 did not block the locomotor activity stimulant effects of NAc amphetamine. Thus, there was a dissociation between the effects of NPC 15437on place conditioning and locomotor activity.

The place preference procedure used in this study was unbiased, i.e. there were no significant differences in time spent on the two sides during pre-conditioning. If groups were observed to spend less time in the tunnel during the test than during the pre-conditioning sessions, it might be possible to observe an increase in time spent on the drug-paired side from pre-conditioning to test that reflected the decrease in tunnel time. This does not account for the data for four of the groups. There was a marginal increase in tunnel time from pre-conditioning to test for the NPC 15437 (0.5 μg) + amph group ($P = 0.05$). In spite of this increase, this group showed a significant increase in time spent on the drug-paired side from pre-conditioning to test. Thus, differences in tunnel time from pre-conditioning to test cannot account for the observed place preferences.

The observation that NAc injections of amphetamine produced a place preference is in good agreement with a number of previous studies (reviewed in [19]). The present study did not set out to target either the shell or core subregions of the NAc. There is a literature evaluating these two subregions with respect to reward-related learning, reviewed by DiChiara [5], but there is no consensus on the contribution of each. As we did not differentiate these two structures in our surgical procedure and the drugs may have diffused into both subregions, the present results do not contribute to the core versus shell debate.

The finding that co-injection of amphetamine plus NPC 15437 dose dependently blocked the place preference effect is consistent with the hypothesis that PKC inhibition will block the rewarding properties of amphetamine. NPC 15437 may also have blocked learning of the association between amphetamine and the environment. The effect of NPC 15437 cannot be attributed to an aversion as injection of this agent alone had no significant effect on side preference. No claims can be made with respect to state-dependent learning. State-dependent learning could be assessed in test sessions conducted following administration of amphetamine or amphetamine plus NPC 15437.

The present findings are consistent with those of Narita et al. [11] who found that adminstration of the PKC inhibitor calphostin C prior to conditioning attenuated a morphine-induced place preference. Cervo et al. [4] reported that the PKC inhibitor chelerythrine given icv immediately after conditioning with systemic cocaine blocked place preference in rats; H7, that also inhibits PKA, given before conditioning sessions was without effect. These results support a role for PKC in reward-related learning. However, differences in route of administration of the inhibitor (icv versus intra-NAc), the inhibitor itself (H7 versus NPC 15437), route of administration of the psychostimulant (systemic versus intra-NAc) or the psychostimulant itself (cocaine versus amphetamine) may account for the differing results with respect to place preference obtained in the

Table 3
Mean (±S.E.M.) activity counts during conditioning days

	Vehicle day					Drug day				
	1	2	3	4	Mean	1	2	3	4	Mean
Amph (20.0)	279.7 (17.7)	291.6 (20.6)	272.7 (31.0)	279.6 (26.2)	280.9 (23.9)	924.9 (33.0)	945.6 (40.4)	987.1 (34.4)	943.4 (38.8)	950.3[a] (36.7)
NPC (0.1) + amph	259.6 (35.1)	228.7 (27.0)	202.7 (33.4)	299.0 (37.2)	247.5 (33.2)	914.7 (61.1)	894.1 (67.8)	994.0 (28.3)	861.9 (44.4)	915.2[a] (50.4)
NPC (0.5) + amph	312.5 (35.9)	243.9 (28.9)	287.5 (40.0)	278.9 (34.7)	280.7 (34.9)	870.6 (102.3)	1019.8 (77.0)	1004.8 (64.8)	1052.0 (30.8)	986.8[a] (68.7)
NPC (1.0) + amph	335.0 (24.9)	253.6 (28.0)	250.3 (29.9)	276.4 (35.2)	278.8 (29.5)	844.0 (38.4)	956.1 (33.5)	972.0 (41.4)	979.4 (79.0)	937.9[a] (48.1)
NPC (1.0) alone	283.9 (36.6)	257.2 (21.1)	265.1 (39.7)	264.9 (23.9)	267.8 (30.3)	264.7 (34.5)	308.3 (43.72)	310.4 (19.8)	289.8 (35.7)	293.3 (33.4)

Abbreviations: amph: amphetamine, NPC: NPC 15437, S.E.M.: standard error of the mean.

[a] Significantly different from NPC (1.0 μg) alone in post hoc pairwise comparisons following significant group effect in analysis of variance.

current study versus Cervo et al. Although chelerythrine was able to attenuate a cocaine place preference the difference in the timing of the administration (i.e. post conditioning versus prior to conditioning) makes it difficult to compare these results to the current study. Further studies that do not differ on these methodological points need to be done to evaluate these discrepancies between H7 and NPC 15437.

The signaling pathways involved in reward-related learning remain to be elucidated although some progress has been made (e.g. [9,17]). The role of PKC remains to be specified but there are a number of possibilities. Thus, PKC activation inhibited dopamine uptake by the human dopamine transporter and PKC inhibition blocked this effect [21]. Iannazzo et al. [7] showed that PKC inhibition blocked stimulation-induced dopamine release in the presence of the D2 receptor antagonist sulpiride but not without D2 receptor blockade suggesting that PKC activity is regulated by pre-synaptic D2 receptors. Paolillo et al. [14] showed a form of PKC-mediated potentiation of cAMP activity by metabotropic glutamate receptors in striatal cells in vitro. As the cAMP-PKA signaling pathway has been implicated in stimulant-induced reward-related learning [1,18], an interaction of the cAMP-PKA and the diacylglycerol (DAG)-PKC second messenger pathways may provide the basis for the role of PKC in reward-related learning.

Browman et al. [3] showed that application of the PKC inhibitor RO 31-8220, different from the one that was used in the current study, was able to inhibit amphetamine-induced dopamine release in striatal slices. In addition, they demonstrated that RO 31-8220 given 15 min prior to intra-NAc amphetamine (10 μg/0.5 μl/side) attenuated the locomotor stimulant effects of amphetamine. We did not observe the same attenuation of amphetamine-induced locomotor stimulation. However, there were a number of differences between the present study and that Browman et al. [3] including the PKC inhibitor used (NPC 15437 versus RO 31-8220), the timing of PKC inhibition and amphetamine injection (coinjection versus 15-min separation) and the dose of NAc amphetamine (20 μg versus 10 μg). It is important to note that co-administration of PKC and amphetamine may have produced biochemical and/or biophysical interactions that would not have been present using serial administration. Taken together, these differences may have led to the discrepant findings with respect to locomotor activity. Our observation that doses of a PKC inhibitor that significantly decreased the CPP effect failed to alter locomotor activation produced by amphetamine show that the rewarding and stimulating effects of amphetamine are dissociable. We have previously observed a similar dissociation for the effects of a PKA inhibitor on unconditioned versus conditioned activity based on NAc injections of amphetamine [18].

In conclusion, the present results show that PKC in the NAc plays an important role in place conditioning produced by NAc injections of amphetamine. Kötter [10] provides some clues to how PKC may be involved in synaptic plasticity that underlies learning produced by reward. It will be the task of future studies to delineate the signaling pathways through which PKC influences reward-related learning.

Acknowledgements

This research was funded by the Natural Sciences and Engineering Research Council of Canada.

References

[1] Beninger RJ, Nakonechny PL, Savina I. cAMP-dependent protein kinase and reward-related learning: intra-accumbens Rp-cAMPS blocks amphetamine-produced place conditioning in rats. Psychopharmacology, in press.
[2] Brockwell NT, Ferguson DS, Beninger RJ. A computerized system for the simultaneous monitoring of place conditioning and locomotor activity in rats. J Neurosci Methods 1996;64:227–32.
[3] Browman KE, Kantor L, Richardson S, Badiani A, Robinson TE, Gnegy ME. Injection of the protein kinase C inhibitor Ro31-8220 into the nucleus accumbens attenuates the acute response to amphetamine: tissue and behavioral studies. Brain Res 1998;814:112–9.
[4] Cervo L, Mukherjee S, Bertaglia A, Samanin R. Protein kinases A and C are involved in the mechanisms underlying consolidation of cocaine place conditioning. Brain Res 1997;775:30–6.
[5] DiChiara G. Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 2002;137:75–114.
[6] Fordyce DE, Clark VJ, Paylor R, Wehner JM. Enhancement of hippocampally-mediated learning and protein kinase C activity by oxiracetam in learning-impaired DBA/2 mice. Brain Res 1995;672:170–6.
[7] Iannazzo L, Sathananthan S, Majewski H. Modulation of dopamine release from rat striatum by protein kinase C: interaction with presynaptic D2-dopamine-autoreceptors. Br J Pharmacol 1997;122:1561–6.
[8] Kane NS, Robichon A, Dickinson JA, Greenspan RJ. Learning without performance in PKC-deficient *Drosophila*. Neuron 1997;18:307–14.
[9] Kelley AE, Berridge KC. The neuroscience of natural rewards: relevance to addictive drugs. J Neurosci 2002;22:3306–11.
[10] Kötter R. Postsynaptic integration of glutamatergic and dopaminergic signals in the striatum. Prog Neurobiol 1995;44:163–96.
[11] Narita M, Aoki T, Ozaki S, Yajima Y, Suzuki I. Involvement of protien kinase Cγ isoform in morphine-induced reinforcing effects. Neuroscience 2001;103:309–14.
[12] Nogues X, Jaffard R, Micheau J. Investigations on the role of hippocampal protein kinase C on memory processes: pharmacological approach. Behav Brain Res 1996;75:139–46.
[13] Nogues X. Protein kinase C, learning and memory: a circular determinism between physiology and behaviour. Prog Neuropsychopharmacol Biol Psychiatry 1997;21:507–29.
[14] Paolillo M, Montecucco A, Zanassi P, Schinelli S. Potentiation of dopamine-induced cAMP formation by group I metabotropic glutamate receptors via protein kinase C in cultured striatal neurons. Eur J Neurosci 1998;10:1937–45.
[15] Paxinos C, Watson G. The rat brain in sterotaxic coordinates. 4th ed. Orlando, USA: Academic Press; 1998.
[16] Sakaguchi H, Yamaguchi A. Early song-deprivation affects the expression of protein kinase C in the song control nuclei of the zebra finch during a sensitive period of song learning. Neuroreport 1997;8:2645–50.
[17] Sutton MA, Beninger RJ. Psychopharmacology of conditioned reward: evidence for a rewarding signal at D1-like dopamine receptors. Psychopharmacol Berl 1999;144:95–110.

[18] Sutton MA, McGibney K, Beninger RJ. Conditioned locomotion in rats following amphetamine infusion into the nucleus accumbens: blockade by coincident inhibition of protein kinase A. Behav Pharmacol 2000;11:365–76.
[19] Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 1998;56:613–72.
[20] Van der Zee EA, Kronforst-Collins MA, Maizels ET, Hunzicker-Dunn M, Disterhoft JF. Gamma isoform-selective changes in PKC immunoreactivity after trace eyeblink conditioning in the rabbit hippocampus. Hippocampus 1997;7:271–85.
[21] Zhang L, Coffey LL, Reith ME. Regulation of the functional activity of the human dopamine transporter by protein kinase C. Biochem Pharmacol 1997;53:677–88.