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Cyclooxygenase inhibitor modulation of dopamine-related behaviours

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Abstract

The sequential action of phospholipase A_2 and cyclooxygenase leads to the production of prostaglandins in the brain, an event hypothesised to cause dopaminergic stimulation. To investigate this further, we examined the effect of the nonselective cyclooxygenase inhibitors indomethacin and piroxicam on several indices of dopaminergic function in adult male rats. Both drugs inhibited catalepsy induced by the dopamine D1-like receptor antagonist R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH23390), the dopamine D2-like receptor antagonist raclopride and by haloperidol, findings in agreement with a dopaminergic effect of cyclooxygenase inhibitors. However, neither cyclooxygenase inhibitor had an effect upon disruption of prepulse inhibition of the auditory startle reflex by amphetamine or on the rate of amphetamine self-administration. Both drugs reduced amphetamine-stimulated locomotor activity. Our data indicate that the mechanism by which cyclooxygenase inhibitors alter motor behaviour is unlikely to be due to a simple direct action at the dopaminergic synapse. Their apparent ability to antagonise hypoactivity without generalised dopaminergic stimulation suggests that other, possibly multiple, neurotransmitter systems may be involved.

Keywords: Catalepsy; Locomotor activity; Prepulse inhibition; Amphetamine; Dopamine; Parkinson's disease; Progressive supranuclear palsy

1. Introduction

Cyclooxygenase inhibitors comprise a class of drugs conventionally used in the treatment of pain and inflammation. Cyclooxygenase comprises two forms, cyclooxygenase-1 and cyclooxygenase-2, both of which are present in brain. Although the enzymes are expressed in a number of brain structures including the cortex and basal ganglia (Kawasaki et al., 1993; Tocco et al., 1997), their role in brain function and animal behaviour is unclear. Cyclooxygenase catalyses the oxidation of unesterified polyunsaturated fatty acids within the cell membrane to form prostaglandins, a class of eicosanoids, such as prostacyclin and prostaglandin D_2 (Smith et al., 1992). Prostaglandins are transported out of the cell and, in general, act as rapidly inactivated "local hormones" exerting an effect very near to where they are synthesised. Operating through G-protein linked cell-surface receptors they act as regulators of second messengers such as cAMP, inositol phosphates and diacylglycerol, in turn affecting a variety of cellular processes including calcium mobilisation, protein kinase activity and protease activity (Wise, 1997). Since cyclooxygenase requires the free fatty acid as substrate, hydrolysis of the phospholipid must first take place. This is accomplished by either phospholipase A_2, or by the combined action of phospholipase C and diacylglycerol lipase (Ross and Kish, 1994). Since various neurotransmitters, including dopamine, glutamate and serotonin, can vary the activity of phospholipase A_2 and/or phospholipase C (Smith et al., 1992), it is apparent that cyclooxygenase comprises part of a signalling cascade working by means of modification of the lipid component of the cell membrane.

Anecdotal clinical reports have suggested that cyclooxygenase inhibitors can cause drug-induced psychosis in healthy subjects (Hoppmann et al., 1991). Due to the

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putative involvement of the dopamine system in psychosis associated with both schizophrenia, a disorder thought to be associated with hyper-dopaminergic activity, and chronic amphetamine use, a drug-causing dopamine release, it was hypothesised that cyclooxygenase inhibitors act as stimulators of dopaminergic transmission (reviewed in Ross, 2000). An effect of cyclooxygenase inhibitors on the dopamine system is also supported by experimental animal-based investigations which report that indomethacin antagonises haloperidol (a dopamine receptor antagonist)-induced catalepsy (a form of hypolocomotion), increases amphetamine (a dopamine-releasing agent)-induced circling in animals with unilateral 6-hydroxy dopamine lesions of the striatum and potentiates the ability of amphetamine to reduce the rate of responding in animals trained to bar-press for food pellets (operant suppression) (Nielsen and Sparber, 1984; Ono et al., 1986, 1992; Schwartz et al., 1982). Interpretation of these studies is made difficult since, with the exception of the operant suppression study, high doses of indomethacin were used, well above the drugs LD_{50} and that required to significantly inhibit brain cyclooxygenase activity (Schwartz et al., 1982; Ono et al., 1992). Furthermore, the limited nature of the catalepsy studies raised the question of whether indomethacin simply reduced uptake of haloperidol into the brain. A dopaminergic mechanism is, however, consistent with the recent observation that cyclooxygenase inhibitors reduce haloperidol-induced orofacial dyskinesia, an animal model of tardive dyskinesia (Naidu and Kulkarni, 2001). Both phospholipase A_2 and cyclooxygenase inhibitors also inhibit behavioural sensitisation to amphetamine and cocaine (Reid et al., 1996, 2002).

In vitro studies also suggest a link between dopamine and phospholipase A_2/cyclooxygenase. Thus, in dopamine receptor expressing Chinese hamster ovary cells, D2 and D4 receptor agonists augment adenosine-stimulated phospholipase A_2 activity (Chio et al., 1994; Vial and Piomelli, 1995). Furthermore, chronic administration of the dopamine transporter inhibitor cocaine leads to a downregulation of striatal (a brain region with high dopamine receptor density) phospholipase A_2 activity in both rodents and human users (Ross et al., 1996; Ross and Turenne, in press), while striatal phospholipase A_2 activity is increased in the hypo-dopamine disorder Parkinson's disease (Ross et al., 2001).

The phospholipase A_2/cyclooxygenase signalling system has, however, been linked to the functioning of other neurotransmitters. For example, serotonin 5HT1A and 5HT2 receptors activate phospholipase A_2 activity in both cell lines and rat hippocampal slices cultures (Felder et al., 1990; Claustre et al., 1991; Berg et al., 1998; Tournois et al., 1998), whereas glutamate *N*-methyl-D-aspartate (NMDA) receptors stimulate eicosanoid synthesis and fatty acid release in cerebellar neurons and astroglia, respectively (Lazarewicz et al., 1990; Mollace et al., 1995). Furthermore, the analgesic effects of cyclooxygenase inhibitors are mediated, in part, via inhibition of gamma-aminobutyric acid (GABA)-ergic neurotransmission in anti-nociceptive circuits (Vaughan et al., 1997).

In order to further investigate the putative dopaminergic properties of cyclooxygenase inhibitors, we examined the effect of two drugs of this type, the non-cyclooxygenase-1 and cyclooxygenase-2 selective inhibitors piroxicam and indomethacin, upon several measures considered to have a strong dopaminergic component.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats (250–350 g) were housed in pairs and given ad lib access to food and water throughout the experiments. The room temperature was 21 ± 1 °C with a 12-h light/dark schedule, lights out at 1900 h. All training and testing was carried out during the light phase. Animals were allowed to habituate to housing conditions and were handled daily for 7 days before experimental testing began. All procedures were approved by the institutions animal ethics committee and were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and the National Institutes of Health guide for the care and use of laboratory animals.

2.2. Drugs

d-Amphetamine (Sigma, US), raclopride (Astra, Sweden) and SCH23390 (Sigma) were dissolved in 0.9% saline while apomorphine (Sigma) was dissolved in 0.9% saline/0.1% ascorbic acid solution. Haloperidol (Sabex) solution was diluted in distilled water. Indomethacin (Sigma) was dissolved in an alkaline sodium carbonate vehicle, the pH of which was adjusted to 8.6 with 1 M HCl. Piroxicam (Pfizer, US) was dissolved in 10 mM phosphate buffer containing 137 mM saline, the pH of which had been adjusted to pH 12.5 with 2.5 M NaOH. After the drug was dissolved, the pH of the solution was then adjusted to 8.0 with 1 M HCl. All drugs were newly prepared each test day and the vehicle used for each served as the control injection.

2.3. Behavioural measures

The brain contains two major dopamine systems, the nigra-striatal which is involved in control of motor behaviour, and the mesocorticolimbic which is critical for the rewarding aspects of dopaminergic agents such as amphetamine. Behavioural measures were utilised which examined both systems. Thus, catalepsy was induced using the dopamine D1-like (D1, D3 and D5) receptor anatagonist R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH23390) and the dopamine D2-like (D2 and D4) receptor antagonist raclopride. Dopamine D1-like and D2-like receptor antagonists produce catalepsy by

inhibiting dopaminergic neurotransmission from the substantia nigra to the striatum (Marin et al., 1993), although they act via the "indirect" and "direct" motor pathways, respectively (Marsden and Obseo, 1994). Secondly, we utilised the ability of amphetamine to stimulate locomotor activity, an effect most likely mediated via stimulation of dopaminergic transmission between the ventral tegmental area and the nucleus accumbens, part of the mesocorticolimbic system (Wise and Rompre, 1989; Amalric and Koob, 1993). Thirdly, the ability to alter the rewarding action of amphetamine was assessed using a self-administration paradigm (Wise and Rompre, 1989; Fletcher et al., 1999). Finally, the effect of cyclooxygenase inhibitors upon prepulse inhibition of the acoustic startle reflex was investigated. Prepulse inhibition is the normal inhibition of auditory startle that occurs when a smaller sub-threshold noise is presented shortly before the startle stimuli (Swerdlow et al., 1994). Prepulse inhibition is reduced in patients with schizophrenia, an effect that can be reproduced in rats by administering dopaminergic agents such as amphetamine and the dopamine D1/D2 receptor agonist apomorphine (Swerdlow et al., 1994).

2.3.1. Catalepsy

Catalepsy was measured using the bar test method. The animal's two front paws were gently placed on a cylindrical plastic rod (1.5 cm in diameter) positioned 8 cm above the tabletop. The time (latency score) the animal's paws remained on the bar was measured and a trial was concluded when the animals placed both paws on the tabletop. If the animal's paws remained on the bar for 5 min, the trial was concluded by the experimenter and a score of 300 s was recorded. The experimenter was blind to treatment group.

Each experiment consisted of 48 rats randomised into four groups receiving (1) dopamine receptor antagonist/vehicle, (2) dopamine receptor antagonist/cyclooxygenase inhibitor, (3) vehicle/cyclooxygenase inhibitor or (4) vehicle/vehicle. In order to account for the differences in onset of catalepsy produced by the different dopamine receptor antagonists, haloperidol was administered 2 h before behavioural testing, while 30- and 15-min pretreatment times were used for raclopride and SCH23390, respectively. Cyclooxygenase inhibitors were administered 60 min before SCH23390 or raclopride, but 60 min after haloperidol due to the much slower rate of onset of catalepsy induced by the latter.

2.3.2. Locomotor activity

Activity was measured in four ENV-515 Open Field Test Environments (Med Associates, St. Albans, VT, USA). Each monitor consisted of a Plexiglas chamber measuring 40 cm long, 40 cm wide and 28 cm high. Two arrays of 16 infrared beams detected horizontal movements. The chambers were connected to an IBM-type personal computer for data collection. Ambulatory counts were calculated from the number of interrupted photo beams.

Animals were pretreated with either indomethacin or piroxicam 60 min before amphetamine injections. Forty minutes following cyclooxygenase inhibitor pretreatment, the animals were placed in the activity chamber for a habituation period of 20 min and then injected with amphetamine and immediately replaced in the chamber. Ambulatory counts were then monitored for an additional 50 min.

2.3.3. Prepulse inhibition of the auditory startle reflex

Three startle chambers (SR-LAB, San Diego Instruments) were used. Each sound-attenuated chamber contained a Plexiglas cylinder (8.2-cm diameter) in which the animal was placed during testing. A piezoelectric accelerator was affixed underneath the cylinder that detected and transduced motion from the animal to the SR-LAB computer software. Acoustic startle stimuli and background white noise were presented via a speaker on the sidewall, 24 cm above the cylinder. Startle amplitude was determined by the average of 150 1-ms readings collected at the beginning of the stimulus onset.

Animals were habituated to the test chambers with 65-dB background white noise before testing for 10 min. Animals were tested in four sessions, each separated by 7 days. Each session included 41 trials separated by 20–40 s (average 30 s) inter-trial intervals and preceded by a 10 min acclimatisation period to background white noise. Four different trial types were presented in random order: (1) no stimulus, (2) startle alone, (3) prepulse alone and (4) prepulse-startle. A 65-dB background white noise was presented throughout the testing session. Each trial type was presented 10 times with one additional startle alone trial presented at the very beginning of the session. Startle parameters were startle 110 dB (40-ms duration), prepulse 75 dB (20-ms duration), prepulse-startle time interval was 100 ms.

Animals were separated into two experimental groups ($n = 15$): (1) apomorphine and (2) amphetamine. Within each experimental group, animals were tested four times, receiving either (1) vehicle/saline, (2) vehicle/dopamine agonist, (3) cyclooxygenase inhibitor/saline or (4) cyclooxygenase inhibitor/dopamine agonist. The four drug combinations were administered in counterbalanced order across the four testing sessions. Rats were pretreated with indomethacin or piroxicam 60 min before apomorphine or amphetamine, and testing began 10 min after apomorphine or amphetamine administration. Percentage of prepulse inhibition was calculated using the formula: (startle − (prepulse/startle))/startle∗100.

2.3.4. Self-administration of amphetamine

Testing was conducted in operant chambers (Med Associates) measuring 28 cm long, 21 cm wide and 21 cm high. Each chamber contained a food pellet dispenser, two response levers and a stimulus light located 6 cm above each lever. The jugular catheters were connected by Tygon tubing to a syringe pump located outside the chamber which

dispensed the drug. The data was collected by an IBM-type personal computer.

Animals were implanted with an indwelling jugular catheter (Fletcher et al., 1999). Just prior to surgery, the rats were food restricted (20 g food per day) and trained to lever press for 45 mg food pellets according to a fixed ratio 1 schedule (FR1). Following recovery from surgery, the rats were trained to self-administer infusions (approximately 0.1 ml over 5 s) of *d*-amphetamine (60 μg/kg) during 3-h sessions according to a fixed ratio 1 schedule as previously described in detail (Fletcher et al., 1999). Each infusion was signalled by a stimulus light, which remained illuminated for a 20-s timeout period, during which responses were recorded but not reinforced. On test days, the animals were pretreated with cyclooxygenase inhibitors or vehicle 60 min before amphetamine (60 μg/kg) self-administration testing. Cyclooxygenase inhibitor or vehicle testing was spaced at least 2 days apart. After this, each animal was shifted to a different dose of *d*-amphetamine (30 or 120 μg/kg), until responding was stable on three consecutive days, and tested again following injections of either cyclooxygenase inhibitor or vehicle.

2.4. Measurement of brain levels of amphetamine and haloperidol

Animals were euthanised by decapitation, and their brains rapidly dissected into cerebellar and cerebral cortices and striatum, and stored at −80 °C until required.

2.4.1. Haloperidol

Brain tissue was disrupted by sonication in water (10% w/v). A D8 haloperidol internal standard was added to 250 μl of tissue sonicate, followed by the sequential addition of 1 ml 0.25 M sodium hydroxide and 10 ml of hexane:isoamyl alcohol (98.5:1.5 v/v). Samples were shaken for 20 min, followed by placing at −70 °C for 10 min to facilitate phase separation. The organic phase was then removed and 0.1 M hydrochloric acid was added and the aqueous phase discarded. Samples were shaken for a further 20 min followed by 10 min at −70 °C. The organic phase was discarded and aqueous phase thawed at 30 °C, followed by the addition of 500 μl 4 M ammonium hydroxide and 200 μl butyl acetate. Samples were then vortexed for 2 min and centrifuged for 10 min at 3000 × g_{av}. The butyl acetate layer was then analysed using gas chromatography mass spectroscopy using a Thermo TRIO-1000 equipped with a quadropole system and an AS800 autosampler. Electron energy was set to 70 eV. Temperature of the source was set to 250 °C with a carrier gas flow rate of 4 ml/min. A splitless injector was used with an injector temperature of 240 °C. Analyses were performed using an 8 m DB-5 column with an initial oven temperature of 140 °C, rising at 20 °C/min to 280 °C. Under these conditions, the retention time for haloperidol was 6.7 min. Base peaks at *m*/*z* 224 and 237 were monitored for haloperidol; 232 and 245 were used for D8 haloperidol. Quantification was performed by calculating the ratio of the protium haloperidol over the deuterated internal standard. Haloperidol levels were calculated by comparison to a 0–60 nM haloperidol standard curve run in parallel to tissue samples.

2.4.2. Amphetamine

Brain tissue (100 mg) was homogenized in 2 ml 100 mM phosphate buffer pH 6.0. Deuterated-analogues of methamphetamine and amphetamine were added for internal standards along with 3 ml 100 mM phosphate buffer pH 6.0 and vortexed. Samples were centrifuged at 3000 rpm for 5–10 min and pH adjusted to 6.0, if necessary. Samples were extracted using Worldwide Monitoring CSDAU020 Clean Screen solid phase extraction columns following the recommended procedure. The samples were then evaporated to dryness under nitrogen at room temperature, derivatized with 50 μl of heptafluorobutyric acid anhydride, capped under nitrogen and incubated at 70 °C for 20 min. Samples were evaporated to dryness at 40 °C and reconstituted with 50 μl ethyl acetate. Samples were analyzed by gas chromatography mass spectroscopy using a Varian Saturn 2000 ion

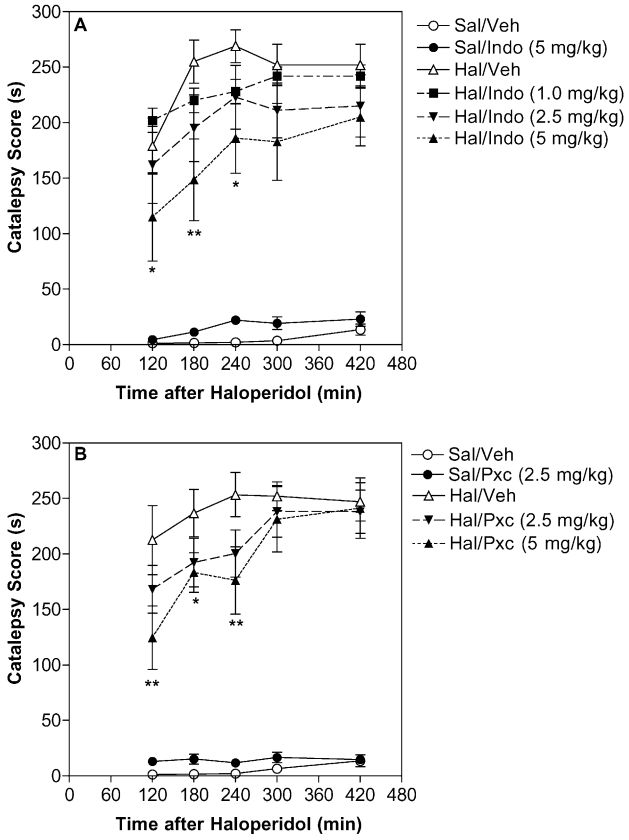

Fig. 1. Haloperidol (Hal)-induced catalepsy is inhibited by (A) indomethacin (Indo) and (B) piroxicam (Pxc). Animals ($n = 12$ per group) were injected with haloperidol (0.5 mg/kg i.p.) followed 60 min later by cyclooxygenase inhibitor (1, 2.5 or 5 mg/kg i.p. as indicated). Graphs show mean catalepsy scores; bars indicate S.E.M. * and ** indicate statistical significance of one-way ANOVA ($P < 0.05$) with *: $P < 0.05$, **: $P < 0.01$ by post-hoc LSD comparing Hal/cyclooxygenase inhibitor with Hal/Veh.

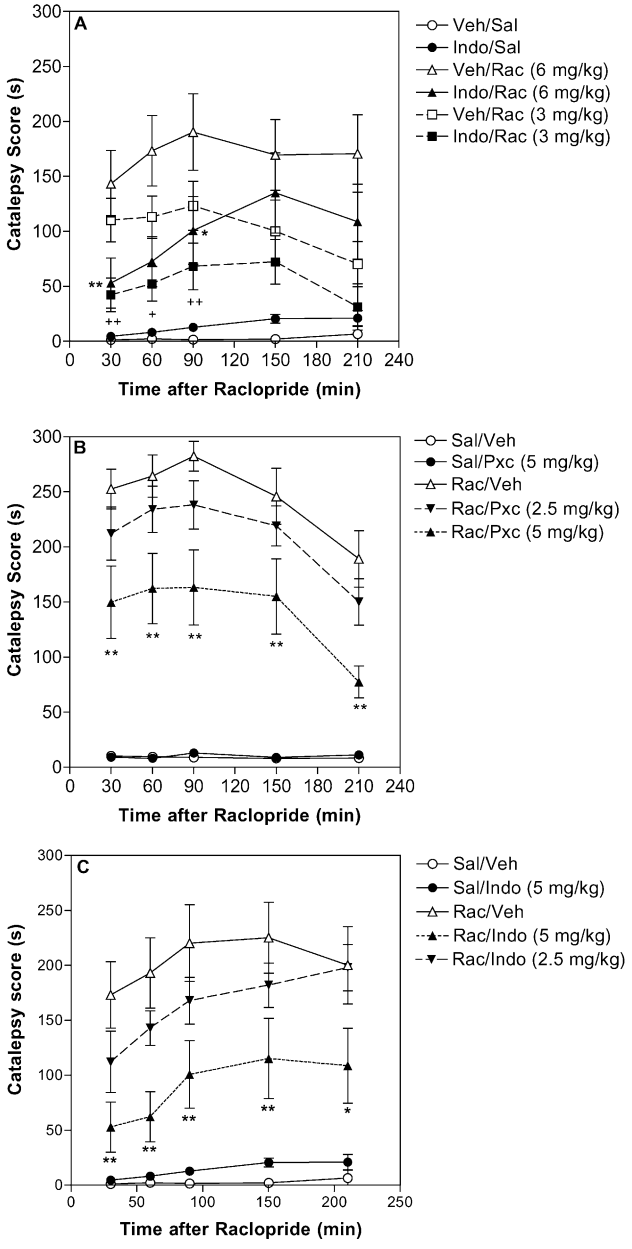

Fig. 2. Catalepsy induced by raclopride (Rac) is inhibited by indomethacin (Indo) and piroxicam (Pxc). Animals ($n = 12$ per group) were injected with cyclooxygenase inhibitor or vehicle (Veh) followed 60 min later by raclopride. (A) Indomethacin (5 mg/kg i.p.) significantly inhibited catalepsy induced by 3 or 6 mg/kg i.p. raclopride. * and ** indicate statistical significance of one-way ANOVA ($P < 0.05$, **: $P < 0.01$ by post-hoc LSD comparing Indo/Rac (6 mg/kg) with Veh/Rac (6 mg/kg) and +: $P < 0.05$, ++: $P < 0.01$ by post-hoc LSD comparing Indo/Rac (3 mg/kg) with Veh/Rac (3 mg/kg). (B–C) Comparison of the effects of two doses (2.5 and 5 mg/kg i.p.) of piroxicam or indomethacin upon 6 mg/kg i.p. raclopride-induced catalepsy. * and ** indicate statistical significance of one-way ANOVA ($P < 0.05$) with *: $P < 0.05$, **: $P < 0.01$ by post-hoc LSD comparing Rac/cyclooxygenase inhibitor with Rac/Veh. Graphs show mean catalepsy scores; bars indicate S.E.M.

trap mass spectrometer interfaced with a Varian 3400 gas chromatograph equipped with a J & W Scientific DB5-MS capillary column (15 m × 0.25 mm ID, 0.25-μm film thickness) using a helium carrier at a flow rate of 1 ml/min. On-column injections of 1 μl of sample were made with the gas chromatograph oven temperature programmed from 75 °C

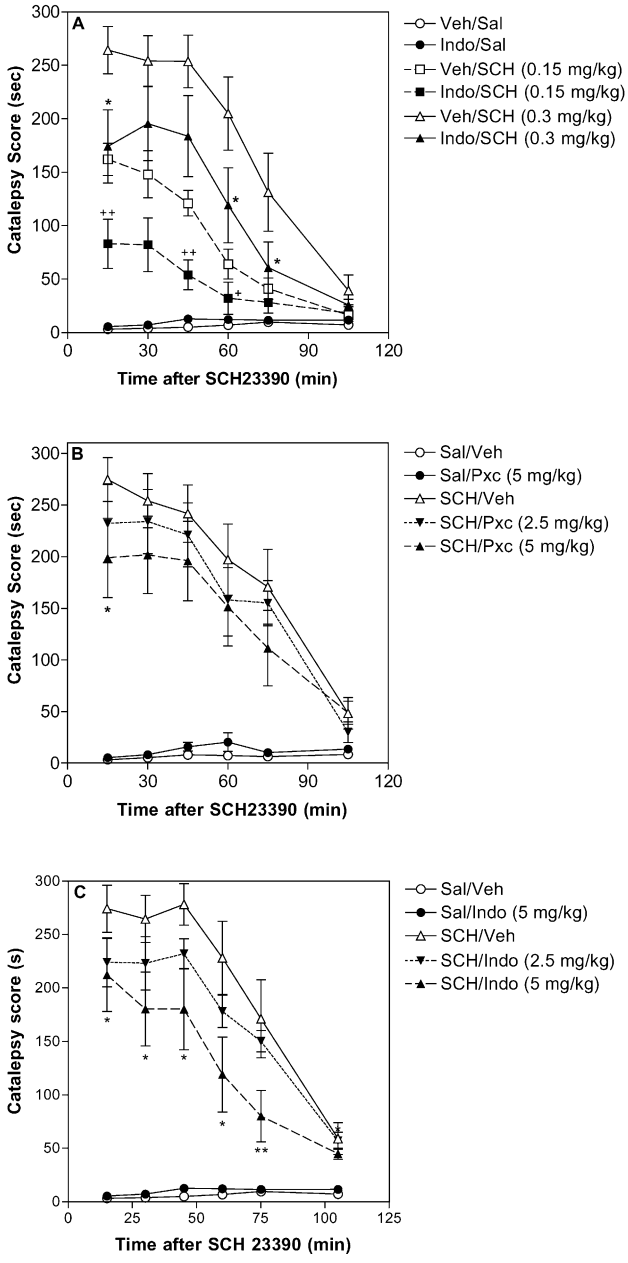

Fig. 3. Catalepsy induced by SCH23390 (SCH) is inhibited by indomethacin (Indo) and piroxicam (Pxc). Animals ($n = 12$ per group) were injected with cyclooxygenase inhibitor or vehicle (Veh) followed 60 min later by SCH23390. (A) Indomethacin (5 mg/kg i.p.) significantly inhibited catalepsy induced by 0.15 or 0.3 mg/kg i.p. SCH23390. *: $P < 0.05$ by one-way ANOVA and post-hoc LSD comparing Indo/SCH (0.3 mg/kg) with Veh/SCH (0.3 mg/kg); +: $P < 0.05$, ++: $P < 0.01$ by post-hoc LSD comparing Indo/SCH (0.15 mg/kg) with Veh/SCH (0.15 mg/kg). (B–C) Comparison of the effects of two doses (2.5 and 5 mg/kg i.p.) of piroxicam or indomethacin upon 0.3 mg/kg i.p. SCH23390-induced catalepsy. * and ** indicate statistical significance of one-way ANOVA ($P < 0.05$) with *: $P < 0.05$, **: $P < 0.01$ by post-hoc LSD comparing SCH/cyclooxygenase inhibitor with SCH/Veh. Graphs show mean catalepsy scores; bars indicate S.E.M.

(hold time 1 min) to 180 °C at 15 °C/min then to 300 °C at 50 °C/min. The injector was set at 150 °C, the transfer lines at 270 °C and the trap temperature was 200 °C. Ions were monitored at *m/z* 254, 210, 118 for methamphetamine and *m/z* 240, 118, 117 for amphetamine. Concentrations were determined based on a standard curve run with each set of samples that included three calibrators as well as a positive control and a negative control.

2.5. Statistical analyses

Data was analysed using the appropriate analysis of variance (ANOVA), which if significant at $P = 0.05$, was followed up using post-hoc LSD comparisons.

3. Results

3.1. Catalepsy

Both indomethacin (5 mg/kg i.p.) and piroxicam (5 mg/kg i.p.) significantly inhibited haloperidol-induced catalepsy (one-way ANOVA, $P < 0.05$; post one-way ANOVA LSD; indomethacin/haloperidol vs. vehicle/haloperidol; $P < 0.05$) (Fig. 1). Indomethacin, 1 and 2.5 mg/kg i.p., and 2.5 mg/kg piroxicam also reduced haloperidol (0.5 mg/kg i.p.)-induced catalepsy, although this did not reach statistical significance (Fig. 1). Raclopride (3 and 6 mg/kg i.p.)-induced catalepsy and that induced by SCH23390 (0.3 and 0.15 mg/kg i.p.) were also significantly reduced by indomethacin (5 mg/kg i.p.) and piroxicam (5 mg/kg i.p.) (Figs. 2 and 3). A lower dose of each cyclooxygenase inhibitor (2.5 mg/kg i.p.) reduced both raclopride (6 mg/kg i.p.)- and SCH23390-induced catalepsy (0.3 mg/kg i.p.), although these effects did not reach statistical significance (Figs. 2 and 3).

3.2. Locomotor activity

As illustrated in Fig. 4A and B, both piroxicam (5 mg/kg i.p.) and indomethacin (5 mg/kg i.p.) significantly inhibited amphetamine (0.3 mg/kg i.p.)-stimulated locomotor activity 20 min (one-way ANOVA, $P < 0.05$; post-ANOVA LSD; indomethacin/amphetamine vs. vehicle/amphetamine; $P < 0.01$) after amphetamine administration; indomethacin also had a significant effect ($P < 0.05$) at the 40-min time point. A lower dose of both cyclooxygenase

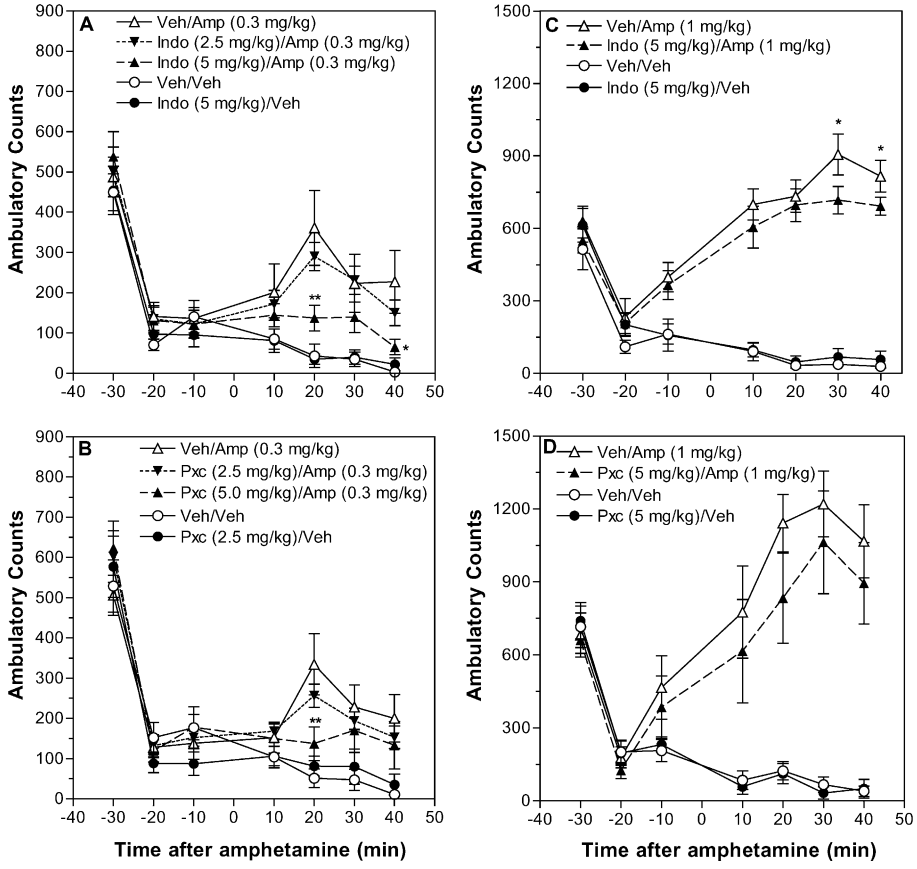

Fig. 4. Inhibition of amphetamine-stimulated locomotor activity by (A and C) indomethacin (Indo) and (B and D) piroxicam (Pxc). Animals ($n = 12–16$ per group) were injected with cyclooxygenase inhibitor (2.5 or 5 mg/kg i.p. as indicated) followed 60 min (time = 0 on figure) later by (A and B) 0.3 mg/kg i.p. or (C and D) 1.0 mg/kg i.p. amphetamine. Graphs show mean locomotor activity; bars indicate S.E.M. * and ** indicate statistical significance of one-way ANOVA ($P < 0.05$) with *: $P < 0.05$, **: $P < 0.01$ by post-hoc LSD comparing cyclooxygenase inhibitor/Amp with Veh/Amp.

inhibitors (2.5 mg/kg i.p.) had no significant effect upon 0.3 mg/kg i.p. amphetamine-stimulated locomotor activity. Indomethacin also inhibited locomotor activity stimulated by 1.0 mg/kg i.p. amphetamine at 30 min ($P<0.05$) and 40 min ($P<0.05$) after injection of amphetamine (Fig. 4C). No significant effect was observed with piroxicam, however, using the higher dose of amphetamine (Fig. 4D). Neither piroxicam (5 mg/kg i.p.) nor indomethacin (5 mg/kg i.p.) had a significant effect upon locomotor activity recorded during (a) habituation trials (Fig. 4), (b) following vehicle injections (Fig. 4) or (c) novelty-stimulated activity (Fig. 5).

3.3. Prepulse inhibition

Preliminary experiments utilising 5 mg/kg i.p. cyclooxygenase inhibitor showed no effect in this model (data not shown), leading to our use of a higher 10 mg/kg i.p. dose. A one-way repeated measures ANOVA on drug treatment revealed a significant effect ($P<0.01$) of 0.25 and 0.5 mg/kg i.p. apomorphine and 2.5 and 5 mg/kg i.p. amphetamine on inhibition of prepulse inhibition as would be expected. Post-hoc LSD tests indicated that indomethacin alone had no significant ($P>0.05$) effect on prepulse inhibition, as well as no effect on apomorphine- or amphetamine-induced inhibition of prepulse inhibition (Fig. 6), while piroxicam (10 mg/kg i.p.) produced a small potentiation of the effect produced by 0.25 mg/kg i.p. apomorphine ($P<0.05$; Fig. 6). Neither amphetamine nor apomorphine administered either alone or in the presence of cyclooxygenase inhibitor significantly altered the response to the startle stimulus alone (data not shown).

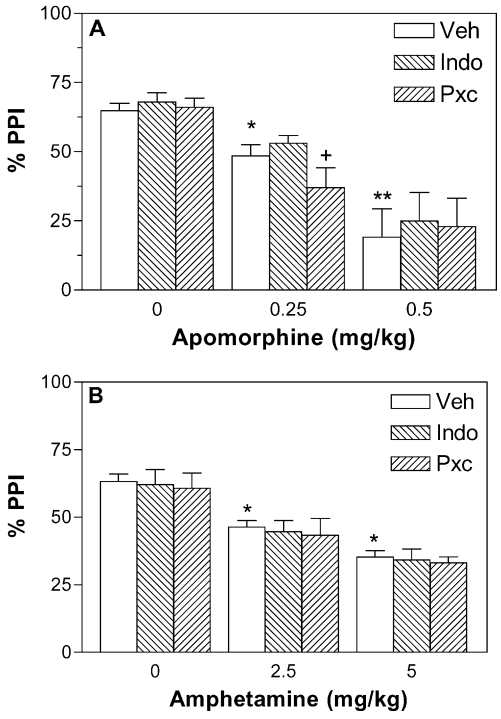

Fig. 6. No effect of indomethacin (Indo) and piroxicam (Pxc) on reduction of prepulse inhibition of the auditory startle reflex (PPI) by (A) apomorphine and (B) amphetamine. Animals ($n=12$ per group) were treated with either vehicle (Veh), 10 mg/kg i.p. indomethacin or 10 mg/kg i.p. piroxicam, followed after 60 min by either 0.25 or 0.5 mg/kg i.p. apomorphine or 2.5 or 5 mg/kg i.p. amphetamine (Amp). Graphs show mean activity scores; bars indicate S.E.M. * and ** indicate statistical significance of one-way ANOVA ($P<0.05$) with *: $P<0.05$, *: $P<0.01$ by post-hoc LSD comparing Veh/Veh with Veh/challenge drug. +: $P<0.05$ by post-hoc LSD comparing Pxc/Apo with Veh/Apo.

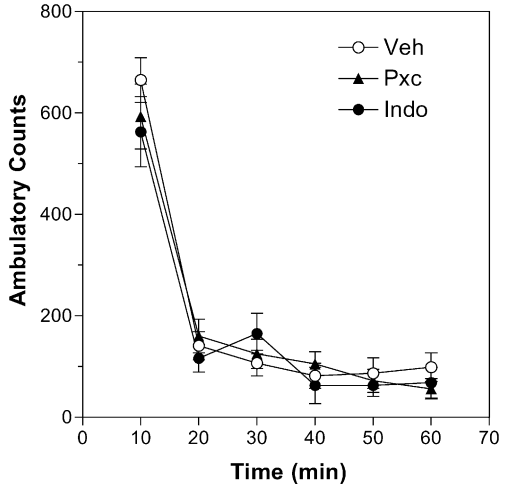

Fig. 5. Cyclooxygenase inhibitors do not effect novelty-stimulated locomotor activity. Animals ($n=8$ per group) were treated with either vehicle (Veh), 5 mg/kg i.p. indomethacin (Indo) or piroxicam (Pxc). After 60 min, animals were transferred to the activity recording apparatus (time = 0 on figure). Graphs show mean activity scores; bars indicate S.E.M.

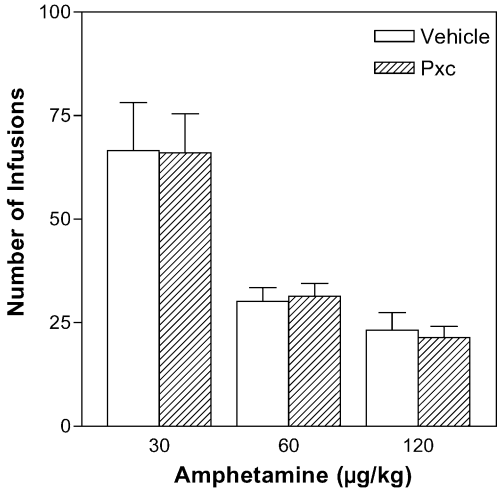

Fig. 7. Cyclooxygenase inhibitors have no effect upon self-administration of amphetamine. 10 mg/kg i.p. piroxicam (Pxc) was administered to animals ($n=8-12$ per group) trained to self-administer amphetamine. Self-administration sessions commenced 60 min after cyclooxygenase inhibitor administration using varying doses of amphetamine as indicated. Graphs show mean number of infusions per session; bars indicate S.E.M.

3.4. Amphetamine self-administration

Piroxicam (10 mg/kg i.p.) did not significantly ($P>0.05$) alter the rate of amphetamine self-administration at any of the infusion doses (30, 60 and 120 μg) utilised (Fig. 7). Furthermore, indomethacin (10 mg/kg i.p.) had no significant effect upon self-administration of the 60-μg infusion dose (mean number of infusions were 37 ± 5 and 33 ± 3 in the vehicle and indomethacin groups ($n=8$), respectively).

3.5. Brain levels of haloperidol and amphetamine after pretreatment with cyclooxygenase inhibitors

We considered that our results may have been due to cyclooxygenase inhibitors altering brain uptake and/or metabolism of the challenge drugs. To address this possibility, brain levels of amphetamine or haloperidol were compared between cyclooxygenase inhibitor treated and untreated animals. The time interval between haloperidol or amphetamine administration and brain level measurement was chosen to coincide with the maximal cataleptic and locomotor stimulatory effect of each drug as appropriate.

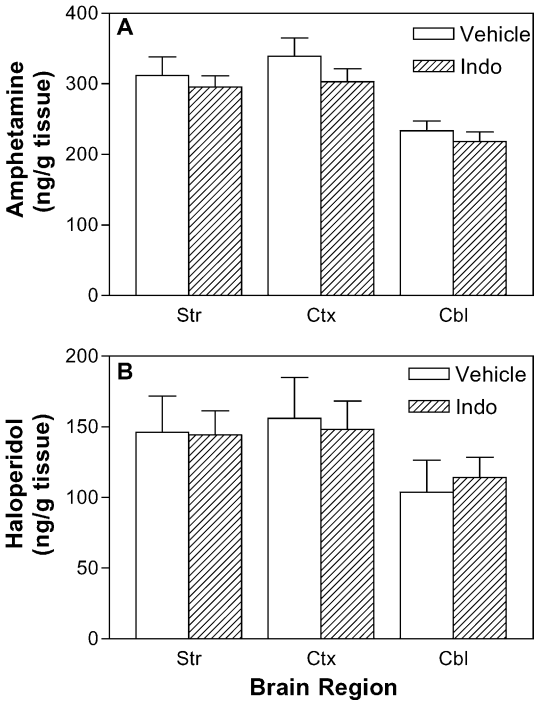

Fig. 8. Pretreament with indomethacin (Ind) does not alter brain levels of either (A) amphetamine or (B) haloperidol. The time course of injections was chosen to coincide with the maximal change in behavioural effects produced by cyclooxygenase inhibitors (see Figs. 1 and 4). For amphetamine: animals were injected with indomethacin (5 mg/kg i.p.) or vehicle ($n=6$ per group) followed 60 min later by amphetamine (0.3 mg/kg i.p.). After further 15 min, animals were euthanised and their brains removed. For haloperidol: animals were injected with haloperidol (0.5 mg/kg i.p.) or vehicle ($n=6$ per group) followed 60 min later by indomethacin (5 mg/kg i.p.). Animals were euthanised 120 min later. Graphs show mean drug level; bars indicate S.E.M. Str: striatum; Ctx: cortex; Cbl: cerebellum.

Indomethacin (5 mg/kg i.p.) did not significantly alter levels of either drug in the cerebral cortex, cerebellar cortex or striatum (Fig. 8).

4. Discussion

The objective of our study was to determine whether the cyclooxygenase inhibitors indomethacin and piroxicam had a generalised dopaminergic effect upon behaviours affected by dopamine receptor agonists or antagonists. In agreement with an earlier investigation (Ono et al., 1992), we observed that both cyclooxygenase inhibitors reduced haloperidol-induced catalepsy. Moreover, brain levels of haloperidol were not affected by administration of indomethacin suggesting that cyclooxygenase inhibitors do not alter the pharmacokinetics of haloperidol (see Fig. 8). Importantly, the cyclooxygenase inhibitor dose was much lower than that used in many earlier studies minimising the possibility that our results are due to a nonspecific effect resulting from drug toxicity (Schwartz et al., 1982; Ono et al., 1992). Nevertheless, the dose of indomethacin used in the present experiments (5 mg/kg i.p.) results in a 50–60% reduction in brain prostaglandin levels 1-h post-administration (Abdel-Halim et al., 1978; Kleven et al., 1983). We have extended the anti-cataleptic effects of cyclooxygenase inhibitors to include catalepsy produced by other dopamine receptor antagonists. Thus, both piroxicam and indomethacin inhibited catalepsy induced by SCH23390 and raclopride, antagonists of the dopamine D1-like and D2-like receptors, respectively, findings consistent with a dopaminergic effect of cyclooxygenase inhibitors. This is unlikely to involve a modulation of post-synaptic dopaminergic signalling given that each class of dopamine receptor has an opposing metabolic effect upon adenylyl cyclase. Moreover, cell culture models utilising transfected dopamine receptors suggest that dopamine D2 receptor blockade should actually decrease prostaglandin synthesis (McAllister et al., 1993; Vial and Piomelli, 1995), an effect that would be expected to be *enhanced* by inhibition of cyclooxygenase. Indeed, our data suggest that if the stimulation of phospholipase A_2 activity by dopamine D2 receptors in these cells predicts a physiologically relevant mechanism, then any functional effects are unlikely to involve prostaglandins. In addition, modulation of dopamine release and uptake rates, i.e. presynaptic control, is also unlikely given the differential effect of cyclooxygenase inhibitors upon catalepsy and amphetamine self-administration (assuming that the neurochemistry of the nigra-striatal dopamine system is similar to that in the mesocorticolimbic). Nevertheless, a direct action by free unmetabolised arachidonic acid, e.g. regulation of the dopamine transporter (Zhang and Reith, 1996; Ingram and Amara, 2000), is not ruled out by our data.

A second mechanism by which cyclooxygenase may modulate dopaminergic transmission has been suggested

by in vitro findings indicating that cyclooxygenase may act as a form of dopamine oxidase (Hastings, 1995). Such an action might be expected to increase presynaptic dopamine levels by reducing dopamine oxidation, thereby increasing dopaminergic signalling. However, results from the other models utilised in our study do not support a dopaminergic mechanism for the antagonism of catalepsy by cyclooxygenase inhibitors. Thus, it would be expected that a stimulator of dopaminergic neurotransmission should (a) increase basal or amphetamine-stimulated locomotor activity, (b) increase disruption of prepulse inhibition by apomorphine or amphetamine, or disrupt prepulse inhibition in its own right and (c) decrease self-administration of amphetamine. We observed, however, that both piroxicam and indomethacin had no effect upon amphetamine self-administration. Furthermore, cyclooxygenase inhibitors significantly *inhibited* amphetamine-stimulated locomotor activity (although to a lesser extent than their effect upon catalepsy) under conditions in which brain levels of amphetamine were unchanged by the presence of the cyclooxygenase inhibitor (see Figs. 4 and 8). The reduction in amphetamine-stimulated activity is also unlikely to be a general sedative effect of cyclooxygenase inhibitors since novelty-stimulated activity was unaltered (see Fig. 5). In addition, although piroxicam potentiated the effects of apomorphine upon prepulse inhibition, no such potentiation was observed using either a higher dose of apomorphine or with amphetamine, or when piroxicam was replaced by indomethacin. It is therefore unlikely that cyclooxygenase inhibitors have a simple generalised dopaminergic action. Indeed, cyclooxygenase inhibitors do not affect the firing rate of mesoaccumbens dopamine neurons, although a modulatory effect of cyclooxygenase inhibitors upon morphine stimulation of these cells is reported (Mellis et al., 2000). Moreover, effects of indomethacin previously thought to be due to dopaminergic stimulation, namely the ability to enhance operant suppression by amphetamine in rats (Nielsen and Sparber, 1984), the inhibition of haloperidol orofacial dyskinesia (Naidu and Kulkarni, 2001) and the anecdotal clinical reports of cyclooxygenase inhibitors inducing psychosis (Hoppmann et al., 1991), are likely to involve cyclooxygenase inhibitor modulation of other nondopaminergic neurotransmitter systems.

Cyclooxygenase has been implicated in the signalling mechanisms of receptors other than dopamine including glutamate and GABA, both of which are known to play important roles in motor behaviour. Thus, the glutamate receptor agonist NMDA stimulates eicosanoid synthesis (Lazarewicz et al., 1990; Mollace et al., 1995), whereas NMDA-induced allodynia and brain c-*fos* expression are both prostaglandin-dependent processes (Lerea et al., 1997; Dolan and Nolan, 1999). Given that the glutamate NMDA receptor antagonists dizocilpine (MK 801) and D(−)-2-amino-5-phosphonopentanoic acid (AP-5) decrease dopamine antagonist-induced catalepsy when administered both systemically and into the striatum or entopeduncular nucleus, whereas infusion of NMDA into the striatum results in catalepsy (Schmidt and Bury, 1988; Yoshida et al., 1994; Kaur et al., 1997), the possibility must be considered that cyclooxygenase inhibitors antagonise catalepsy by inhibiting NMDA receptor-initiated processes. An alternative explanation is suggested by a recent report showing that quinolinic acid lesion of the output nuclei of the basal ganglia, the globus pallidus, have two quite opposite effects, namely antagonising both raclopride and SCH23390 induced catalepsy, and inhibiting locomotor stimulation by amphetamine (Haber et al., 1998). This outcome is similar to that occurring after systemic administration of cyclooxygenase inhibitors, with the exception that globus pallidus lesions normally produce an overall decrease in locomotor activity. Furthermore, given the major role of GABA in the control of motor behaviour by the globus pallidus, e.g. the $GABA_A$ receptor antagonist picrotoxin inhibits dopamine antagonist-induced catalepsy when infused into this structure (Ossowska et al., 1984), and that cyclooxygenase inhibitors have been shown to decrease GABAergic signalling in nociceptive circuits (Vaughan et al., 1997), one must also consider that they influence the motor system by modulating GABAergic transmission. In this regard, it is notable that GABA receptor agonists can have both hypo- and hyper-locomotory effects depending on the dose of drug used when infused into the ventral pallidum (Swerdlow and Koob, 1984; Swerdlow et al., 1986).

In summary, our data suggests that the anti-cataleptic effects of cyclooxygenase inhibitors are not due exclusively to a simple dopaminergic effect of these compounds. Indeed they have the unique effect of both inhibiting hypoactivity (catalepsy) and, albeit to a lesser extent, amphetamine-stimulated hyperactivity. It seems likely that their mechanism of action involves multiple transmitter systems and/or brain structures. Further study of the nature of the interaction between prostaglandins and the brain's motor system appears warranted, especially given that the possibility of an extra-striatal site of action offers the potential for therapeutic approaches in movement disorders, e.g. progressive supranuclear palsy, in which striatally acting drugs such as 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) are largely ineffective (Pahwa, 1999).

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