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Evidence for the involvement of cyclooxygenase activity in the development of cocaine sensitization

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Abstract

Phospholipase A2 (PLA_2) activation generates the release of arachidonic acid (AA) and platelet-activating factor (PAF), two compounds which may be involved in neuroplasticity. In previous studies, we found that PLA_2 activation is involved in the development of stimulant sensitization. In the present study, we have examined the roles of AA and PAF in the development of stimulant sensitization using agonists and antagonists selective for PAF receptors or the induction of various AA cascade-mediated eicosanoids. Sprague–Dawley rats were treated for 5 days with cocaine (30 mg/kg) or D-amphetamine (1 mg/kg) preceded 15 min earlier by various antagonists, and then tested following a 10-day withdrawal period for cocaine (15 mg/kg) or D-amphetamine (0.5 mg/kg)-induced locomotion. Consistent with our earlier work, pretreatment with the PLA_2 inhibitor quinacrine (25 mg/kg) blocked the development of cocaine and amphetamine sensitization. The lipoxygenase (LOX) inhibitors nordihydroguaiaretic acid (NDGA) (5–10 mg/kg) and MK-886 (1 mg/kg) had no effect on cocaine sensitization. The PAF receptor antagonist WEB 2086 (5–10 mg/kg) reduced the development of cocaine sensitization. The cyclooxygenase (COX) inhibitors indomethacin (1–2 mg/kg), piroxicam (0.5–1 mg/kg), 6-methoxy-2-napthylacetic acid (6-MNA; 0.5–1 mg/kg), and NS-398 (0.5–1 mg/kg) blocked the development of cocaine sensitization. The COX inhibitors indomethacin (2 mg/kg) and 6-MNA (1 mg/kg) also reduced the development of amphetamine sensitization. Rats were administered bilateral intraventral tegmental area (VTA) injections of D-amphetamine (5 μg/side) or saline coadministered with indomethacin (0.5 μg/side) or vehicle three times over 5 days and were then tested after a 10-day withdrawal for D-amphetamine (0.5 mg/kg ip)-induced locomotion. Intra-VTA amphetamine induced a robust form of amphetamine sensitization, which was blocked by coadministration of indomethacin. Unilateral intra-VTA injections of PAF (1 μg) did not significantly alter cocaine (15 mg/kg ip)-induced locomotion when tested after a 3-day withdrawal. These findings suggest that COX, and possibly PAF, activity is involved in the development of stimulant sensitization. Neuroanatomical studies demonstrate that this may occur at the level of the VTA.

1. Introduction

It is well documented that the behavioral response to psychostimulants, such as amphetamine and cocaine, is enhanced with repeated administration (Downs and Eddy, 1932; Kalivas et al., 1988; Post and Rose, 1976). This phenomenon, known as stimulant sensitization, is long lasting and can increase the susceptibility to stimulant self-administration in rats (Horger et al., 1992; Piazza et al., 1990; Robinson and Becker, 1986; Robinson and Berridge, 1993). A large body of evidence suggests that mesolimbic

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; AA, arachidonic acid; cAMP, cyclic AMP; COX, cyclooxygenase; LPS, lipopolysaccharide; LOX, lipoxygenase; LTD, long-term depression; LTP, long-term potentiation; 6-MNA, 6-methoxy-2-naphthylacetic acid; NDGA, nordihydroguaiaretic acid; NMDA, *N*-methyl-D-aspartate; PLA_2, phospholipase A2; PAF, platelet-activating factor; VTA, ventral tegmental area.

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dopaminergic neurotransmission is directly involved in the development and expression of stimulant sensitization (Kalivas, 1995; Kalivas and Stewart, 1991; Robinson and Berridge, 1993) and the dopaminergic cell bodies of the ventral tegmental area (VTA) are critical in the development of sensitization. Repeated microinjections of amphetamine into the VTA, but not in the mesolimbic terminal regions such as the nucleus accumbens or prefrontal cortex, produce behavioral sensitization in rats (Cador et al., 1995; Dougherty and Ellinwood, 1981; Kalivas and Weber, 1988; Vezina, 1996; Vezina and Stewart, 1990). Furthermore, intra-VTA injections of D1 dopamine receptor antagonists (Bijou et al., 1996; Stewart and Vezina, 1989), *N*-methyl-D-aspartate (NMDA) receptor antagonists (Kalivas and Alesdatter, 1993), or protein synthesis inhibitors (Sorg and Ulibarri, 1995) significantly reduce the development of amphetamine sensitization. Even electrical stimulation of the VTA (Ben-Shahar and Ettenberg, 1994) or local administration of drugs known to activate VTA neurons such as morphine or substance P analogs (Eison et al., 1982; Joyce and Iversen, 1979) will also induce stimulant sensitization.

In addition to dopaminergic involvement in the development of stimulant sensitization, numerous studies indicate the potential involvement of several other neurotransmitter systems. For example, glutamate systems have been implicated by the finding that pretreatment with NMDA (Karler et al., 1989) or non-NMDA (Karler et al., 1991) glutamate receptor antagonists blocks the development of cocaine and amphetamine sensitization. Furthermore, nitric oxide (Itzhak, 1997; Pudiak and Bozarth, 1993) and protein (Karler et al., 1993) synthesis inhibitors also block the development of cocaine and amphetamine sensitization.

We have recently investigated the role of phospholipase A_2 (PLA_2) in the development of stimulant sensitization (Reid et al., 1996). PLA_2 activation, which induces the release of platelet-activating factor (PAF) and the arachidonic acid (AA) cascade of eicosanoids (see Fig. 1), is hypothesized to be involved in numerous neuroplastic phenomena including long-term depression (LTD) in *Aplysia*, hippocampal long-term potentiation (LTP), and the facilitation of neurotransmitter release (Desnos et al., 1992; Koltai et al., 1991; Lynch et al., 1994; Schacher et al., 1993). The AA eicosanoid cascade may produce several bioactive proteins that are potentially relevant to sensitization, such as leukotrines and hepoxilins, mediated by the lipoxygenase (LOX) pathway, and thromboxanes and prostaglandins, mediated by the cyclooxygenase (COX) pathway (Wolfe and Horrocks, 1994). The COX pathway actually comprises two enzyme forms: COX-1, the constitutive form, and COX-2, the inducible form. Studies on COX expression and activity in the brain suggest that COX-2 is the predominant form in neurons and that COX-2 mediates prostaglandin signaling in the brain as well as neurodegeneration (Kaufman et al., 1996; Tocco et al., 1997; Yamagata et al., 1993). In our previous studies on the role of PLA_2 in stimulant sensitization, we found

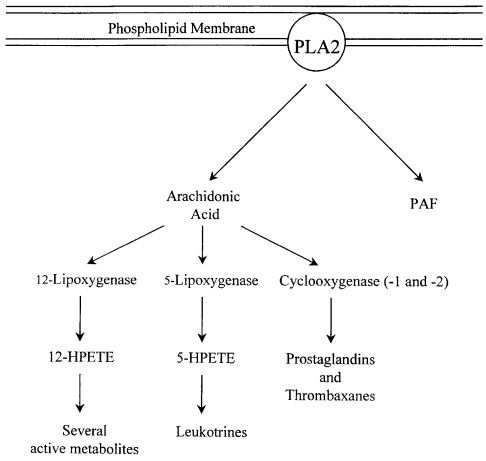

Fig. 1. Schematic diagram of cystosolic PLA_2 and the release of PAF and AA, with the subsequent eicosanoid cascade pathway. AA and PAF are liberated from phospholipid membranes through receptor-mediated activation of cystosolic PLA_2, which cleaves AA and lyso-PAF from esterified phospholipids, phosphatidylcholine, and phosphatidylinositol. Quinacrine inhibits PLA_2 by binding directly to the enzyme and interacting with acidic phospholipids. Once released, AA is metabolized through several pathways, three of which are well documented in the brain tissue and are shown in this figure. This eicosanoid cascade, mediated by LOX and COX enzyme activity, may produce several bioactive proteins that are potentially relevant to sensitization. The LOX pathways consist of 12- and 5-LOX, which catalyze the production of leukotrienes, hepoxilins, and other bioactive proteins. The COX pathway includes a constitutive form of the enzyme (COX-1) and an inducible form of the enzyme (COX-2) both of which catalyze the production of thromboxanes and prostaglandins. Pharmacological inhibition of these pathways is achieved with drugs selective for each of the LOX and COX enzymes (see Methods section for drug selectivity).

that pretreatment with the PLA_2 inhibitor quinacrine dose-dependently blocked the development of behavioral sensitization to cocaine and amphetamine and that intra-VTA injections of the PLA_2 stimulatory drug melittin sensitized animals to both the behavioral and dopamine releasing effects of cocaine (Reid et al., 1996). In the present study, we have further examined the involvement of PLA_2 in the development of cocaine and amphetamine sensitization by investigating the effects of a PAF receptor agonist and antagonist, and several COX and LOX selective enzyme inhibitors on the development of stimulant sensitization. The effects of both peripherally and intra-VTA administered compounds were tested.

2. Methods

2.1. Locale

The studies were performed at the San Francisco, Cincinnati, and New York V.A. Medical Centers. At each site, control groups, vehicle plus cocaine and vehicle plus saline were tested and used for comparison with experimental treatment groups where appropriate.

2.2. Animals

Male Sprague–Dawley rats weighing 260–300 g were used in all experiments. All rats were obtained from Simonsen Animals, Gilroy, CA, except those tested at the New York V.A. Medical Center which were obtained from Charles River, Bloomington, MA. Animals were housed two to a cage in a temperature and humidity-controlled environment, on a 12-h light/dark cycle (6:00 a.m. on, 6:00 p.m. off) with food and water available ad lib. Rats were allowed at least one week of habituation to their housing prior to experimentation.

2.2.1. Cocaine and amphetamine sensitization

2.2.1.1. Pretreatment. Animals were treated in their home cages for 5 consecutive days according to the following schedule: antagonist or vehicle injection, wait 15 min in home cage, cocaine (30 mg/kg), D-amphetamine (1 mg/kg) or saline injection, and then return to home cage. The 15-min interval between the first (vehicle or antagonist/inhibitor) and second (saline or stimulant) injection was chosen based on our previous study on PLA_2 mechanisms in sensitization (Reid et al., 1996). Treatment was given once a day. All drugs were administered intraperitoneally at a volume of 1 ml/kg body weight. The antagonists tested, and their receptor/enzyme selectivity, were as follows:

Antagonist	Selectivity
Quinacrine (25 mg/kg ip)	PLA_2
WEB 2086 (5–10 mg/kg ip)	PAF receptor
Indomethacin (1–2 mg/kg ip)	COX-1 > COX-2
Piroxicam (0.5–1 mg/kg ip)	COX-1
6-MNA (0.5–1 mg/kg ip)	COX-2 > COX-1
NS-398 (0.5–1 mg/kg ip)	COX-2
Nordihydroguaiaretic acid (NDGA) (5–10 mg/kg ip)	5-LOX > 12-LOX
MK-886 (1 mg/kg ip)	5-LOX

2.2.1.2. Study compounds. Quinacrine, cocaine, and D-amphetamine (Sigma, St. Louis, MO), indomethacin, MK-886, 6-MNA, NS-398, and piroxicam (BIOMOL Research Laboratories, Plymouth Meeting, PA), and WEB 2086 (Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT) were prepared daily in either deionized H_2O, 8% EtOH (with 1 drop DMSO/ml), or 0.1 M Na_2HPO_4. None of the above vehicles were found to alter subsequent cocaine responsiveness following the 5-day treatment with a 10-day withdrawal (data not shown). Vehicle/saline-pretreated groups presented in the Results section include animals that were given deionized H_2O or 10% EtOH (with 1 drop DMSO/ml) as the vehicle. Cocaine and D-amphetamine were dissolved in saline.

The selectivity of quinacrine (Blackwell et al., 1977; Chan et al., 1982), indomethacin (Laneuville et al., 1994; Meade et al., 1993; Salari et al., 1984), piroxicam (Laneuville et al., 1994; Meade et al., 1993), 6-MNA (Meade et al., 1993), NS-398 (Futaki et al., 1994), NDGA (Aktan et al., 1993; Salari et al., 1984), MK-886 (Gillard et al., 1988), and WEB 2086 (Casals-Stenzel et al., 1987) are based on previous in vitro studies. Dose selections for the current study were based on compound solubility, the reported IC_{50} for each enzyme inhibitor, the affinity of WEB 2086 for the PAF receptor, and a review of previous in vivo studies in mice, rats, or pigs as follows. Quinacrine pretreatment blocks the development of stimulant sensitization (8–25 mg/kg ip) (Reid et al., 1996) and stress-induced β receptor down-regulation (10–20 mg/kg ip) (Torda et al., 1981). Indomethacin pretreatment reduces lipopolysaccharide (LPS)-induced sickness (3–30 mg/kg ip) (Fishkin and Winslow, 1997) and ischemic neuronal damage (1–10 mg/kg ip) (Sasaki et al., 1988). Piroxicam pretreatment reduces convulsion-induced PGE_2 in the brain (0.1–0.4 mg/kg po) (Engelhardt et al., 1996) and ischemic-induced neuronal damage (10 mg/kg ip) (Nakagomi et al., 1989). 6-MNA inhibits gastric prostaglandin synthesis (1–20 mg/kg ip) (Dandona and Jeremy, 1990) and inhibits hindpaw inflammation/hyperalgesia (10–100 mg/kg im) (Clarke et al., 1994). NS-398 is analgesic in arthritic, edema, and acetic acid-induced pain models (0.2–8.2 mg/kg po) (Futaki et al., 1993) and inhibits LPS-induced plasma PGE_2 (1.5 mg/kg po) (Futaki et al., 1997). NDGA pretreatment reduces cold-induced cerebral edema (3–6 mg/kg ip) (Yen and Lee, 1990) and ischemia-induced PGE_2 in the brain (0.1 mg/kg ip) (Aktan et al., 1993). MK-886 pretreatment blocks LPS-induced leukotriene, but not PGE_2, levels in the hypothalamus (1 mg/kg ip) (Paul et al., 1999). WEB 2086 inhibits intravenous PAF-induced platelet aggregation (0.1–2.0 mg/kg po or 0.01–0.1 mg/kg iv) (Casals-Stenzel et al., 1987).

2.2.1.3. Behavioral testing. Animals were given a 10-day withdrawal before being tested. On the test day, the animals were placed into an activity monitor (Columbus Instruments, Columbus, OH) and allowed to habituate for 60 min. Following the end of habituation, a test dose of cocaine (15 mg/kg ip) or D-amphetamine (0.5 mg/kg ip) was administered and the animals were placed back in the activity monitors for another 60 min. Horizontal locomotion was measured every 10 min during the last 30 min of habituation (preinjection) and the entire 60 min cocaine testing period (postinjection).

2.2.2. Acute cocaine effects

2.2.2.1. Behavioral testing. Animals were tested for the effects of quinacrine, indomethacin, or WEB 2086 on acute, cocaine-induced locomotion. Drugs were prepared as described above. Briefly, each animal was placed into an activity monitor (Columbus Instruments) and allowed to habituate for 60 min. Following the end of habituation, the animal received an injection of vehicle, quinacrine (25 mg/kg

ip), indomethacin (2 mg/kg ip), or WEB 2086 (10 mg/kg ip) and then, 15 min later, an injection of cocaine (30 mg/kg ip). The animals were then placed back in the activity monitors for another 60 min and horizontal locomotion was measured every 10 min.

2.2.2.2. Neurochemical testing. The day before testing, each animal was anesthetized with a 1.5% isoflourane mixture in oxygen, placed on a stereotax, and unilaterally implanted with a microdialysis probe (CMA12, CMA/Microdialysis, Acton, MA) into the left nucleus accumbens (core region) (AP: 1.0, ML: 1.3, DV: −8.3, 2 mm probe membrane), which was anchored to the skull with dental cement (all coordinates from bregma according to Paxinos and Watson, 1986). On the following day, each rat was placed in an awake animal microdialysis chamber (CMA/Microdialysis) and connected to a perfusion line, which was perfused with artificial cerebrospinal fluid (CSF) (125 mM NaCl, 0.5 mM NaH_2PO_4, 2.5 mM Na_2HPO_4, 1.2 mM $CaCl_2$, 2.5 mM KCl, 1 mM $MgCl_2$, pH 7.4) at 3 μl/min. Each animal was perfused for 2 h before samples were collected. The testing schedule consisted of a 60-min baseline period followed by an injection of vehicle, quinacrine (25 mg/kg ip) or indomethacin (2 mg/kg ip), and then 20 min later an injection of cocaine (30 mg/kg ip) was given (the cocaine injection is not made 15 min later, as in the behavioral studies, in order to obtain two complete 10-min perfusate samples prior to cocaine injection). Ten-minute perfusate samples were collected during the 60-min baseline period, following antagonist injection, and during a 60-min postcocaine injection period. Following completion of the experiment, each animal was anesthetized, sacrificed, and visually analyzed to verify proper probe placement in the nucleus accumbens using a freezing vibratome set to cut 100-μm sections.

2.2.3. Intra-VTA amphetamine-induced sensitization

2.2.3.1. Surgery. Rats were anesthetized with ketamine (80 mg/kg ip) and xylazine (12 mg/kg ip) and placed in a stereotaxic instrument (Kopf, Tujunga, CA). Bilateral 26-gauge stainless steel microinjection guide cannulae, fitted with 33-gauge obturators (Plastics One, Roanoke, VA), were implanted into the VTA (A/P: −5.3 mm, M/L: ±0.6 mm, D/V: −8.3 mm from bregma according to Paxinos and Watson, 1986) and secured to the skull with skull screws and dental acrylic. Following surgery, dust caps were attached to the guide cannulae and rats were allowed to recover for at least 1 week before beginning the experiment. During this time, they were habituated to handling.

2.2.3.2. Pretreatment. The treatment schedule and dose of intra-VTA amphetamine were chosen based on previous studies using similar protocols (Fraser et al., 1993; Stewart and Vezina, 1989; Tocco et al., 1997). On days 1, 3, and 5, rats were habituated to the activity monitors (Columbus Instruments) for 60 min and then were given bilateral microinjections into the VTA. Briefly, dust caps and obturators were removed and replaced with 33-gauge injection cannulae (Plastics One), which were connected to 10-μl

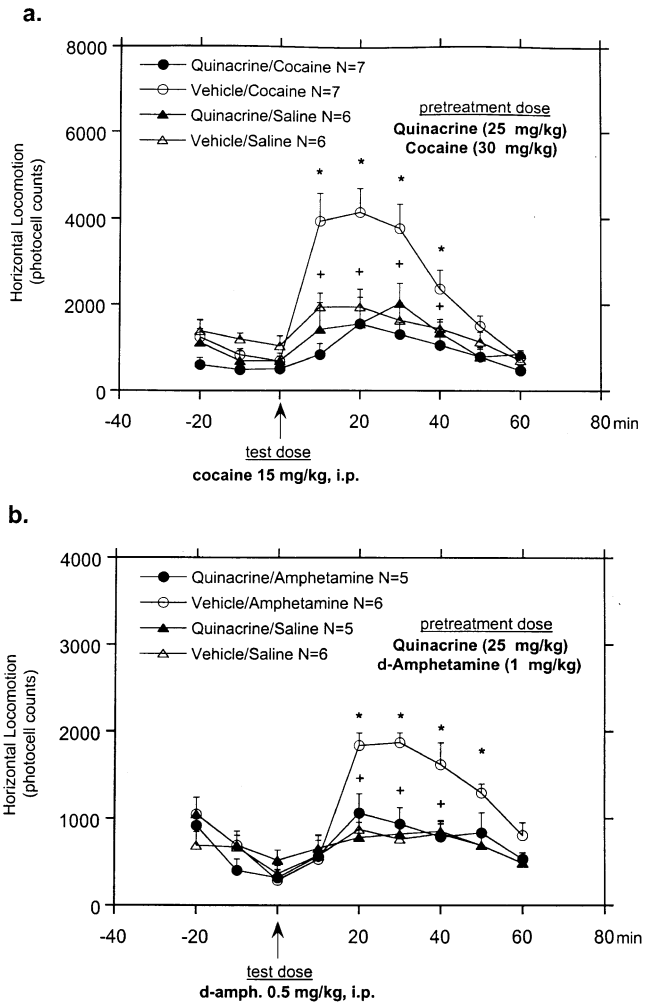

Fig. 2. The effects of quinacrine pretreatment on the development of (a) cocaine or (b) amphetamine locomotor sensitization. In (a) animals were pretreated with quinacrine (25 mg/kg ip) followed 15 min later by cocaine (30 mg/kg ip) for 5 consecutive days and then tested for cocaine (15 mg/kg ip)-induced horizontal locomotion following a 10-day withdrawal. In (b), animals were pretreated with quinacrine (25 mg/kg ip) followed 15 min later by D-amphetamine (1 mg/kg ip) for 5 consecutive days and then tested for D-amphetamine (0.5 mg/kg ip)-induced locomotion following a 10-day withdrawal. The data are shown as mean ± S.E.M. of horizontal locomotion scores obtained in 10-min recording intervals following the test injection of (a) cocaine or (b) D-amphetamine. Repeated-measures ANOVA (mixed factorial design), followed by post hoc Newman–Keuls tests, was used to analyze time-course locomotor activity data. Statistics for (a): overall $F(15,110) = 5.455$, $P < .01$; quinacrine/cocaine vs. vehicle/cocaine $F(5,60) = 11.842$, $P < .01$; quinacrine/saline vs. vehicle/saline $F(5,50) = 1.370$, $P = .254$. Statistics for (b): overall $F(15,90) = 3.474$, $P < .01$; quinacrine/amphetamine vs. vehicle/amphetamine $F(5,45) = 8.644$, $P < .01$; quinacrine/saline vs. vehicle/saline $F(5,45) = 0.989$, $P = .588$. * $P < .05$ for comparison of individual time points with vehicle/saline group and $^{+}$ $P < .05$ for comparison of individual time points with vehicle/cocaine group.

Hamilton syringes. A total volume of 0.5 μl of saline or drug was infused over 60 s per side (volumes were confirmed by tracing the movement of an air bubble introduced into each injection line approximately 10 μl distal to the injection cannulae). Approximately 2 min was allowed for diffusion of the drugs before removal of the injection cannulae and obturator replacement. The microinjections contained: (1) D-amphetamine (5 μg/side) plus indomethacin (0.5 μg/side), (2) D-amphetamine (5 μg/side), (3) indomethacin (0.5 μg/side), or (4) saline. Drugs were prepared daily and delivered in an isotonic saline solution, though the indomethacin vehicle also contained 1 mM Na_2HPO_4. Following the microinjections, the rats were placed back into the activity monitors for 60 min before being returned to their home cages.

2.2.3.3. Behavioral testing. Following a 10-day withdrawal after the last intra-VTA microinjection, rats were tested with saline and D-amphetamine. On the test day, animals were placed into an activity monitor (Columbus Instruments) and allowed to habituate for 60 min. Following the end of habituation, an injection of saline (1 ml/kg ip) was administered and the animals were placed back in the activity monitor for another 60 min. Following the end of the saline test period, the animals received an injection of D-amphetamine (0.5 mg/kg ip) and were placed back in the activity monitor for another 120 min. Horizontal locomotion was measured every 10 min during the 60-min habituation, 60-min saline test, and 120-min D-amphetamine testing periods. After testing, the animals were returned to their home cages.

2.2.3.4. Histology. Within 7 days of completing the experiment, each animal was anesthetized with ketamine (100 mg/kg ip), perfused via the ascending aorta with 4% paraformaldehyde in 0.1 M phosphate buffer (PBA), and decapitated. Whole brains were removed and stored in PBA until sectioned using a vibratome (Lancer, St. Louis, MO). Coronal sections (100 μm) were mounted on gel-coated slides, stained with 2% cresyl violet, and examined under a

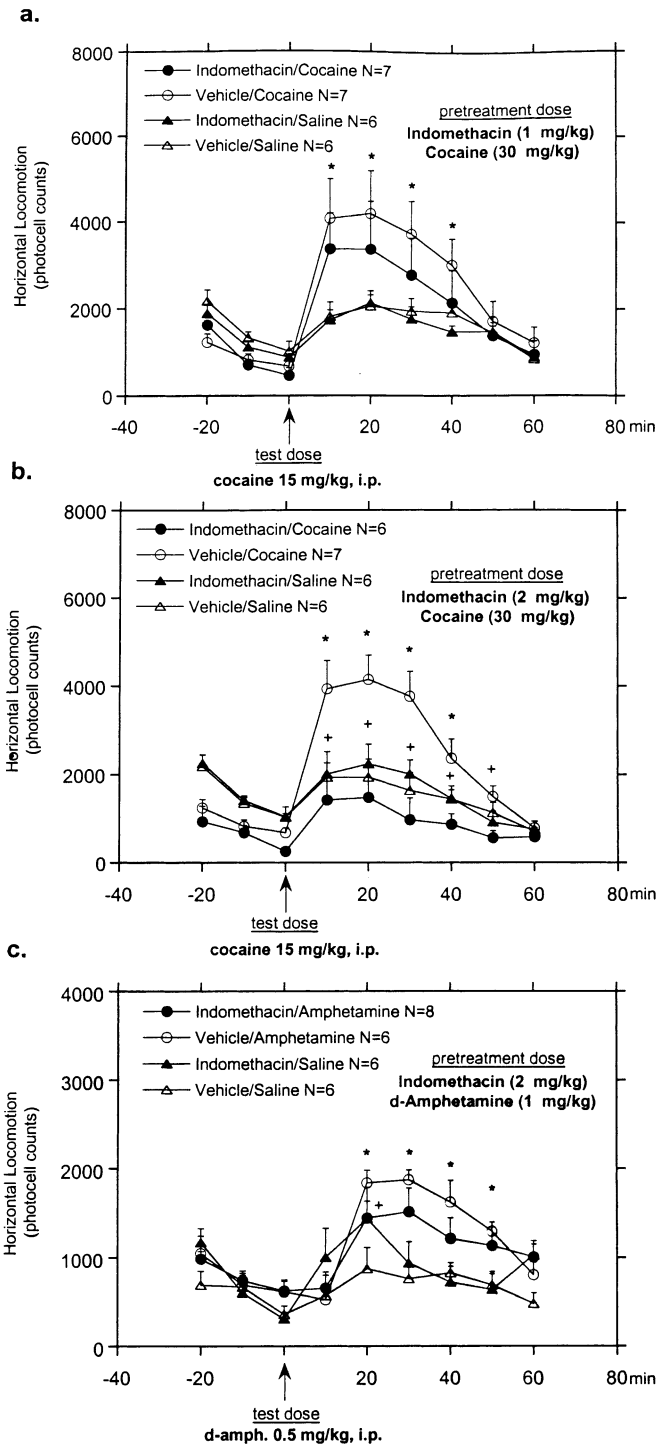

Fig. 3. The effects of indomethacin pretreatment on the development of cocaine (a and b) or amphetamine (c) locomotor sensitization. In (a) and (b), animals were pretreated with indomethacin (1–2 mg/kg ip) followed 15 min later by cocaine (30 mg/kg ip) for 5 consecutive days and then tested for cocaine (15 mg/kg ip)-induced horizontal locomotion following a 10-day withdrawal. In (c), animals were pretreated with indomethacin (2 mg/kg ip) followed 15 min later by D-amphetamine (1 mg/kg ip) for 5 consecutive days and then tested for D-amphetamine (0.5 mg/kg ip)-induced locomotion following a 10-day withdrawal. The data are shown as mean ± S.E.M. of horizontal locomotion scores obtained in 10-min recording intervals following the test injection of (a and b) cocaine or (c) D-amphetamine. Repeated-measures ANOVA (mixed factorial design), followed by post hoc Newman–Keuls tests, was used to analyze time-course locomotor activity data. Statistics for (a): overall $F(15,110)=3.581$, $P<.01$; indomethacin (1 mg/kg)/cocaine vs. vehicle/cocaine $F(5,60)=0.394$, $P=.851$; indomethacin (1 mg/kg)/saline vs. vehicle/saline $F(5,55)=0.556$, $P=.733$. Statistics for (b): overall $F(15,105)=3.250$, $P<.01$; indomethacin (2 mg/kg)/cocaine vs. vehicle/cocaine $F(5,55)=6.884$, $P<.01$; indomethacin (2 mg/kg)/saline vs. vehicle/saline $F(5,50)=0.915$, $P=.479$. Statistics for (c): overall $F(15,110)=4.302$, $P<.01$; indomethacin (2 mg/kg)/amphetamine vs. vehicle/amphetamine $F(5,60)=2.475$, $P<.05$ (Time × Drug interaction) and $F(1,12)=0.691$, $P=.422$ (main effect of drug); indomethacin (2 mg/kg)/saline vs. vehicle/saline $F(5,45)=2.334$, $P=.056$. * $P<.05$ for comparison of individual time points with vehicle/saline group and $^{+}$ $P<.05$ for comparison of individual time points with vehicle/cocaine group (a and b) or vehicle/amphetamine group (c).

light microscope. Microinjection tract and injection locations were determined according to the atlas of Paxinos and Watson (1986).

2.2.4. Intra-VTA PAF-induced sensitization

2.2.4.1. Pretreatment and testing protocol. The intra-VTA PAF sensitization protocol was modeled after our previous study on the cocaine sensitizing effects of intra-VTA melittin microinjections (Reid et al., 1996). PAF dose selection was based on our previous intra-VTA studies (Reid et al., 1996), as well as prior studies on local PAF (0.3–1.0 μg) effects on cutaneous inflammation and eosinophil induction (Juhlin and Pihl-Lundin, 1992; Sciberras et al., 1987). Briefly, all rats were initially habituated to the testing chambers on day 1 (60 min in testing apparatus, saline injection, and then another 60 min in testing apparatus), given an intra-VTA injection of PAF or saline on day 2, and then tested for cocaine (15 mg/kg)-induced locomotor activity on day 5.

2.2.4.2. Surgery. On day 2, the rats were anesthetized with ketamine (80 mg/kg ip) and xylazine (12 mg/kg ip) and placed in a stereotaxic instrument (Kopf). A 26-gauge needle, with angle cut tip oriented so that the opening faced medially, was unilaterally lowered (left side) into the VTA (AP: −5.3, ML: +0.6, DV: −8.7 from bregma according to Paxinos and Watson, 1986) and PAF (Sigma) (1 μg/0.5 μl, prepared daily in isotonic saline) or saline (0.5 μl) was infused into the VTA over 60 s. Approximately 2 min was allowed for diffusion of the drug before removal of the injection syringe, after which the skull was sealed with bone wax, skin was sutured, and the animal was given 3 days recovery before testing.

2.2.4.3. Behavioral testing. On day 5, the animals were placed in an activity monitor (Columbus Instruments) and allowed to habituate for 60 min. Following the end of habituation, a test dose of cocaine (15 mg/kg ip) was administered and the animals were placed back in the activity monitors for another 60 min. Horizontal locomotion was measured every 10 min during the last 30 min of

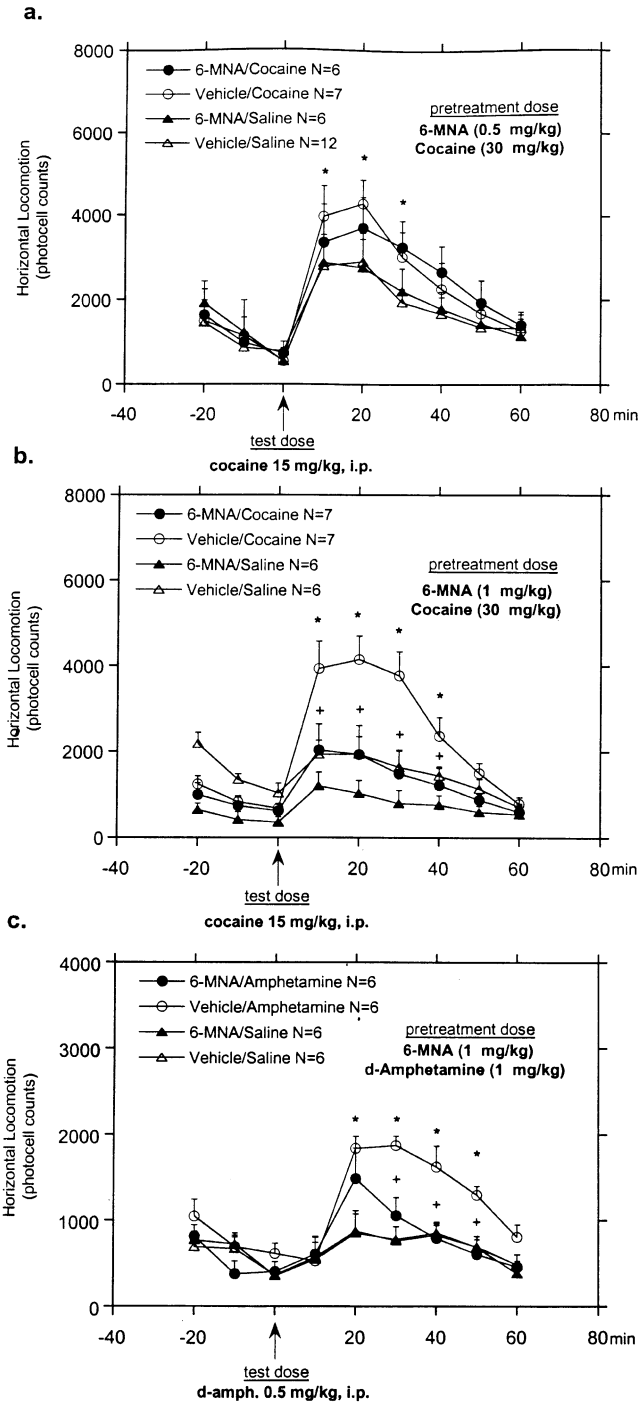

Fig. 4. The effects of 6-MNA pretreatment on the development of cocaine (a and b) or amphetamine (c) locomotor sensitization. In (a) and (b), animals were pretreated with 6-MNA (0.5–1 mg/kg ip) followed 15 min later by cocaine (30 mg/kg ip) for 5 consecutive days and then tested for cocaine (15 mg/kg ip)-induced horizontal locomotion following a 10-day withdrawal. In (c), animals were pretreated with 6-MNA (1 mg/kg ip) followed 15 min later by D-amphetamine (1 mg/kg ip) for 5 consecutive days and then tested for D-amphetamine (0.5 mg/kg ip)-induced locomotion following a 10-day withdrawal. The data are shown as mean ± S.E.M. of horizontal locomotion scores obtained in 10-min recording intervals following the test injection of cocaine (a and b) or (c) D-amphetamine. Repeated-measures ANOVA (mixed factorial design), followed by post hoc Newman–Keuls tests, was used to analyze time-course locomotor activity data. Statistics for (a): overall $F(15,135)=1.787$, $P<.05$; 6-MNA (0.5 mg/kg)/cocaine vs. vehicle/cocaine $F(5,55)=0.407$, $P=.798$; 6-MNA (0.5 mg/kg)/saline vs. vehicle/saline $F(5,80)=0.289$, $P=.964$. Statistics for (b): overall $F(15,110)=2.603$, $P<.01$; 6-MNA (1 mg/kg)/cocaine vs. vehicle/cocaine $F(5,60)=2.938$, $P<.05$; 6-MNA (1 mg/kg)/saline vs. vehicle/saline $F(5,50)=2.267$, $P=.063$. Statistics for (c): overall $F(15,100)=3.024$, $P<0.01$; 6-MNA(1 mg/kg)/amphetamine vs. vehicle/amphetamine $F(5,50)=2.797$, $P<.05$; 6-MNA (1 mg/kg)/saline vs. vehicle/saline $F(5,50)=0.089$, $P=.994$. * $P<.05$ for comparison of individual time points with vehicle/saline group and $^{+}$ $P<.05$ for comparison of individual time points with vehicle/cocaine group (a and b) or vehicle/amphetamine group (c).

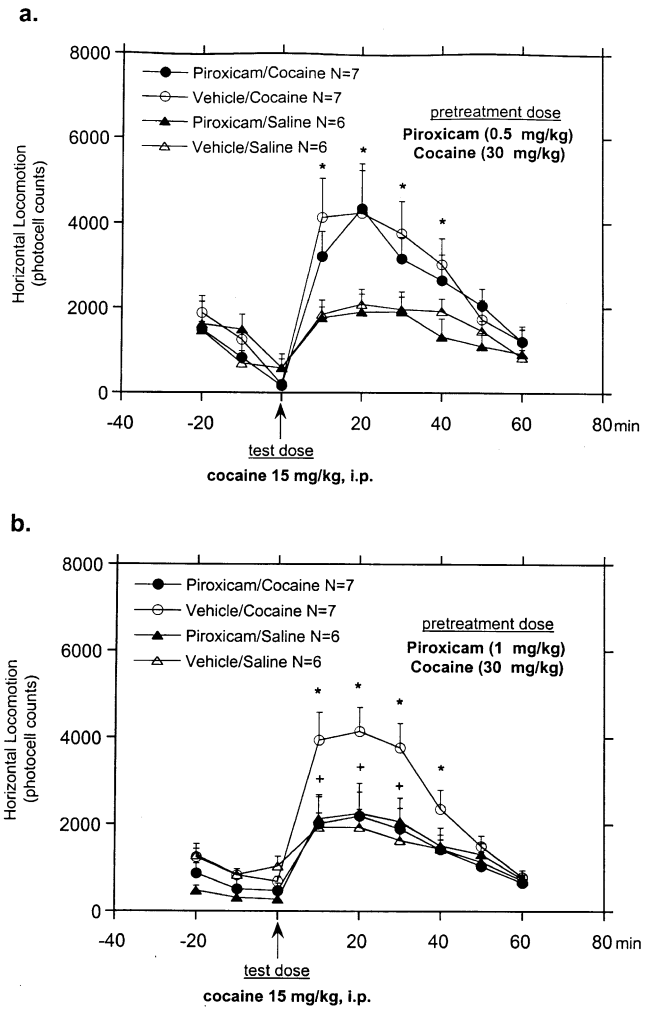

Fig. 5. The effects of piroxicam pretreatment on the development of locomotor sensitization to cocaine. Animals were pretreated with (a) 0.5 mg/kg or (b) 1 mg/kg piroxicam (ip) followed 15 min later by cocaine (30 mg/kg ip) for 5 consecutive days and then tested for cocaine (15 mg/kg ip)-induced horizontal locomotion following a 10-day withdrawal. The data are shown as mean ± S.E.M. of horizontal locomotion scores obtained in 10-min recording intervals following the test injection of cocaine. Repeated-measures ANOVA (mixed factorial design), followed by post hoc Newman–Keuls tests, was used to analyze time-course locomotor activity data. Statistics for (a): overall $F(15,110)=2.205$, $P<.05$; piroxicam (0.5 mg.kg)/cocaine vs. vehicle/cocaine $F(5,60)=0.661$, $P=.654$; piroxicam (0.5 mg/kg)/saline vs. vehicle/saline $F(5,50)=1.119$, $P=.363$. Statistics for (b): overall $F(15,110)=2.730$, $P<.01$; piroxicam (1 mg/kg)/cocaine vs. vehicle/cocaine $F(5,60)=3.698$, $P<.05$; piroxicam (1 mg/kg)/saline vs. vehicle/saline $F(5,50)=0.435$, $P=.822$. * $P<.05$ for comparison of individual time points with vehicle/saline group and $^{+}$ $P<.05$ for comparison of individual time points with vehicle/cocaine group.

habituation (preinjection) and the entire 60-min cocaine testing period (postinjection).

2.2.4.4. Histology. Within 7 days of completing the experiment, each animal was sacrificed, its brain fixed, and sections were prepared and stained exactly as described above for the intra-VTA amphetamine-sensitized animals.

2.3. Behavioral and neurochemical assessments

2.3.1. Quantification of locomotion

Locomotor activity was recorded in 10-min intervals. Each of the locomotor activity monitors (Opto-varimex Activity Meter, Columbus Instruments) consisted of a plexiglass cage $42 \times 42 \times 20$ cm in width, length, and

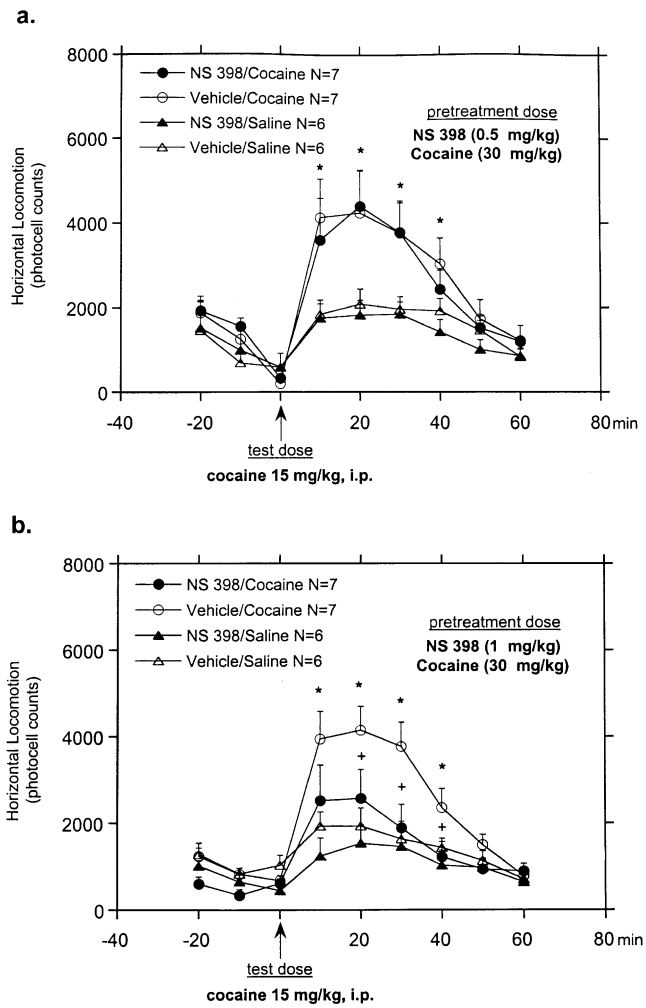

Fig. 6. The effects of NS-398 pretreatment on the development of locomotor sensitization to cocaine. Animals were pretreated with (a) 0.5 mg/kg or (b) 1 mg/kg NS-398 (ip) followed 15 min later by cocaine (30 mg/kg ip) for 5 consecutive days and then tested for cocaine (15 mg/kg ip)-induced horizontal locomotion following a 10-day withdrawal. The data are shown as mean ± S.E.M. of horizontal locomotion scores obtained in 10-min recording intervals following the test injection of cocaine. Repeated-measures ANOVA (mixed factorial design), followed by post hoc Newman–Keuls tests, was used to analyze time-course locomotor activity data. Statistics for (a): overall $F(15,110)=2.310$, $P<.01$; NS-398 (0.5 mg/kg)/cocaine vs. vehicle/cocaine $F(5,60)=0.257$, $P=.935$; NS-398 (0.5 mg/kg)/saline vs. vehicle/saline $F(5,50)=0.934$, $P=.466$. Statistics for (b): overall $F(15,110)=2.693$, $P<.01$; NS-398 (1 mg/kg)/cocaine vs. vehicle/cocaine $F(5,60)=2.923$, $P<.05$; NS-398 (1 mg/kg)/saline vs. vehicle/saline $F(5,50)=0.871$, $P=.444$. * $P<.05$ for comparison of individual time points with vehicle/saline group and $^{+}$ $P<.05$ for comparison of individual time points with vehicle/cocaine group.

height, respectively, with 15 photocell detectors along two perpendicular sides of the chamber and 15 light-emitting diodes (LEDs) along the other two sides for the measurement of horizontal activity. Photocell counts produced by interruption of the LED beam by movement of the animal were quantified and saved on computer by the microprocessor every 10 min.

2.3.2. High-performance liquid chromatography (HPLC)

Perfusate samples were analyzed for dopamine content by reverse-phase HPLC connected to an electrochemical detector (LC-4B, BAS, West Lafayette, IN). The reverse-phase, ion-pairing column (100 × 3.2) was prepacked with Phase II ODS 3 μm particulate, the mobile phase contained 0.06 M Na_2HPO_4, 0.09 M EDTA, and 1.3 mM 1-octanesulfonic acid in an 18% methanol solution adjusted to pH 2.95 with phosphoric acid. Electrochemical detection was done using a dual glassy carbon electrode (BAS-LC4B) set at 0.65 V. The limit of detection for dopamine was approximately 5 fmol. Perfusate samples were analyzed for glutamate content by reverse-phase HPLC connected to a fluorescence detector (CMA 280, CMA/Microdialysis) for the detection of an *o*-pthalaldehyde/mercaptoethanol derivative. The reverse-phase, ion-pairing column (100 × 2.1) was prepacked with Phase II ODS 5 μm particulate, the mobile phase contained 0.1 M sodium acetate in 10% methanol at pH 6.0 and was pumped at 0.7 ml/min. The derivatization reagent was 50% 0.1 M borate buffer (pH 9.5) containing 20 mM *o*-phthalaldehyde and 40 mM mercaptoethanol and 50% methanol. The derivatization was done automatically with a microautosampler (CMA 200, CMA/Microdialysis) maintained at 4 °C. The fluorescence detector (CMA 280, CMA/Microdialysis) was set with an excitation wavelength of 330 nm and an emission cut-off filter set at 418 nm. The limit of detection of glutamate was 0.05 pmol.

2.4. Data analysis

Repeated-measures ANOVA (mixed factorial design), followed by post hoc Newman–Keuls tests, was used to analyze time-course locomotor activity data (SuperANOVA, Abacus Concepts, Berkeley, CA and SPSS 9.0, SPSS, Chicago, IL). Overall interaction, drug interaction with stimulant pretreatment, and drug interaction with saline pretreatment were analyzed. Reported *F* values are from Time × Drug group analyses (unless otherwise noted). In all cases of significant or nonsignificant difference, both the Time × Drug and the main effect of drug analyses were in agreement except for two groups: WEB 2086 (10 mg/kg)/cocaine and indomethacin (2 mg/kg)/amphetamine vs. vehicle/cocaine and vehicle/amphetmine, respectively (for these groups both the Time × Drug and the main effect of drug analyses are reported). Total (60 min) locomotor activity scores following acute cocaine injections were analyzed by one-way ANOVA (SuperANOVA, Abacus Concepts). Maximal changes in extracellular nucleus accumbens dopamine or glutamate following cocaine injection were analyzed by one-way ANOVA (SuperANOVA, Abacus Concepts). Due to the variability of glutamate and dopamine levels between different animals, the changes in these neurotransmitter levels (postcocaine injection) are expressed as a percentage of control value (the control value was obtained from the final sample prior to cocaine

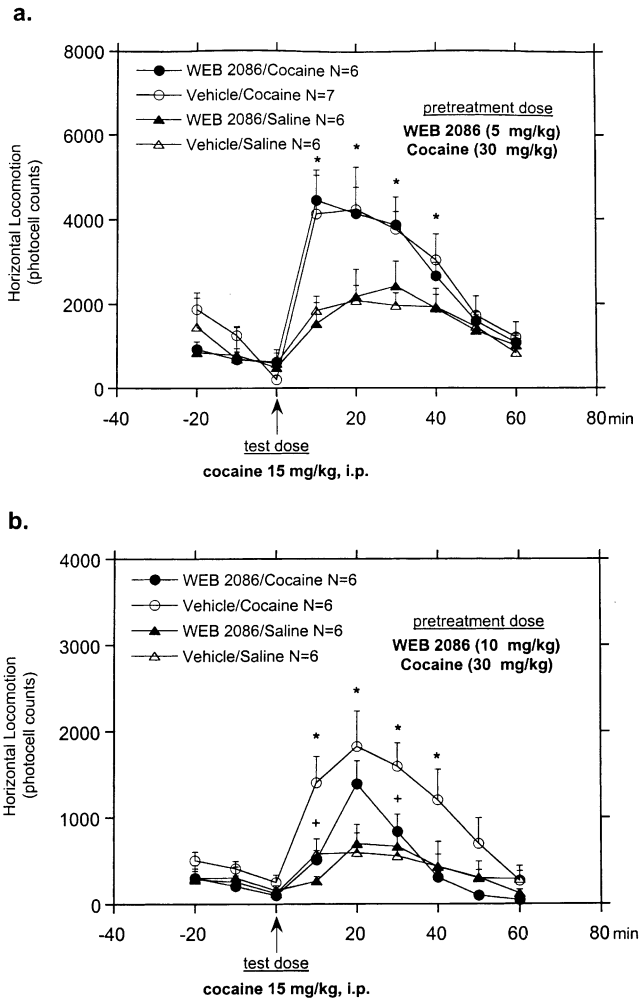

Fig. 7. The effects of WEB 2086 pretreatment on the development of locomotor sensitization to cocaine. Animals were pretreated with (a) 5 mg/kg or (b) 10 mg/kg WEB 2086 (ip) followed 15 min later by cocaine (30 mg/kg ip) for 5 consecutive days and then tested for cocaine (15 mg/kg ip)-induced horizontal locomotion following a 10-day withdrawal. The data are shown as mean ± S.E.M. of horizontal locomotion scores obtained in 10-min recording intervals following the test injection of cocaine. Repeated-measures ANOVA (mixed factorial design), followed by post hoc Newman–Keuls tests, was used to analyze time-course locomotor activity data. Statistics for (a): overall $F(15,105) = 4.348$, $P < .01$; WEB 2086 (5 mg/kg)/cocaine vs. vehicle/cocaine $F(5,55) = 0.143$, $P = .981$; WEB 2086 (5 mg/kg)/saline vs. vehicle/saline $F(5,50) = 2.359$, $P = .053$. Statistics for (b): overall $F(15,100) = 3.055$, $P < .01$; WEB 2086 (10 mg/kg)/cocaine vs. vehicle/cocaine $F(5,50) = 1.190$, $P = .328$ (Time × Drug interaction) and $F(1,10) = 5.965$, $P < .05$ (main effect of drug); WEB 2086 (10 mg/kg)/saline vs. vehicle/saline $F(5,50) = 0.965$, $P = .448$. * $P < .05$ for comparison of individual time points with vehicle/saline group and $^{+}$ $P < .05$ for comparison of individual time points with vehicle/cocaine group.

administration). The statistics (F values) are shown in the figure and table legends.

3. Results

3.1. Cocaine and amphetamine sensitization

Cocaine sensitization was tested with all of the above listed antagonists. Amphetamine sensitization was tested with select compounds that were found to block cocaine sensitization; quinacrine, indomethacin, and 6-MNA.

Five-day pretreatment with quinacrine (25 mg/kg) followed 15 min later by cocaine (30 mg/kg) completely blocked the development of locomotor sensitization to cocaine (15 mg/kg) (Fig. 2a). Similarly, 5-day pretreatment with quinacrine (25 mg/kg) followed 15 min later by D-amphetamine (1 mg/kg) completely blocked the development of locomotor sensitization to D-amphetamine (0.5 mg/kg) (Fig. 2b). In the control groups, 5-day pretreatment with quinacrine (25 mg/kg) alone did not alter the subsequent locomotor responsive to cocaine (15 mg/kg) or D-amphetamine (0.5 mg/kg) (Fig. 2a,b).

Five-day pretreatment with indomethacin (1–2 mg/kg) followed 15 min later by cocaine (30 mg/kg) produced a dose-related reduction in the development of locomotor sensitization to cocaine (15 mg/kg). Pretreatment with the lower dose (1 mg/kg) resulted in a nonsignificant reduction in sensitization (Fig. 3a), while the higher dose (2 mg/kg) completely blocked sensitization (Fig. 3b). Five-day pretreatment with the high dose of indomethacin (2 mg/kg) followed 15 min later by D-amphetamine (1 mg/kg) produced a moderate reduction in the development of locomotor sensitization to D-amphetamine (0.5 mg/kg) (Fig. 3c). This effect on amphetamine sensitization was significant by Time × Drug interaction, but not by main effect of drug. In the control groups, 5-day pretreatment with indomethacin alone did not alter the subsequent locomotor responsive to cocaine (15 mg/kg), however, a trend towards an increase in the locomotor response to D-amphetamine (0.5 mg/kg) was noted (Fig. 3a–c).

Five-day pretreatment with 6-MNA (0.5–1 mg/kg) followed 15 min later by cocaine (30 mg/kg) produced a dose-related reduction in the development of locomotor sensitization to cocaine (15 mg/kg). Pretreatment with the lower dose (0.5 mg/kg) had no effect on sensitization (Fig. 4a), while the higher dose (1 mg/kg) completely

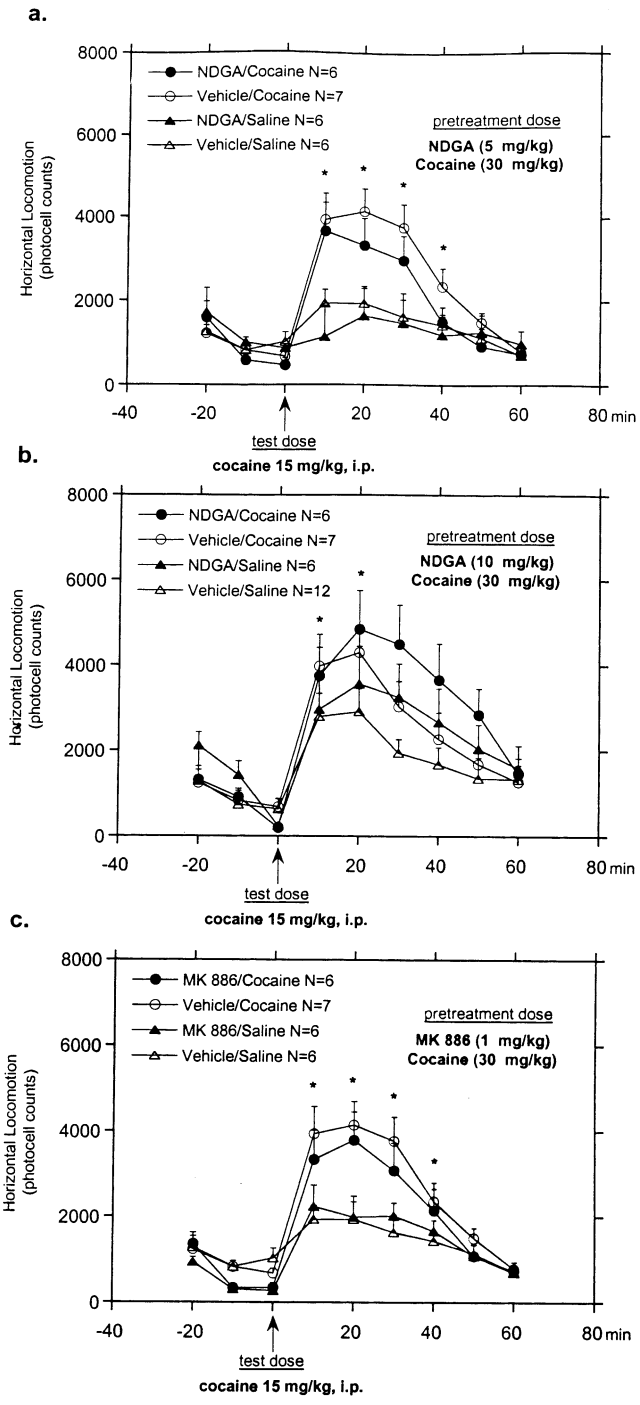

Fig. 8. The effects of NDGA or MK-886 pretreatment on the development of locomotor sensitization to cocaine. Animals were pretreated with (a) 5 mg/kg NDGA (ip), (b) 10 mg/kg NDGA (ip), or (c) 1 mg/kg MK-886 (ip) followed 15 min later by cocaine (30 mg/kg ip) for 5 consecutive days and then tested for cocaine (15 mg/kg ip)-induced horizontal locomotion following a 10-day withdrawal. The data are shown as mean ± S.E.M. of horizontal locomotion scores obtained in 10-min recording intervals following the test injection of cocaine. Repeated-measures ANOVA (mixed factorial design), followed by post hoc Newman–Keuls tests, was used to analyze time-course locomotor activity data. Statistics for (a): overall $F(15,105)=3.503$, $P<.01$; NDGA (5 mg/kg)/cocaine vs. vehicle/cocaine $F(5,55)=1.272$, $P=.285$; NDGA (5 mg/kg)/saline vs. vehicle/saline $F(5,50)=1.798$, $P=.163$. Statistics for (b): overall $F(15,135)=1.785$, $P<.05$; NDGA (10 mg/kg)/cocaine vs. vehicle/cocaine $F(5,55)=0.777$, $P=.570$; NDGA (10 mg/kg)/saline vs. vehicle/saline $F(5,50)=1.240$, $P=.348$. Statistics for (c): overall $F(15,105)=2.165$, $P<.05$; MK-886 (1 mg/kg)/cocaine vs. vehicle/cocaine $F(5,55)=1.387$, $P=.312$; MK-886 (1 mg/kg)/saline vs. vehicle/saline $F(5,50)=0.896$, $P=.748$. * $P<.05$ for comparison of individual time points with vehicle/saline group.

Table 1
Effects of PLA_2 and COX inhibition, or PAF receptor blockade, on acute cocaine (30 mg/kg)-induced locomotion and neurotransmitter release

Pharmacological pretreatment	Total locomotion	Maximal increase nucleus accumbens glutamate levels (%)	Maximal increase nucleus accumbens dopamine levels (%)
Vehicle	18,491 ± 3977 (n = 7)	221 ± 47 (n = 6)	208 ± 35 (n = 6)
Quinacrine (25 mg/kg)	16,916 ± 2680 (n = 7)	202 ± 55 (n = 6)	164 ± 28 (n = 6)
Indomethacin (2 mg/kg)	15,041 ± 3822 (n = 10)	273 ± 125 (n = 5)	153 ± 16 (n = 6)
WEB 2086 (10 mg/kg)	17,191 ± 3826 (n = 7)	207 ± 51 (n = 4)	181 ± 58 (n = 3)

All animals were pretreated with vehicle, quinacrine (25 mg/kg ip), indomethacin (2 mg/kg ip), or WEB 2086 (10 mg/kg) and then given an injection of cocaine (30 mg/kg ip). In the behavioral tests, cocaine was given 15 min later, while in the neurotransmitter release tests, cocaine was given 20 min later. Total locomotion scores represent data from a 60-min postcocaine testing period, with measures taken at 10-min intervals. Statistics comparing vehicle vs. quinacrine (25 mg/kg) [$F(1,12) = 0.156$, $P = .700$], indomethacin (2 mg/kg) [$F(1,15) = 0.489$, $P = .494$], or WEB 2086 (10 mg/kg) [$F(1,12) = 0.065$, $P = .803$] pretreatment revealed no differences. Extracellular levels of nucleus accumbens glutamate and dopamine values are expressed as percentage of control value (the control value was designated as the final baseline microdialysis sample prior to cocaine injection). The maximal change (increase or decrease) observed during 60 min following cocaine (30 mg/kg ip) injection is shown. The maximal change usually occurred within the first 20–30 min, however, the timing of this effect was not similar for all animals in each group. Data are presented as means ± S.E.M. Statistics comparing dopamine release responses following vehicle vs. quinacrine (25 mg/kg) [$F(1,10) = 1.072$, $P = 0.327$], indomethacin (2 mg/kg) [$F(1,10) = 0.454$, $P = .519$], or WEB 2086 (10 mg/kg) [$F(1,7) = 0.873$, $P = .447$] pretreatment, and comparing glutamate release responses following vehicle vs. quinacrine (25 mg/kg) [$F(1,10) = 0.210$, $P = .656$], indomethacin (2 mg/kg) [$F(1,9) = 0.182$, $P = .680$], or WEB 2086 (10 mg/kg) [$F(1,8) = 0.043$, $P = .841$] pretreatment, revealed no differences.

blocked sensitization (Fig. 4b). Five-day pretreatment with 6-MNA (1 mg/kg) followed 15 min later by D-amphetamine (1 mg/kg) significantly reduced the development of locomotor sensitization to D-amphetamine (0.5 mg/kg) (Fig. 4c). In the control groups, 5-day pretreatment with 6-MNA alone did not alter the subsequent locomotor response to D-amphetamine (0.5 mg/kg) or cocaine (15 mg/kg) (Fig. 4a–c).

Five-day pretreatment with piroxicam (0.5–1 mg/kg) followed 15 min later by cocaine (30 mg/kg) produced a dose-related reduction in the development of locomotor sensitization to cocaine (15 mg/kg). Pretreatment with the lower dose (0.5 mg/kg) had no effect on sensitization (Fig. 5a), while the higher dose (1 mg/kg) completely blocked sensitization (15 mg/kg) (Fig. 5b). In the control groups, 5-day pretreatment with piroxicam alone (Fig. 5a,b) did not alter the subsequent locomotor response to cocaine (15 mg/kg).

Five-day pretreatment with NS-398 (0.5–1 mg/kg) followed 15 min later by cocaine (30 mg/kg) produced a dose-related reduction in the development of locomotor sensitization to cocaine (15 mg/kg). Pretreatment with the lower dose (0.5 mg/kg) had no effect on sensitization (Fig. 6a), while the higher dose (1 mg/kg) completely blocked sensitization (Fig. 6b). In the control groups, 5-day pretreatment with NS-398 alone (Fig. 6a,b) did not alter the subsequent locomotor response to cocaine (15 mg/kg).

Five-day pretreatment with WEB 2086 (5–10 mg/kg) followed 15 min later by cocaine (30 mg/kg) produced a dose-related reduction in the development of locomotor sensitization to cocaine (15 mg/kg). Pretreatment with the lower dose (5 mg/kg) had no effect on sensitization (Fig. 7a), while the higher dose (10 mg/kg) partially blocked sensitization (Fig. 7b). The effect of the high dose (10 mg/kg) on cocaine sensitization was significant by main effect of drug, but not by Time × Drug interaction. In the control groups, 5-day pretreatment with WEB 2086 alone (Fig. 7a,b) did not significantly alter the subsequent locomotor response to cocaine (15 mg/kg), though a

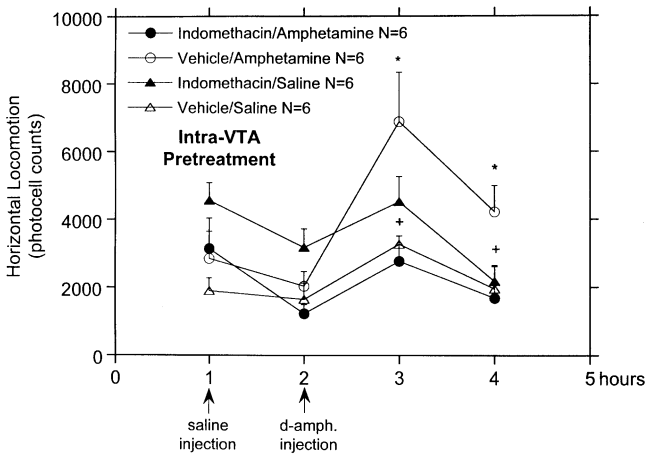

Fig. 9. The effects of repeated intra-VTA injections of D-amphetamine (5 μg/side) or saline coadministered with indomethacin (0.5 μg/side) or vehicle on the development of amphetamine sensitization. The pretreatment regimen consisted of bilateral intra-VTA injections given three times (Monday, Wednesday, and Friday) over 5 days. D-Amphetamine (0.5 mg/kg ip)-induced locomotion was tested following a 10-day withdrawal. Horizontal locomotor measures were taken at 10-min intervals. Total locomotion scores from 60-min recording periods: baseline, postsaline injection, postamphetamine injection (min 0–60 and 60–120) are shown. The data are shown as mean ± S.E.M. Repeated-measures ANOVA (mixed factorial design), followed by post hoc Newman–Keuls tests, was used to analyze locomotor activity data. Main effect of treatment on baseline activity: $F(3,20) = 4.738$, $P < .05$. Individual group effect for indomethacin/saline vs. vehicle/saline on baseline activity: $F(1,22) = 6.546$, $P < .05$. Main effect of treatment for the response to saline: $F(3,60) = 1.585$, $P = .224$. Individual group effects for the response to amphetamine (0.5 mg/kg ip): vehicle/amphetamine vs. vehicle/saline $F(3,15) = 4.971$, $P < .01$; vehicle/amphetamine vs. indomethacin/amphetamine $F(3,15) = 6.030$, $P < .05$; indomethacin/saline vs. vehicle/saline $F(3,15) = 2.491$, $P = .075$. * $P < .05$ for comparison of 60 min time points with vehicle/saline group and $^{+}$ $P < .05$ for comparison of 60 min time points with vehicle/amphetamine group.

trend towards enhanced responding in the 5-mg/kg group was noted.

Five-day pretreatment with NDGA (5–10 mg/kg) or MK-886 (1 mg/kg) followed 15 min later by cocaine (30 mg/kg) did not significantly reduce the development of locomotor sensitization to cocaine (15 mg/kg) (Fig. 8a–c). In the control groups, 5-day pretreatment with NDGA (5–10 mg/kg) or MK-886 (1 mg/kg) alone (Fig. 8a–c) did not significantly alter the subsequent locomotor response to cocaine (15 mg/kg). A trend towards enhanced cocaine responsivity in animals pretreated with 10 mg/kg NDGA, either alone or with cocaine, was noted.

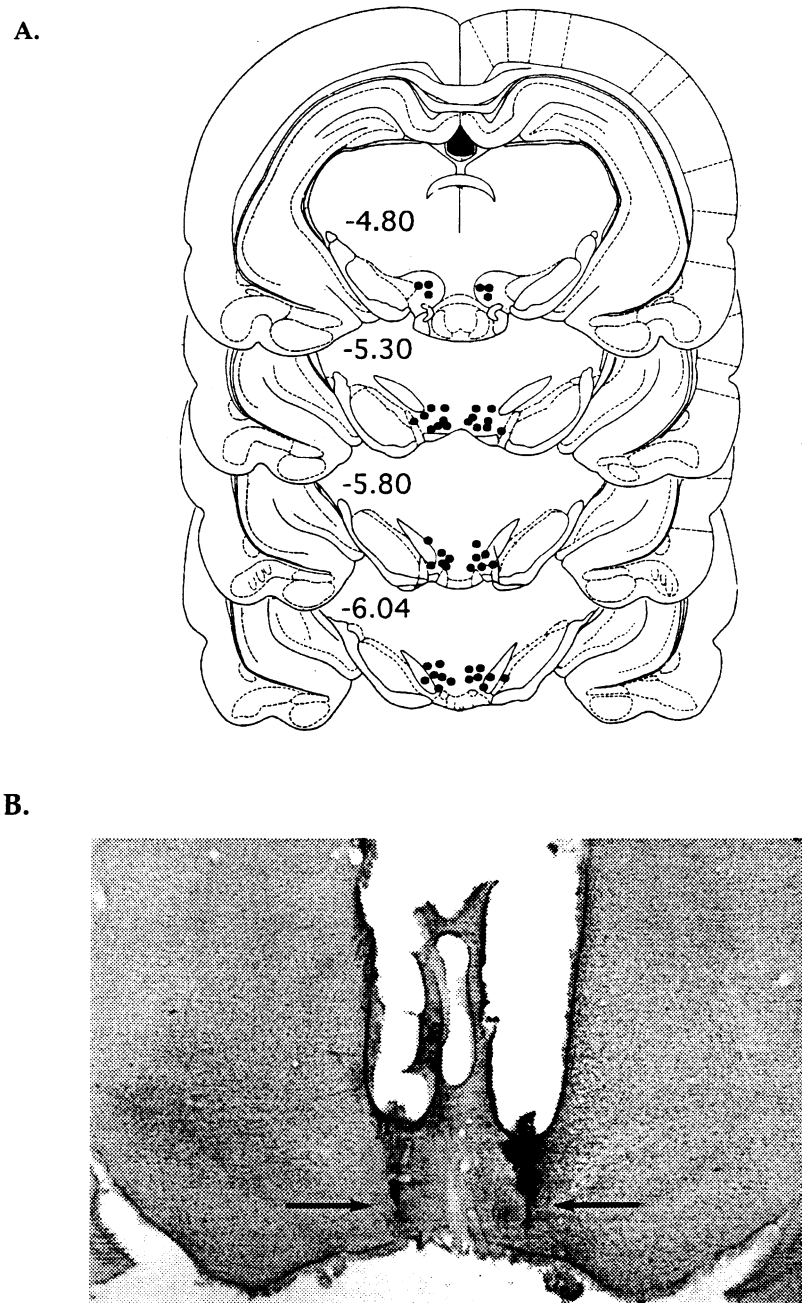

Fig. 10. Histological verification of local injection sites in bilateral VTA amphetamine- and indomethacin-injected rats (Study III). In (A), a schematic drawing depicting the ventral tip of the injection cannulae tracts for the injections of D-amphetamine (5 μg/side) or saline coadministered with indomethacin (0.5 μg/side) or vehicle into the VTA are shown. Filled circles represent accepted cannula placements in the VTA from −4.80 to −6.04 mm from the bregma according to the atlas of Paxinos and Watson (1986). In (B), a photomicrograph of a 100-μm section stained with cresyl violet depicting the location of the bilateral guide cannulae and injection tracts in the VTA is shown. Black arrows indicate location of injection cannula tracts. This section is typical of the accuracy and related tissue damage observed in the animals that are presented in this study.

3.2. Acute cocaine effects

The effects of quinacrine, indomethacin, or WEB 2086 pretreatment on the acute neurochemical and locomotor responses to cocaine are shown in Table 1. Total (60 min) locomotor scores for cocaine (30 mg/kg)-induced locomotion were not affected by quinacrine (25 mg/kg), indomethacin (2 mg/kg), or WEB 2086 (10 mg/kg) pretreatment. Cocaine-induced changes in extracellular nucleus accumbens dopamine and glutamate levels were compared by averaging the maximal change in neurotransmitter level (+ or −) observed during any of the 10-min sampling periods following cocaine injection. Cocaine (30 mg/kg)-induced dopamine levels were not affected by quinacrine (25 mg/kg), indomethacin (2 mg/kg), or WEB 2086 (10 mg/kg) pretreatment. Similarly, cocaine (30 mg/kg)-induced glutamate levels were not affected by quinacrine (25 mg/kg), indomethacin (2 mg/kg), or WEB 2086 (10 mg/kg) pretreatment.

3.3. Intra-VTA amphetamine-induced sensitization

Analysis of the total (60 min) locomotor scores from Day 1 of intra-VTA drug injection protocol revealed that bilateral microinjections of D-amphetamine (5 μg/side) [$t(10) = 0.510$, $P = .621$], indomethacin (0.5 μg/side) [$t(10) = 0.471$, $P = .648$], or the combination of D-amphetamine (5 μg/side) and indomethacin (0.5 μg/side) [$t(10) = 0.738$, $P = .478$] did not elicit an acute locomotor response (data not shown). Repeated-measures analysis of the effects of intra-VTA D-amphetamine (5 μg/side) treatment over the course of days 1, 3, and 5 of treatment revealed no augmentation of the locomotor response [$F(2,20) = 1.885$, $P = .30$] (data not shown). These data are consistent with similarly treated animals presented in our previous study (see Piomelli and Greengard, 1990).

Following a 10-day withdrawal, all intra-VTA injection pretreated rats were tested for their behavioral response to peripherally administered saline and D-amphetamine (see Fig. 9). Baseline levels of locomotor activity were significantly affected by the pretreatment group such that indomethacin pretreatment resulted in higher baseline activity. The locomotor response to saline injection, however, was not affected by the pretreatment group. D-Amphetamine (0.5 mg/kg)-induced locomotor activity was sensitized in the intra-VTA D-amphetamine vs. saline-pretreated animals. Coadministration of indomethacin with intra-VTA D-amphetamine completely blocked this development of sensitization. Pretreatment with intra-VTA indomethacin alone did not significantly alter D-amphetamine (0.5 mg/kg)-induced locomotor activity, though a slight enhancement of the locomotor response was noted.

Histological analyses of the bilateral cannulae tract and injection sites were employed to verify microinjection location within the VTA. Injection sites in the VTA were considered accurate if (1) the bilateral cannulae hit *both* the left and right hemisphere VTA nuclei and (2) the tracts were located along the rostrocaudal axis −4.80 to −6.00 mm from the bregma according to the atlas of Paxinos and Watson (1986). Any animal with tract placements that did not meet these criteria was excluded from the behavioral analysis [approximately 30% of animals tested were excluded based on this criteria ($n = 3$)]. Representative injection locations and a photomicrograph of a cresyl violet-stained, 100-μm section showing the location and tissue damage associated with a typical intra-VTA cannulation are shown in Fig. 10A,B.

3.4. Intra-VTA PAF-induced sensitization

Animals that received single, unilateral, intra-VTA injections of PAF or saline were tested for cocaine-induced locomotor activity 3 days later. Though a trend suggesting enhanced activity was seen, cocaine (15 mg/kg)-induced locomotor activity was not significantly altered in PAF (1 μg) vs. saline-pretreated animals (Fig. 11). Histological analyses of the unilateral tract and injection sites were employed to verify microinjection location within the left VTA. Injection sites in the VTA were considered accurate if (1) the tip of the tract terminated within the VTA and (2) the tract was located along the rostrocaudal axis −4.80 to −6.00 mm from the bregma according to the atlas of Paxinos and Watson (1986). Any animal with tract placements that did not meet these criteria was excluded from the behavioral analysis [approximately 30% of animals tested were excluded based on these criteria ($n = 4$)]. Representative injection locations and a photomicrograph of a 100-μm cresyl violet-stained section showing the

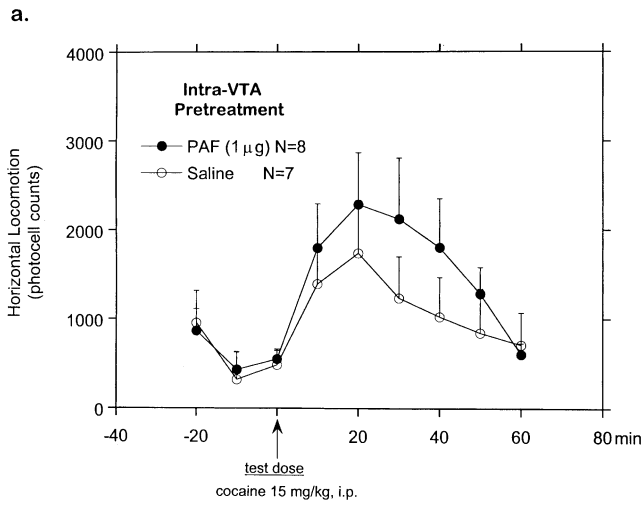

Fig. 11. The effects of local injections of PAF into the VTA on cocaine-stimulated locomotor activity. Animals were pretreated with unilateral injections of PAF (1 μg/0.5 μl) or saline (0.5 μl) into the VTA, while under anesthesia, and then tested with cocaine (15 mg/kg ip) 72 h later. Data are presented as mean ± S.E.M. Repeated-measures ANOVA (mixed factorial design), followed by post hoc Newman–Keuls tests, was used to analyze time-course locomotor activity data: PAF vs. saline $F(5,65) = 1.096$, $P = .372$.

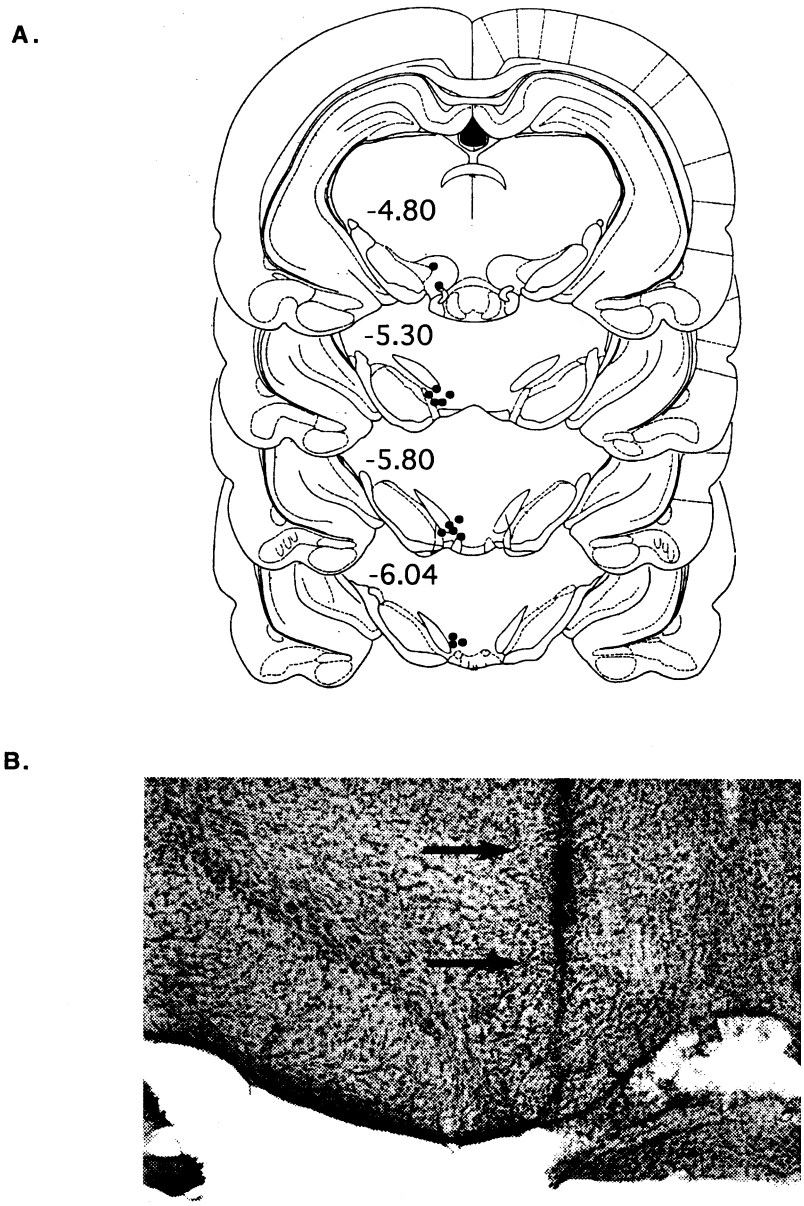

Fig. 12. Histological verification of local injection sites in unilateral VTA PAF-injected rats (Study IV). In (A), a schematic drawing depicting the ventral tip of the injection cannulae tracts for the injections of PAF (1 μg/0.5 μl) or saline (0.5 μl) into the VTA are shown. Filled circles represent accepted cannula placements in the VTA from −4.80 to −6.00 mm from the bregma according to the atlas of Paxinos and Watson (1986). In (B), a photomicrograph of a 100-μm section stained with cresyl violet depicting the location of an injection tract in the VTA is shown. The black arrows indicate the injection cannula tract. This section is typical of the accuracy and related tissue damage observed in the animals that are presented in this study.

location and tissue damage associated with a typical intra-VTA cannulation are shown in Fig. 12A,B.

4. Discussion

The results in this study confirm and extend previous evidence for involvement of PLA_2 activity in the development of stimulant sensitization (Reid et al., 1996). By testing a variety of agonist and antagonists selective for PAF receptors or the induction of AA cascade-mediated eicosanoids, the present study attempted to pharmacologically characterize potential downstream mediators of PLA_2 involvement in stimulant sensitization. The results indicate involvement of the COX-mediated AA cascade pathway, and possibly PAF, in the development of stimulant sensitization. Local injection studies demonstrate that this may occur at the level of the VTA.

The results from the quinacrine pretreatment studies confirmed our previous finding that quinacrine blocks the development of stimulant sensitization (Reid et al., 1996). The protocol used in the present study differed from those in the previous study, which included 1- and 10-day pretreatment protocols for cocaine and amphetamine, respectively.

Thus, evidence for the effects of quinacrine on stimulant sensitization are now more broad-based. Furthermore, both condition-dependent (1 day) and -independent (5 days) models of cocaine sensitization have now been shown to be blocked by quinacrine pretreatment. These results further strengthen the hypothesis for PLA_2 involvement in stimulant sensitization.

Stimulation of cytostolic PLA_2, a calcium-dependent phospholipase, induces the release of PAF and cleaves AA from membrane phospholipid. The release of AA, and the subsequent activation of a complex free fatty acid and lysophospholipid cascade system for which it is the substrate, produces several compounds that are potentially relevant to synaptic plasticity and/or stimulant sensitization (Harish and Poo, 1992; Schacher et al., 1993; Schaechter and Benowitz, 1993; Schweitzer et al., 1990). AA and its metabolites have been shown to stimulate protein kinase C activity in cortex synaptosomes (Schaechter and Benowitz, 1993), modulate G protein activity in neuromuscular junctions (Harish and Poo, 1992), enhance glutamate release from hippocampal synaptosomes (Lynch and Voss, 1990), reduce glutamate uptake in neurons and astrocytes (Chan et al., 1983; Volterra et al., 1992), and modulate calcium, sodium, and M channel current (Anderson and Welsh, 1990; Fraser et al., 1993; Keyser and Alger, 1990; Schweitzer et al., 1990). More recent studies on *Xenopus* oocytes have reported that AA stimulates a novel, cocaine-sensitive form of cation conductance associated with the dopamine transporter (Ingram and Amara, 2000; Reith et al., 1997). Similar to AA, PAF has also been suggested to be involved in synaptic plasticity (Kornecki and Ehrlich, 1988). PAF has been shown to increase intracellular calcium levels (Kornecki and Ehrlich, 1988; Yue et al., 1991), enhance evoked excitatory synaptic transmission (Bazan et al., 1993), and stimulate phosphoinositide metabolism (Yue et al., 1991) in neuronal cell cultures, and to sensitize dopamine and serotonin release from platelets (Kornecki and Ehrlich, 1988). Both AA and PAF have been proposed as a potential retrograde messenger mediating a presynaptic component of LTP (Kato et al., 1994).

The potential involvement of PAF in the development of cocaine sensitization was tested in two separate experiments, each of which investigated a different model of sensitization. The PAF receptor antagonist WEB 2086 (5–10 mg/kg) was tested in the same manner as the AA cascade antagonists, a 5-day pretreatment protocol with testing after a 10-day withdrawal. It was found that the high dose of WEB 2086 moderately reduced the development of cocaine sensitization, though this effect was significant only by main effect of treatment. The ability of intra-VTA PAF injections to produce cocaine sensitization was tested using a protocol from a previous study (Reid et al., 1996), which employed a shorter pretreatment and withdrawal period. In that previous study, a unilateral intra-VTA injection of the PLA_2 stimulator melittin was found to produce a robust form of cocaine sensitization (Reid et al., 1996). In the present study, a unilateral intra-VTA injection of PAF did not produce cocaine sensitization, although a trend towards locomotor sensitization was observed. This weak affect might suggest that a higher dose or bilateral injections of PAF are necessary to produce cocaine sensitization. Alternatively, PAF involvement in cocaine sensitization might occur in other brain structures. This weak enhancement of locomotor responsivity is consistent with the moderate reduction in cocaine sensitization seen with WEB 2086 in the 5-day pretreatment study. Taken together, these results indicate that PAF-mediated mechanisms may possibly be involved in the development of cocaine sensitization.

In the brain, the AA-mediated eicosanoid cascade is composed of two main pathways, which are mediated by LOX and COX, respectively (Wolfe and Horrocks, 1994). In the present study, we examined the effects of compounds selective for each of these pathways on cocaine sensitization. The involvement of the LOX pathways was tested with NDGA, a nonselective 12- and 5-LOX inhibitor with weak activity on PLA_2 and COX (Aktan et al., 1993; Meade et al., 1993), and the selective 5-LOX inhibitor MK-886 (Gillard et al., 1988). NDGA (5–10 mg/kg) did not affect the development of cocaine sensitization in our 5-day pretreatment paradigm. In fact, a moderate increase in cocaine sensitivity was indicated in animals receiving NDGA (10 mg/kg). MK-886 (1 mg/kg) also did not affect the development of cocaine sensitization. The inability of either drug to reduce the response to cocaine indicates that LOX activity is not involved in the development of cocaine sensitization. This interpretation must, however, be tempered by the finding that animals receiving the high dose of NDGA (10 mg/kg) showed a trend towards increased cocaine sensitivity, regardless of cocaine pretreatment. Also, it is possible that the 15-min pretreatment interval employed in the present study was too brief for effective enzyme inhibition to have occurred prior to cocaine administration. Previous studies with intraperitoneally administered NDGA and MK-886 employed pretreatment intervals of 1 h or greater (Paul et al., 1999; Yen and Lee, 1990). However, in a study on hypoxia-induced decrease in cerebral oxygen consumption, intravenous NDGA (3 mg/kg) attenuated this effect when administered only 15 min prior to the hypoxic event (Goperlud et al., 1995). Based on these considerations, it is suggested that the current findings indicate that LOX activity is not critical for the development of stimulant sensitization. Further studies confirming these results, as well as investigations on endogenous enzyme activity, would be necessary to conclusively rule out LOX activity in the development of stimulant sensitization.

The involvement of the COX pathway was tested using inhibitors with varying selectivity for either COX-1 or COX-2. Indomethacin (1–2 mg/kg), a well-studied nonsteroidal anti-inflammatory drug which reversibly inhibits the ability of COX to convert AA into prostaglandin G2

(Meade et al., 1993), was initially found to completely block the development of cocaine sensitization. In similar tests with amphetamine, a partial reduction in the development of sensitization was found. Subsequent tests with more selective COX inhibitors were performed to determine the relative contribution of COX-1 and COX-2 to the development of stimulant sensitization. 6-MNA (0.5–1 mg/kg), which affects both COX-1 and COX-2 though with higher affinity at COX-2 (Meade et al., 1993), blocked the development of both cocaine and amphetamine sensitization. Piroxicam (0.5–1 mg/kg), a COX-1 selective inhibitor (Laneuville et al., 1994), and NS-398 (0.5–1 mg/kg), a COX-2 selective inhibitor (Futaki et al., 1994), both blocked the development of cocaine sensitization (amphetamine sensitization was not tested with these compounds). These findings indicate that COX activity is critical for the development of stimulant sensitization. Studies on COX expression and activity in the brain suggest that COX-2 is the predominant form of this enzyme in neurons and that COX-2 mediates prostaglandin signaling in the brain as well as neurodegeneration (Kaufman et al., 1996; Tocco et al., 1997; Yamagata et al., 1993). However, the present results do not indicate differential involvement of COX-1 and COX-2 in stimulant sensitization. This suggests the need for further investigation of COX-2 in the neuronal adaptations to repeated cocaine.

PLA_2 and COX inhibitors that blocked cocaine sensitization were also tested on amphetamine sensitization. Not all COX inhibitors were tested, since these follow-up studies were not intended to characterize the involvement of COX-1 or COX-2 in amphetamine sensitization. Rather, these tests were performed in order to confirm the involvement of PLA_2 and COX in stimulant sensitization. The selection of quinacrine and indomethacin was consistent with the focus in the present study on the interaction of these drugs with stimulant sensitization. The selection of 6-MNA was made based on its high affinity for COX (COX-2 > COX-1), and the fact that it is the active metabolite of a clinically available nonsteroidal anti-inflammatory medication, nabumetone. We found that quinacrine, indomethacin, and 6-MNA inhibited the development of amphetamine sensitization. This broadens the evidence supporting our hypothesis that PLA_2 and COX are involved in stimulant sensitization. However, it must be recognized that amphetamine sensitization was not fully blocked by the COX inhibitors, particularly indomethacin. This discrepancy might reflect the properties of amphetamine, which are dissimilar from cocaine, such as its ability to reverse the dopamine transporter and induce release from a nonvesicular pool of dopamine. Alternatively, this might reflect the ability of indomethacin alone to enhance the locomotor effects of amphetamine (see indomethacin/saline group in Fig. 3c).

Quinacrine, indomethacin, and WEB 2086 had no effects on the acute behavioral effects of cocaine, nor did they affect cocaine-stimulated nucleus accumbens dopamine or glutamate levels. These data indicate that the mechanisms whereby quinacrine, indomethacin, and WEB 2086 modulate cocaine sensitization are not directly involved in the acute behavioral and neurochemical responses to cocaine. Interestingly, cocaine, amphetamine, AA, and PLA_2 activation have all been shown to enhance glutamate release (Sasaki et al., 1988; Aronica et al., 1992; Karler et al., 1989; Reid et al., 1997), yet neither quinacrine nor indomethacin affected stimulant-induced glutamate levels as might be expected. We suggest that PLA_2 involvement in cocaine sensitization occurs via a gradual process, possibly via up-regulation of signal transduction mechanisms.

Repeated intra-VTA injections of D-amphetamine produced sensitization to a peripheral injection of D-amphetamine given after a 10-day withdrawal. When indomethacin was coadministered with D-amphetamine into the VTA, this form of sensitization was blocked. These data demonstrate a potential neuroanatomical substrate for COX involvement in the development of stimulant sensitization, and are consistent with the previous finding that intra-VTA injections of the PLA_2 stimulator melittin produce cocaine sensitization (Reid et al., 1996). Interestingly, the intra-VTA injections of indomethacin alone produced a moderate increase in basal activity levels relative to the other groups, though the response to saline or D-amphetamine was unaffected. This might suggest that indomethacin alone can up-regulate behavioral activity and is consistent with the moderately higher response to cocaine in the 5-day indomethacin/saline-pretreated group in Study 1.

Based on the present findings, it is conceivable that bioactive eicosanoids produced in the brain by COX-mediated pathways, such as prostaglandins and thromboxanes, are involved in cocaine sensitization. Their functions in the CNS are numerous. Both thromboxane and prostaglandins can regulate hemostasis and cerebrovascular integrity and, as such, may be involved in cerebral inflammatory processes. With regard to neuronal regulatory properties, prostaglandins can inhibit calcium conductance and have been shown to modulate electrically stimulated release of norepinephrine and dopamine in the rat cortex and striatum (see Wolfe and Horrocks, 1994). Consistent with the suggestion that prostaglandins may be stimulated by cocaine, in vitro studies have demonstrated cocaine-induced prostaglandin release in blood vessel preparations (Cejtin et al., 1990). Prostaglandins are also well known to stimulate cyclic AMP (cAMP) production in the cerebral cortex (see Nathonson and Greengard, 1977). Since cAMP systems are known to be involved in the development of stimulant sensitization (Nestler et al., 1990; Steketee and Kalivas, 1991; Tolliver et al., 1996, 1999), this could represent a critical mechanism for cross talk between the AA and cAMP signal transduction cascades in the regulation of stimulant sensitization. These findings, and those in the present study, support the suggestion that prostaglandins may be involved in the development of cocaine sensitization. Further studies on the effects of acute and chronic

cocaine on prostaglandin levels in the brain are needed to further investigate this possibility.

The present study has shown that pretreatment with COX-1 or COX-2 inhibitors, or a PAF receptor antagonist, inhibits the development of stimulant sensitization. These results are consistent with previous work showing that quinacrine blocks the development of cocaine sensitization (Reid et al., 1996), thus, providing further evidence for the hypothesis that PLA_2 activity is involved in the development of stimulant sensitization. Furthermore, the local injection studies indicate that this effect may occur within the mesolimbic dopamine system. Preclinical findings support the suggestion that PLA_2 activity is involved in mediating dopamine-related behaviors (Chio et al., 1994; L'hirondel et al., 1995; McAllister et al., 1993). Furthermore, a recent postmortem study of chronic cocaine users has shown that PLA_2 activity is selectively decreased in the putamen (Ross et al., 1996). Based on these and our own findings, it may be suggested that PLA_2 activation in dopamine neurons may be involved in the neuroadaptations that result from chronic cocaine use. We propose that PLA_2-activated COX, and possibly PAF, pathways are critically involved in this neuronal process.

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