Spontaneous and Drug-Stimulated Locomotor Activity after the Administration of Pertussis Toxin into the Ventral Tegmental Area

Shridhar Narayanan, B Pharm, Lane Wallace, PhD, Norman Uretsky, PhD
Division of Pharmacology, College of Pharmacy, Ohio State University, Columbus, Ohio, USA

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Pertussis toxin (PTX) injected into the ventral tegmental area (VTA) produces an enhanced locomotor response to amphetamine. In the present study, we have evaluated the role of dopamine receptors on spontaneous locomotor activity and the enhanced locomotor response to dopaminergic agonists after the administration of PTX into the VTA. PTX injected into the VTA of rats produced a delayed increase in spontaneous locomotor activity with a latency of 4 d. This activity was markedly increased by day 6 and remained elevated for at least 28 d after PTX treatment. This increased spontaneous locomotor activity of PTX-treated animals was antagonized by the administration of the D_1 receptor antagonist SCH23390 (0.03 and 0.1 mg/kg sc), but not by the D_2 receptor antagonist eticlopride (0.1 and 0.3 mg/kg sc). After adaptation to the locomotor cages, the animals showed a markedly enhanced motor response to amphetamine (0.5 mg/kg ip) and apomorphine (5 mg/kg sc). The heightened locomotor responses to these dopaminergic agonists could be elicited for at least 2 mo after PTX administration. The enhanced response to amphetamine was antagonized by the administration of SCH23390 (0.03 and 0.1 mg/kg sc), but not by eticlopride (0.1 mg/kg). The increased response to apomorphine in PTX-treated animals was inhibited by SCH23390 (0.1 mg/kg sc) and partially inhibited by eticlopride (0.1 mg/kg sc). Both of these antagonists inhibited the spontaneous and the drug-induced locomotor responses in vehicle-treated control animals. These results suggest that the administration of PTX into the VTA leads to an increase in spontaneous and drug-induced locomotor activity in which D_1 receptors seem to play an important role.

Key Words: pertussis toxin, amphetamine, dopamine receptors, locomotor activity

INTRODUCTION

Psychostimulant drugs like amphetamine and cocaine produce a locomotor stimulant response that becomes progressively enhanced on repeated administration. This change in response, which has been termed behavioral sensitization, may last for several months after the drug has been discontinued and is related to an increase in dopaminergic neurotransmission within the mesolimbic dopamine system (Robinson and Becker 1986; Kalivas and Stewart 1991). Behavioral sensitization consists of 2 phases: the developmental phase is characterized by changes occurring in somatodendritic dopamine receptors located in the VTA, while the expression phase involves an enhanced dopaminergic neurotransmission in the nucleus accumbens (NAc) (Kalivas and

Address reprint requests to: Dr Norman J Uretsky, Ohio State University, College of Pharmacy, 500 West 12th Avenue, Columbus, Ohio 43210 USA.
e-mail: uretsky@dendrite.pharmacy.ohio-state.edu

Weber 1988; Kalivas and Stewart 1991; Paulson and Robinson 1991; Wolf and others 1993).

Several electrophysiological and behavioral studies have shown that the development of sensitization to psychostimulant drugs involves a subsensitivity of somatodendritic D_2 dopaminergic receptors (Kalivas and Weber 1988; Kalivas and Stewart 1991; Steketee and Kalivas 1991; Wolf and others 1993). Thus, the initial dose of psychostimulant drug releases dopamine from dendrites and/or cell bodies of the VTA, which activates the somatodendritic D_2 receptors, resulting in an inhibition of dopaminergic neuronal activity. After repeated administration, however, the ability of psychostimulant drugs to inhibit the dopamine neuronal firing rate is reduced, producing an enhanced release of dopamine (DA) from nerve terminals in the NAc. This enhancement in DA release, which is reflected in an increase in the extracellular concentration of DA as measured by microdialysis, is postulated to play an important role in the expression of sensitization to psychostimulant drugs (Kalivas and Stewart 1991).

The subsensitivity of somatodendritic D_2 receptors produced by the repeated administration of amphetamine is associated with a decrease in G_i/G_o protein that is linked with these receptors (Innis and Aghajanian 1987), but not with a decrease in receptor number (Nestler and others 1990; Steketee and Kalivas 1991). This observation suggests that the decrease in G_i/G_o proteins in the VTA is involved in the development of sensitization. In support of this concept is the finding that the administration into the VTA of pertussis toxin, a bacterial toxin that inactivates G_i/G_o proteins, produced a sensitized behavioral response to amphetamine or cocaine (Steketee and Kalivas 1991). The sensitization to these psychostimulant drugs after PTX administration resembled that produced by the repeated administration of psychostimulant drugs in that it was long-lasting (through at least 26 d following PTX administration) and was correlated with an increased concentration of extracellular dopamine in the NAc after psychostimulant drug administration (Steketee and Kalivas 1991). The sensitized response in PTX-treated animals was specific to drugs like amphetamine and cocaine, which act on the dopaminergic system. In contrast, morphine and caffeine, which presumably stimulate locomotor activity via nondopaminergic mechanisms, did not produce an enhanced locomotor response in PTX-treated animals (Steketee and Kalivas 1991). These results suggest that sensitized responses after PTX treatment require an interaction of the drug with the mesolimbic dopaminergic system.

The sensitized behavioral response to amphetamine and cocaine after PTX administration into the VTA could involve an enhancement in the release of DA from nerve terminals in the NAc by these drugs and/or an enhanced postsynaptic effect of released DA. The role of dopamine receptors in the sensitized response of PTX-treated animals to psychostimulants has not been investigated. The purpose of the present study, therefore, was to evaluate the locomotor response to dopaminergic agonists after PTX administration into the VTA and to determine the role of D_1 and D_2 receptors in the locomotor response to these agonists in PTX-treated animals. In our initial studies, we observed that rats injected with PTX into the VTA exhibited a delayed and prolonged increase in spontaneous locomotor activity compared with animals injected with vehicle. Consequently, we have also characterized this increase in spontaneous locomotion in order to obtain information on the neural mechanisms responsible for this effect.

METHOD

Administration of drugs into the brain

Male Sprague-Dawley rats (Harlan Inc, Indianapolis) weighing 250 to 350 g were housed 4 animals per cage in a temperature-controlled environment (23°C ± 1°C) with a 12-h dark-light cycle. The animals were provided with plenty of food and water. All animal-use procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Laboratory Animal Care and Use Committee. Prior to the administration of PTX or vehicle into the brain, the animals were anesthetized with a halothane–oxygen mixture and mounted on a stereotaxic frame (David Kopf Instruments, Tajunga, CA). PTX or vehicle in a volume of 0.3 to 0.5 µl was injected bilaterally into the VTA according to the coordinates described by the atlas of Pellegrino and others (tooth bar + 5 mm; VTA: anterior–posterior (AP) −3.8 mm from bregma, lateral (L) ± 1.8 mm, dorsal–ventral (DV) −8.5 mm from skull surface with needle angled at 6°). The solutions were injected slowly over approximately 1 min, using a 1 µl Hamilton syringe (outer diameter: 0.48 mm) (Hamilton Co, Reno, NV), which was inserted directly into the brain. After injection, the needle was left at the injection site for an additional minute to enable the drug to diffuse from the injection site. After the surgery, rats were placed in their home cage and allowed to recover from the halothane and regain their righting reflex.

Measurement of locomotor activity

The locomotor activity was monitored in activity cages (Columbus Instruments Inc, Columbus, OH), which were ventilated plexiglass boxes measuring 42 cm × 42 cm × 20 cm high and containing a 12 × 12 grid of infrared beams 3.5 cm apart and 5 cm from the bottom of the cages. Ambulatory counts were recorded when at least 2 consecutive beams were interrupted. For the measurement of spontaneous locomotor activity, vehicle- and PTX-treated rats were placed in test

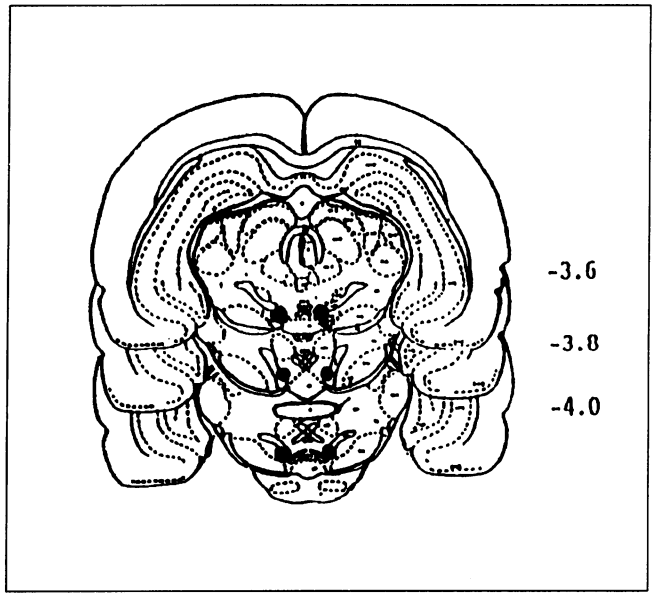

Figure 1. Localization of the injection sites in the VTA. The filled circles represent the region where the needle tips were located. The negative numbers refer to the distance in millimeters posterior to the bregma. (Figure was adapted from the atlas of Pellegrino and others 1976.)

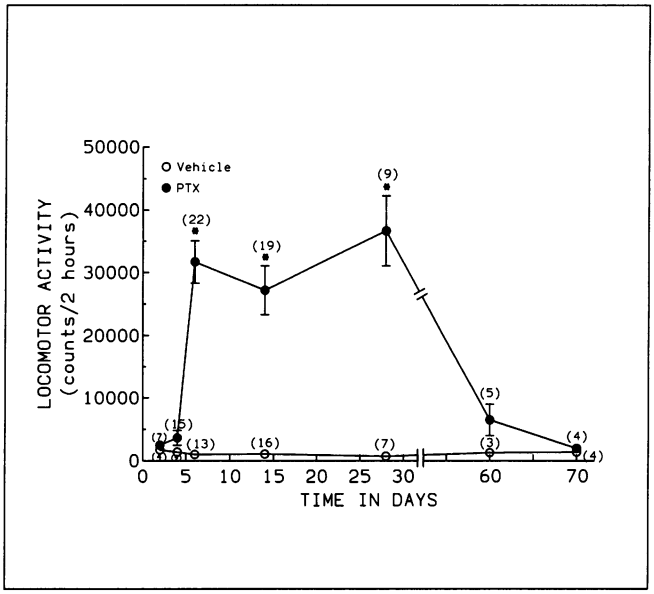

Figure 2. Time response for the spontaneous locomotor activity after the administration of pertussis toxin into the VTA. Rats were treated with PTX or vehicle into the VTA. On different days after PTX administration the animals were placed in the locomotor cages, and the locomotor activity of the animals was recorded every 10 min for 2 h. Each value represents a mean ± SEM of 3 to 22 animals. *$P < 0.01$ (Student's t test). The groups with the large number of animals included the control animals for the drug-induced locomotor activity experiments.

cages, and locomotor activity was recorded for 2 h. In studies involving a D_1 or D_2 antagonist, the drugs were administered 5 min before the animals were placed in the test cages. In the experiments measuring responses to dopaminergic agonists, the animals were adapted to the locomotor cages for 2 h on 3 consecutive days between days 10 and 14 after PTX administration. On the test days, all animals were adapted to the locomotor cages for at least 2 h prior to the injection of drugs. At the end of this period, the spontaneous locomotor activity of PTX-treated rats decreased and was similar to that of vehicle-treated controls; however, the PTX-treated rats were hyperreactive to the handling required for drug injection. In order to facilitate the injection process, therefore, both vehicle and PTX-treated animals were lightly anesthetized with halothane (exposure to halothane was terminated immediately after the righting reflex was lost) prior to drug administration. The rats regained their righting reflex within a minute after removal of halothane, showed no visible adverse effects from the halothane, and moved around the test cages in a coordinated manner. In studies involving antagonists, the antagonists were administered 20 min before the administration of either amphetamine or apomorphine.

Histology

For some experiments, the brains were fixed in 10% formalin for at least 24 h and then frozen. They were sectioned using a Cryo-Cut Microtome (American Optical Corp, Buffalo, NY), and the locations of the needle tracks in unstained sections were determined and recorded to verify injection sites. The tips of the needle tracks, which are depicted as shaded regions in the coronal sections of Figure 1 (Pellegrino and others 1976), are located in the VTA.

Drugs

D-amphetamine sulfate (0.1 to 1 mg/kg ip) was obtained from Sigma Chemicals Co (St Louis, MO). Pertussis toxin (0.03 to 0.5 μg per side into the VTA), apomorphine (5 mg/kg sc), eticlopride HCl (0.1 to 0.3 mg/kg sc), and SCH23390 HCl (0.03 to 0.1 mg/kg sc) were purchased from Research Biochemicals Inc (Natick, MA). Pertussis toxin was reconstituted using sterile water for injection; all the other drug solutions were made in 0.9% saline.

Statistical analysis

Data are expressed as the mean ± SEM. Statistical analysis was done using a 1-way or 2-way ANOVA, and further post hoc analyses were performed using a least significant

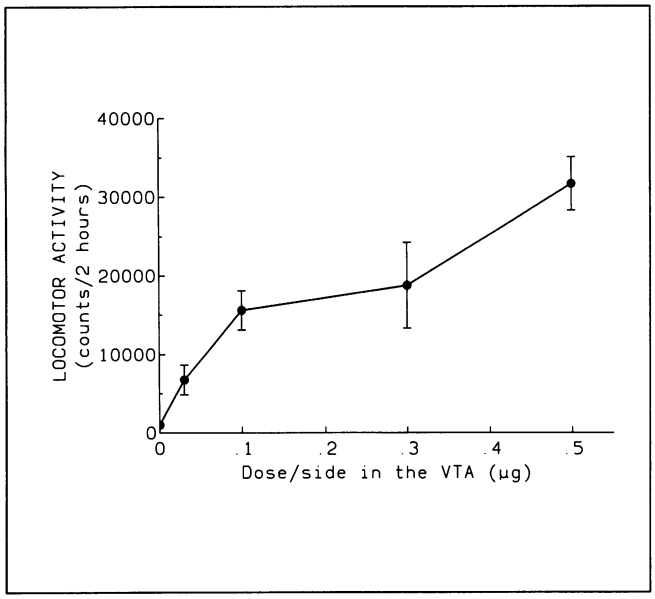

Figure 3. Dose response relationship between PTX administered into the VTA and spontaneous locomotor activity. Rats were treated with PTX 6 d prior to the measurement of locomotor activity. On the test day the rats were placed in the locomotor cages without any treatment, and the locomotor activity was recorded every 10 min for 2 h. Each value represents a mean ± SEM of 4 to 22 animals. Some of the data also appear in Figure 2. The groups with the large number of animals included the control animals for the drug-induced locomotor activity experiments.

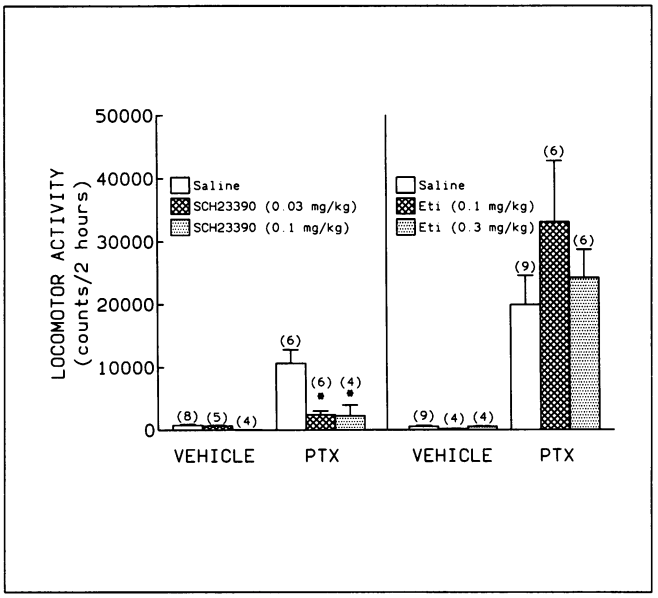

Figure 4. Effect of D_1 antagonist SCH23390 and D_2 antagonist eticlopride on the spontaneous locomotor activity 6 to 10 d after the administration of PTX into the VTA. Rats were treated with SCH23390 (0.03 and 0.1 mg/kg sc) or eticlopride (0.1 and 0.3 mg/kg sc) or vehicle, and the spontaneous locomotor activity of the animals was recorded every 10 min for 2 h. Each value represents a mean ± SEM of 4 to 9 animals. $^*P < 0.05$ (LSD test) when compared with PTX animals injected with saline.

differences (LSD) test or an unpaired Student's *t* test where appropriate.

RESULTS

Effect of PTX administered into the VTA on spontaneous locomotor activity and the role of D_1 and D_2 receptors in this effect

Rats were injected bilaterally into the VTA with either PTX (0.3 µg) or vehicle, and spontaneous locomotor activity was measured at 2-h intervals on various days after PTX administration (Figure 2). The spontaneous locomotor activity of PTX-treated animals was significantly greater than that of vehicle-treated control animals. A between–within ANOVA conducted on these data found significant effects of treatment ($F_{1,6} = 46.0$, $P < 0.001$) and time ($F_{6,123} = 7.13$, $P < 0.001$) and there was a significant treatment × time interaction ($F_{6,123} = 7.69$, $P < 0.001$). A post hoc Student's *t* test showed that there was no significant change in the spontaneous locomotor activity of PTX-treated animals at 2 and 4 d after PTX administration compared with that of vehicle-treated controls. There was, however, a marked and significant increase in locomotion after 4 d, reaching a maximum activity that was several times that of vehicle-treated controls. The spontaneous activity of these animals remained significantly elevated through 28 d after PTX administration. At 60 d after PTX administration, the spontaneous activity of the PTX-treated animals was reduced from the earlier peak activity level and was not significantly higher than that of vehicle-treated control animals. The increased spontaneous locomotor activity of PTX-treated animals determined 6 d after PTX administration was dependent on the dose of PTX administered into the VTA, reaching the highest levels after the administration of 0.5 µg of PTX per side (Figure 3). Many of the animals injected with this dose, however, exhibited seizure activity; therefore, a lower dose of 0.3 µg, which did not produce seizure activity, was used for the remainder of these studies.

In order to determine the roles of D_1 and D_2 receptors in the increased spontaneous activity associated with administration of PTX into the VTA, SCH23390 (0.03 and 0.1 mg/kg sc), a selective D_1 receptor antagonist, or eticlopride (0.1 and 0.3 mg/kg sc), a selective D_2 antagonist, was administered to rats treated 6 to 10 d previously with PTX

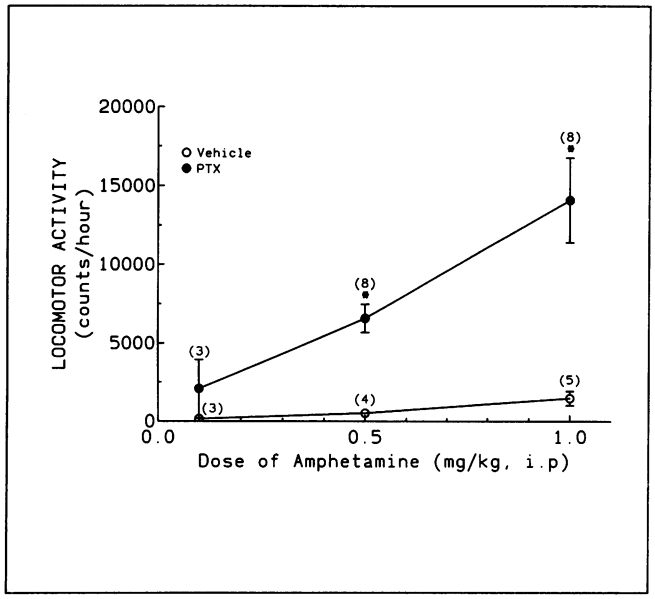

Figure 5. Dose response for amphetamine-stimulated locomotor activity in PTX- and vehicle-treated animals. Rats that had been treated with PTX or vehicle into the VTA were challenged with amphetamine (0.1 to 1 mg/kg ip) after 2 h of adaptation on the test day, and the locomotor activity of the animals was recorded every 10 min for 1 h. Each value represents the mean ± SEM of 3 to 8 animals. *$P < 0.01$ (Student's t test).

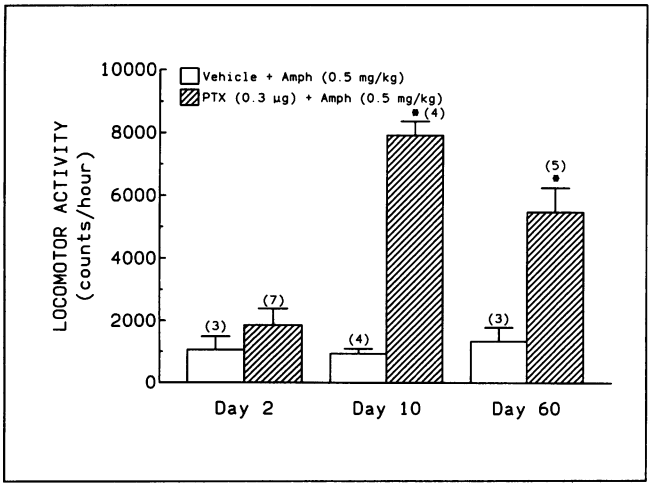

Figure 6. Time response for amphetamine-stimulated locomotor activity in PTX- and vehicle-treated animals. Rats that had been treated with PTX or vehicle into the VTA were challenged with amphetamine (0.5 mg/kg ip) on different days after PTX administration, and the locomotor activity was recorded every 10 min for 1 h. Each value represents a mean ± SEM of 3 to 7 animals. *$P < 0.01$ (Student's t test).

(Figure 4). A 2-way ANOVA showed a significant effect on the locomotor activity of PTX-treated animals compared with vehicle-treated controls ($F_{1,2} = 24.25$, $P < 0.001$), a significant drug effect ($F_{2,27} = 9.27$, $P < 0.001$), and a significant treatment × drug interaction ($F_{2,27} = 8.12$, $P < 0.002$). A post hoc analysis of the effect using the LSD test showed that SCH23390 at both doses produced a significant inhibition of the spontaneous locomotor activity of PTX-treated animals. In the eticlopride study, there was a significant PTX treatment effect, but no significant effect of eticlopride or treatment × drug interaction.

Effect of PTX administration on amphetamine- and apomorphine-stimulated locomotor activity and the role of D_1 and D_2 receptors in this effect

Animals injected 10 to 28 d previously with PTX were exposed to the locomotor test cages for 2 h per day on 3 consecutive days. To test whether the stress of an injection stimulated locomotor activity after this adaptation procedure, PTX- and vehicle-treated animals were injected with saline, and the locomotor activity was recorded. The locomotor activity of PTX-treated animals that received saline was not significantly different from that of vehicle-treated animals. Consequently, this adaptation regimen was used in all studies evaluating the locomotor stimulatory effects of drugs in PTX- and vehicle-treated rats.

Amphetamine (0.1 to 1 mg/kg ip) produced a dose-dependent increase in the locomotor activity of animals injected 14 to 28 d previously with either PTX or vehicle into the VTA (2-way ANOVA: treatment effect $F_{1,2} = 16.37$, $P < 0.001$; dose effect $F_{2,25} = 5.26$, $P < 0.05$; treatment × dose interaction, $F_{2,25} = 3.38$, $P < 0.05$) (Figure 5). The change in the response to amphetamine at different times after PTX administration is shown in Figure 6. At 2 d after treatment with PTX or vehicle, amphetamine (0.5 mg/kg ip) produced a similar degree of stimulation of locomotor activity in both groups of rats. After 10 d of treatment with PTX or vehicle, however, the response to amphetamine of PTX-treated animals (0.5 mg/kg ip) was significantly greater than that of vehicle-treated control animals. At 60 d after treatment with PTX, this enhanced effect of amphetamine was still present. The heightened locomotor response to amphetamine was dependent on the dose of PTX administered into the VTA (Figure 7). The increased response to amphetamine in PTX-treated animals was inhibited by SCH23390 (0.03 and 0.1 mg/kg sc), administered 20 min prior to the injection of amphetamine (0.5 mg/kg ip). In contrast, eticlopride (0.1 mg/kg sc), administered 20 min prior to the administration of amphetamine (0.5 mg/kg ip), did not inhibit the enhanced response to amphetamine. Both of these antagonists inhibited the locomotor response to amphetamine in vehicle-treated control animals (Figure 8).

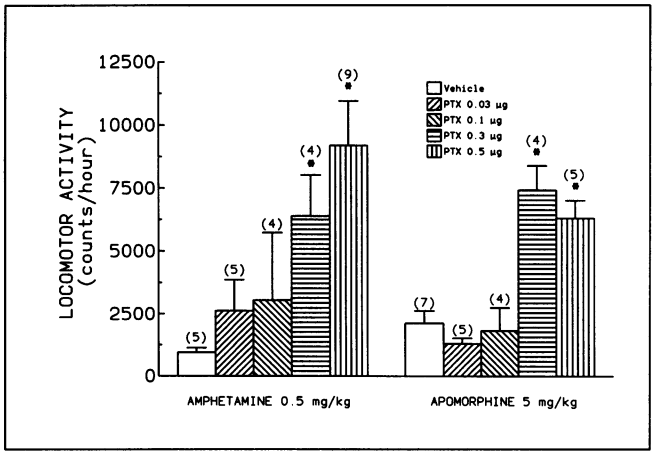

Figure 7. Dose response relationship between PTX injected into the VTA and the locomotor stimulatory effects of amphetamine and apomorphine. Rats were treated with varying doses (0.03 to 0.5 µg) of PTX into the VTA and 14 to 28 d later challenged with either amphetamine (0.5 mg/kg ip) or apomorphine (5 mg/kg sc), and the locomotor activity was recorded every 10 min for 1 h. Each value represents a mean ± SEM of 4 to 9 animals. *$P < 0.05$ (LSD test) when compared with vehicle-treated control animals.

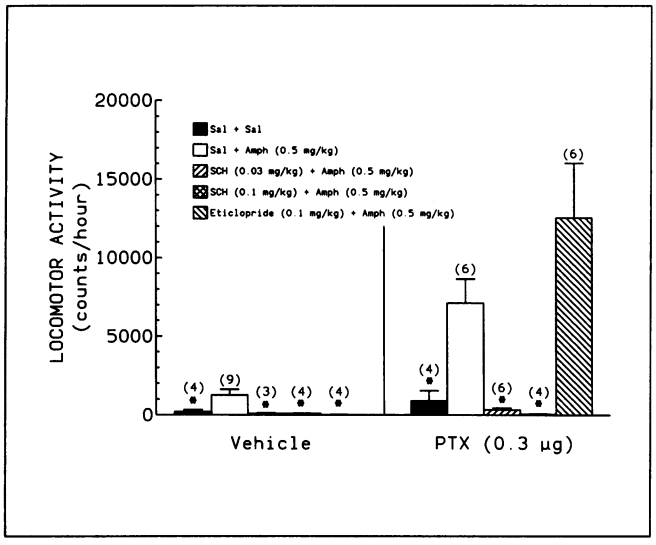

Figure 8. Effect of D_1 antagonist SCH23390 and D_2 antagonist eticlopride on amphetamine-stimulated locomotor activity in PTX- and vehicle-treated animals. Rats were treated with SCH23390 (0.03 and 0.1 mg/kg sc) or eticlopride (0.1 mg/kg sc) or vehicle and 20 min later injected with amphetamine (0.5 mg/kg ip), and the locomotor activity was recorded every 10 min for 1 h. Each value represents a mean ± SEM of 3 to 9 animals. *$P < 0.05$ (LSD test) when compared with either vehicle-treated animals or PTX-treated animals that received saline before amphetamine (0.5 mg/kg ip).

Rats treated with either PTX or vehicle were injected with apomorphine (5 mg/kg sc), which directly activates D_1 and D_2 dopaminergic receptors. Apomorphine stimulated locomotion in both groups of rats, but this response was significantly greater after PTX treatment. At 2 d after treatment with PTX or vehicle, apomorphine produced a similar degree of stimulation of locomotor activity in both groups of rats (Figure 9); however, 15 d after treatment with PTX or vehicle, the response to apomorphine of PTX-treated animals was significantly greater than that of vehicle-treated control animals (see Figure 9). This enhanced effect of apomorphine persisted through 70 d after treatment with PTX and was dependent on the dose of PTX administered into the VTA (see Figure 7). The enhanced response to apomorphine in PTX-treated animals was inhibited by SCH23390 (0.1 mg/kg sc), administered 20 min prior to the injection of apomorphine. Eticlopride (0.1 mg/kg sc), also administered 20 min prior to the injection of apomorphine, partially blocked the enhanced response to apomorphine. Both of these antagonists markedly reduced the locomotor response to apomorphine in vehicle-treated control animals (Figure 10).

DISCUSSION

This study shows that the administration of PTX into the VTA produces a marked, dose-dependent, and long-lasting increase in spontaneous locomotor activity in rats. In addition, after adaptation to the locomotor test cages, PTX-treated animals exhibited an increased locomotor response to both amphetamine, an indirectly acting dopamine agonist, and apomorphine, a direct-acting dopamine agonist. These effects were also long-lasting, and were observed for at least 2 mo after PTX treatment. The increased spontaneous- and drug-stimulated locomotor activity was dependent upon the activation of dopamine receptors, since these effects were antagonized by SCH23390. D_1 receptors appear to be more important in these responses than D_2 receptors, since eticlopride was either ineffective or partially effective in blocking the increased spontaneous locomotor activity or the enhanced drug-induced locomotor responses. These studies suggest that the administration of PTX into the VTA produces long-lasting changes in dopaminergic neurotransmission. In addition, the observation that the locomotor response to apomorphine is enhanced in PTX-treated animals suggests that postsynaptic dopaminergic receptor mechanisms are involved in these changes.

The administration of PTX into the VTA produced a marked increase in spontaneous locomotor activity. This effect of PTX did not occur immediately, but had a latency to onset of 4 to 6 d. This result reflects a 1994 study by Self and others, which reports a similar delay for G_i/G_o inactivation in the NAc after the administration of PTX. Other studies

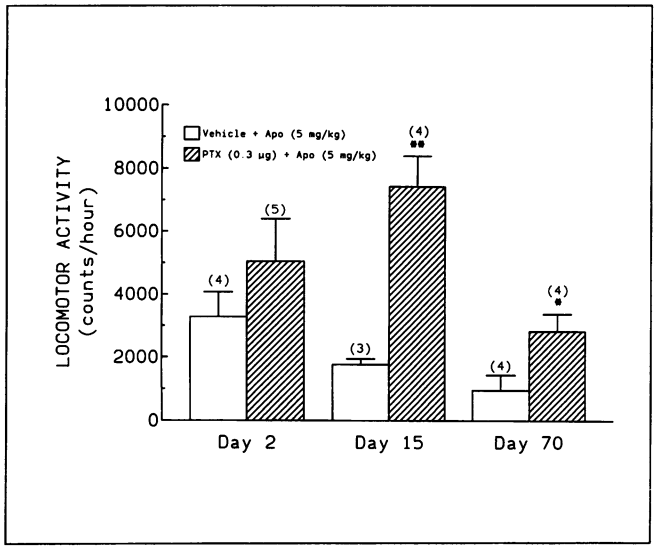

Figure 9. Time response for apomorphine-stimulated locomotor activity in PTX- and vehicle-treated animals. Rats that had been treated with PTX or vehicle into the VTA were challenged with apomorphine (5 mg/kg sc) on different days after PTX administration, and the locomotor activity was recorded every 10 min for 1 h. Each value represents a mean ± SEM of 3 to 5 animals. $^{*}P < 0.05$ and $^{**}P < 0.01$ (Student's *t* test) when compared with vehicle-treated controls.

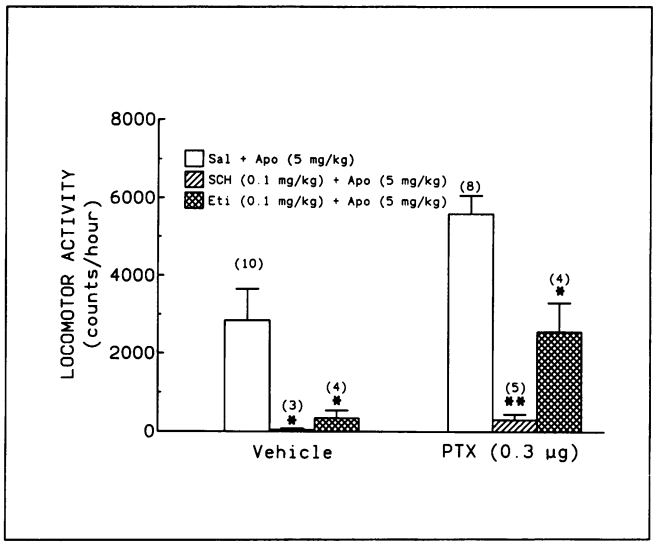

Figure 10. Effect of D_1 antagonist SCH23390 and D_2 antagonist eticlopride on apomorphine-stimulated locomotor activity in PTX- and vehicle-treated animals. Rats were treated with SCH23390 (0.1 mg/kg sc) or eticlopride (0.1 mg/kg sc) and 20 min later treated with apomorphine (5 mg/kg sc), and the locomotor activity was recorded every 10 min for 1 h. Each value represents a mean ± SEM of 3 to 10 animals. $^{*}P < 0.05$ and $^{**}P < 0.01$ (LSD test) when compared with controls that received saline and apomorphine.

involving PTX have also reported a similar latency of response after PTX treatment (Parenti and others 1986; Hoehn and others 1988; Chang and others 1991). The spontaneous locomotor activity remained elevated through 28 d after injection, and was no longer present at 60 d after PTX. The mechanism of this increased spontaneous activity is not clear. The present study demonstrates that the increased spontaneous locomotor activity of PTX-treated animals is antagonized by SCH23390, which blocks D_1 dopaminergic receptors (Iorio and others 1986), but not by the D_2 receptor antagonist eticlopride (Dreher and Jackson 1989), administered at a dose that inhibited the spontaneous locomotor activity and the amphetamine-stimulated response of vehicle-treated animals. These results suggest that the increase in spontaneous locomotion after PTX administration is mediated by the activation of D_1 receptors. It does not seem to be related to an increase in the synaptic dopamine concentration in the NAc. Thus, the metabolism of dopamine in PTX-treated animals was not significantly different from that of vehicle-treated controls at 7 d after PTX (Steketee and others 1991), a time when spontaneous locomotor activity is markedly increased. In addition, microdialysis studies have shown that basal levels of extracellular dopamine in the NAc are not greater than those of vehicle-treated controls at 14 d after PTX administration (Steketee and Kalivas 1991). Taken together, these observations suggest that the increase in spontaneous locomotor activity in PTX-treated animals is not due to an increased synaptic dopamine concentration, but may involve an increase in sensitivity of D_1 receptor-mediated events.

PTX-treated rats, which were adapted to the activity test cages for 2 h a day on 3 consecutive days, exhibited a dose-dependent enhancement in the locomotor response to amphetamine relative to the response of the vehicle-treated controls (see Figure 5). As observed for the increased spontaneous locomotor activity in PTX-treated animals, there was a time lag between PTX administration and the observation of the elevated response to amphetamine (see Figure 6), suggesting that the increase in spontaneous locomotion and the response to amphetamine may be mediated by the same or similar mechanisms. In addition, the enhanced locomotor response to amphetamine was long-lasting, occurring at least 60 d after PTX treatment. Previous studies have suggested that the increased locomotor response to cocaine in PTX-treated animals may be related to an enhanced dopaminergic neurotransmission, as reflected in an intensified cocaine- induced increase in extracellular dopamine in the NAc. Since both amphetamine and cocaine are thought to produce their behavioral effects by their presynaptic actions, the increased locomotor response to amphetamine in PTX-treated

animals may also be related to a greater presynaptic action resulting in enhanced dopaminergic neurotransmission. The present study shows that the augmented locomotor response to amphetamine in PTX-treated animals can be antagonized by the D_1 receptor antagonist SCH23390, but not by the D_2 receptor antagonist eticlopride administered at a dose that antagonized the stimulant effects of amphetamine in the vehicle-treated control rats (see Figure 8). These results suggest that the activation of D_1 receptors mediates the enhanced stimulant effects of amphetamine after PTX-treatment and that the administration of PTX into the VTA has produced a change in the responsiveness of dopamine receptors to agonists and antagonists.

Our studies on the stimulatory effect of apomorphine suggest that postsynaptic receptor mechanisms change after PTX administration. Thus, the locomotor stimulatory response to apomorphine, which directly activates D_1 and D_2 dopamine receptors, is enhanced after PTX administration. The augmented locomotor response of PTX-treated animals to apomorphine, as well as to amphetamine, is not due to a nonspecific change in sensitivity to stimulant drugs, since the locomotor stimulatory effects of caffeine, administered systemically, and morphine, administered into the VTA, are not changed after PTX administration (Steketee and Kalivas 1991). The greater locomotor response to apomorphine in PTX-treated animals was almost completely antagonized by SCH23390 and was partially antagonized by eticlopride, suggesting that both dopaminergic receptors may be involved in the enhanced apomorphine response, but that D_1 receptors may play a more significant role than D_2 receptors (see Figure 10). The site of action of apomorphine for stimulating locomotor activity is generally considered to be the NAc (Swerdlow and Koob 1984). Thus, we postulate that the augmented drug responses after PTX administration involve enhanced D_1 receptor mechanisms in the NAc.

The mechanism by which the administration of PTX into the VTA produces long-term pre and postsynaptic changes in the NAc is not clear. PTX administration has been shown to produce a decrease in the functional levels of G_i/G_o protein and a subsensitivity of D_2 somatodendritic dopaminergic receptors in the VTA. Since activation of somatodendritic D_2 receptors exerts an inhibitory effect on the firing rate of dopaminergic neurons, the changes induced by PTX should increase the activity of dopaminergic fibers, resulting in an increase in dopaminergic neurotransmission in the NAc. As indicated previously, it is likely that such an increased dopaminergic neurotransmission is transient and cannot account for the prolonged increase in spontaneous locomotion or the enhanced locomotor response to amphetamine, since at 14 d after PTX administration, the turnover of dopamine in the NAc has returned to normal levels (Steketee and Kalivas 1991), and the levels of extracellular dopamine were not significantly different in PTX-treated animals compared with vehicle-treated controls (Steketee and Kalivas 1991). Furthermore, G_i/G_o proteins, linked to receptors other than D_2, may mediate the prolonged changes in behavior after PTX treatment. The possibility that PTX injected into the VTA acts outside the VTA can be excluded based on a recent study by Van der Ploeg and others (1991), in which they report that there is minimal diffusion of PTX injected into the lateral ventricles. However, if it is assumed that the critical effect of PTX administration into the VTA is on dopaminergic neurons, then it is possible that the relatively brief increase in dopaminergic transmission in the NAc may trigger secondary events that mediate the long-term behavioral changes produced by PTX administration.

In conclusion, the results of the present study indicate that the administration of PTX into the VTA leads to an alteration in neurotransmission within the mesolimbic dopamine system, which may be related to both pre and postsynaptic mechanisms, and that D_1 receptors play a more important role than D_2 receptors in the enhanced locomotor responses to psychostimulant drugs.

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