Differential Regulation of Dopamine Transporter after Chronic Self-administration of Bupropion and Nomifensine

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ABSTRACT

Inhibition of dopamine (DA) transporter function is thought to be the principal mechanism underlying cocaine's addictive effects. In contrast to cocaine, several other inhibitors of DA transporter function are not considered to possess abuse liability. One of the neuroadaptive changes to chronic cocaine self-administration is the up-regulation of DA transporters. In the present study, we investigated the reinforcing and neuroadaptive effects of two other DA reuptake inhibitors, namely bupropion and nomifensine. Drug-naive rats readily acquired and subsequently maintained consistent self-administration of 3 and 1 mg/kg/infusion doses of bupropion and nomifensine, respectively, during 2-hr daily sessions over a prolonged period. Similarly, self-administration responding at low doses of bupropion (0.75 and 1.5 mg/kg/infusion) and nomifensine (0.1 and 0.3 mg/kg/infusion) showed some consistency during the initial weeks of testing which gradually declined or tended to decline to levels similar to that of the water control group during the later weeks of testing. Bupropion self-administration dose-dependently up-regulated DA transporters in caudate putamen and nucleus accumbens. In contrast, nomifensine self-administration did not alter DA transporter levels. These data provide evidence for heterogeneity among DA reuptake inhibitors, with some of these drugs being able to up-regulate DA transporters after their self-administration, whereas others lack this neuroadaptive response.

The dopamine transporter plays a pivotal role in cocaine's reinforcing effects. There is a large body of animal data which suggest that the reinforcing and behavioral effects of cocaine are caused by its binding to dopamine (DA) transporters leading to inhibition of dopamine reuptake and enhancement of dopaminergic transmission, especially in the mesocorticolimbic system (Johanson and Fischman, 1989; Kuhar et al., 1991; Koob, 1992). However, the clinical evidence does not appear to fully support this hypothesis (see review by Rothman and Glowa, 1995). For example, unlike cocaine, the DA reuptake inhibitors that are used clinically for other central nervous system disorders are not considered to possess abuse potential in humans. The reasons for these differences are not understood. Recent studies have focused on the differences between the binding characteristics of cocaine in contrast to those of several other DA reuptake inhibitors. The evidence favors the existence of multiple binding domains on the DA transporter molecule with different DA reuptake inhibitors possibly binding to different sites (Kitayama et al., 1992; McElvain and Schenk, 1992; Johnson et al., 1992; Wall et al., 1993; Deutsch and Schweri, 1994; Dersch et al., 1994; Akunne et al., 1994; Giros et al., 1994; Rothman et al., 1994). The in vivo pharmacological significance of these multiple binding domains on DA transporters remains to be understood.

The characterization of neuroadaptive changes in the dopaminergic system after chronic exposure to cocaine is important because these may relate to addictive or withdrawal states associated with cocaine abuse (Nestler, 1994). Several previous studies have investigated the changes in DA release, receptors and transporters after chronic exposure to cocaine (Peris et al., 1990; Kalivas and Stewart, 1991; Sharpe et al., 1991). In these studies, cocaine was administered on a noncontingent basis, even though substantial neurochemical, physiological and behavioral differences exist between contingent and noncontingent presentation of drugs (Siegel, 1988; Ator and Griffiths, 1992). Response-dependent presentation of cocaine is reinforcing, whereas response-independent presentation of cocaine is lethal (Dworkin et al., 1995). In this context, intravenous drug self-administration, a response-contingent procedure, is quite similar to the drug abuse pattern in humans (Deneau et al., 1969; Johanson and Balster, 1978; Griffiths et al., 1980). Recent studies with chronic intravenous cocaine self-administration procedures

ABBREVIATIONS: Acb, nucleus accumbens; CPU, caudate putamen; DA, dopamine; GBR-12909, (1-2-(bis(4-fluorophenyl)-methoxy)-ethyl-4-(3-phenylpropyl)piperazine); DAT, DA transporter.
have reported up-regulation of DA transporters (Wilson et al., 1994; Tella et al., 1996). It is not known whether this up-regulation of DA transporters is a direct response to cocaine or a homeostatic response to increases in synaptic DA produced by cocaine. Furthermore, the extent to which increases in DA transporters might be important in the ability of a given drug to maintain self-administration remains to be clarified. To address these issues, we have examined two DA reuptake inhibitors, namely nomifensine and bupropion, for their propensity to be self-administered. In addition, we have assessed possible adaptive changes in DA transporters after the self-administration of these drugs.

Methods

Subjects. Male Sprague-Dawley rats (Charles River Laboratories Inc., Wilmington, DE) weighing 350 to 450 g were used. The rats were individually housed in a temperature- and humidity-controlled room under a 12-hr light and dark cycle.

Intravenous drug self-administration. The procedure used for drug self-administration was the same as that described earlier (Tella, 1995). Animals were initially trained to press a lever for food pellets (45 mg) in standard operant boxes (Med Associates Inc., East Fairfield, VT) equipped with two levers. Responding on one of the levers resulted in delivery of food pellets, whereas responding on the other lever was recorded but had no programmed consequences. Initially each correct lever press was reinforced by food delivery. The number of correct lever presses required to produce a food pellet was gradually increased until stabilized at a response requirement of 10 (10-response fixed ratio, FR10). After training, a small plastic pedestal was surgically mounted on the skull with dental cement and stainless steel screws under pentobarbital anesthesia (55 mg/kg i.p.). A swivel spring was connected to the plastic pedestal during self-administration sessions. After 7 days of postoperative recovery, animals were implanted with polyvinyl chloride catheters into femoral veins under halothane anesthesia (2–3% in medical grade oxygen). Venous catheters were passed subcutaneously and exited the skin at the midscapular region. Animals were allowed to recover for an additional 7 days before initiation of i.v. drug self-administration. During drug self-administration sessions, food pellets were no longer delivered, and instead intravenous injections of drug were delivered by way of the catheter, which was connected to an injection pump outside the experimental chamber by polyvinyl tubing. Each completion of 10 lever press responses (FR10) resulted in a 1-sec i.v. infusion of bupropion (0.75–3 mg/kg/infusion), nomifensine (0.1–1 mg/kg/infusion) or sterile water in a volume of 0.25 ml/kg b.wt. There was a 1-min time-out period, during which the house light was off and responding had no programmed consequences. Experimental sessions were 2 hr in duration and were conducted once daily Monday through Friday. Rats were fed their daily requirement of about 20 g (~5 g/100 g b.wt.) standard rat chow as a single meal after daily sessions.

Seven groups of rats were trained to lever press for food reinforcement before i.v. drug self-administration testing. After lever press training, one group was tested with water and three groups were tested with three different doses, namely 0.1, 0.3 or 1 mg/kg/infusion of nomifensine, whereas the other three groups were tested with 0.75, 1.5 or 3 mg/kg/infusion doses of bupropion self-administration. The doses were chosen based on the published data that these dose ranges are pharmacologically active (Spyraki and Fibiger, 1981; Bergman et al., 1989). Because animals were food shaped for lever press training, one group of rats was tested with water to determine the time course of decline in lever press responding upon introduction of a nonreinforcer. This time course of responding for water served as a control for bupropion and nomifensine self-administration. The present experiment is a part of the series of experiments conducted with several drugs including GBR-12909. Water was used in the control group because of the low solubility of some of the drugs used in these experiments. Animals in the water control group maintained excellent health throughout the study and did not lose weight.

Reinforcing effects. Figure 1 shows the time course of individual and mean self-administration data of groups of rats receiving sterile water or bupropion infusions. Behavioral responding of the water control group has been described previously (Tella et al., 1996). Behavioral responding...
for water declined markedly within the first 3 days of testing and remained low thereafter (fig. 1). The 0.75 and 1.5 mg/kg/infusion doses of bupropion showed stable rates of responding during the initial few weeks of testing. For example, the 0.75 and 1.5 mg/kg/infusion doses of bupropion [(day 15, P = .05; day 16, P = .05; day 17, P = .05; day 18, P = .001) and (day 15, P = .01; day 16, P = .01; day 17, P = .001; day 18, P = .01), respectively] maintained significantly higher rates of responding after 3 weeks of testing as compared with the corresponding responding maintained by the water control group. During subsequent weeks of testing, the 0.75 mg/kg/infusion dose group showed a gradual decline in rates of responding reaching levels similar to that of the water control group. The group self-administering 1.5 mg/kg/infusion dose of nomifensine also showed some small and gradual reduction in rates of responding. Similar to bupropion, the rate of responding was inversely related to the infusion dose of nomifensine.

Biochemical effects. As compared with the water control group, there were no significant changes in [125I]RTI-121 binding in CPu and Acb after chronic self-administration of nomifensine at all three doses studied (fig. 5).

Reinforcing and Biochemical Effects of Nomifensine Self-administration

Reinforcing effects. Similar to bupropion, nomifensine at 1 mg/kg/infusion dose maintained consistent responding in all four animals during the entire testing period (fig. 4). The average daily intake of nomifensine during the last 4 weeks of testing ranged from 7.5 to 10 mg/kg for this group of animals. When water was substituted for this dose of nomifensine, self-administration responding declined over 5 to 10 days. Upon resubstitution of 1 mg/kg/infusion dose of nomifensine, regular self-administration responding was readily restored. Similar to bupropion, the low and medium doses of nomifensine also showed stable rates of responding during the initial weeks of testing. For example, the 0.1 and 0.3 mg/kg/infusion doses of nomifensine [(day 15, P = .05; day 16, P = .05; day 17, P = .09; day 18, P < .05) and (day 15, P = .05; day 16, P = .07; day 17, P < .01; day 18, P = .01), respectively] maintained significantly higher rates of responding after 3 weeks of testing as compared with the corresponding responding maintained by the water control group. During subsequent weeks of testing, the 0.1 mg/kg/infusion dose group showed a gradual decline in rates of responding reaching levels similar to that of the water control group. The group self-administering 0.3 mg/kg/infusion dose of nomifensine also showed some small and gradual reduction in rates of responding. Similar to bupropion, the rate of responding was inversely related to the infusion dose of nomifensine.
Discussion

The results of the present study indicate that rats reliably self-administer both bupropion and nomifensine. The present data also indicate that animals self-administering low doses of bupropion and nomifensine initially maintain stable rates of responding which gradually diminish to levels similar to those of the water control group during prolonged testing. This suggests that the reinforcing effects of low doses of these drugs gradually diminish during prolonged testing. Animals self-administering high doses of bupropion and nomifensine readily extinguish the responding after the substitution of water for these test drugs. The extinction of behavioral responding with water substitution and the subsequent restoration of responding after restimulation of bupropion and nomifensine further emphasize that both these drugs possess reinforcing properties. However, despite their common reinforcing properties, the neuroadaptive changes after their self-administration are clearly different. Self-administration of bupropion dose-dependently up-regulated DA transporters in CPu and Acb, whereas nomifensine did not alter transporter levels. In prior studies that used similar procedures, it has been shown that cocaine self-administration up-regulates DA transporters in these regions, whereas GBR-12909, another DA reuptake inhibitor, lacks this effect (Wilson et al., 1994; Tella et al., 1996). Those reports and the present findings collectively indicate pharmacological heterogeneity among DA reuptake inhibitors and support the idea of the possible existence of at least two distinct classes of DA reuptake inhibitors; the self-administration of one class of DA reuptake inhibitors (cocaine and bupropion) leads to up-regulation of DA transporters, whereas the self-administration of another class (nomifensine and GBR-12909) does not alter transporter levels. These differences in neuroadaptive changes after self-administration of DA reuptake inhibitors do not appear to be related to differences in their selectivity to different monoamine transporters. For example, both GBR-12909 (Van Der Zee et al., 1980) and bupropion (Ferris and Cooper, 1993) are DA-selective reuptake inhibitors, yet bupropion, but not GBR-12909, up-regulated DA transporters. Although cocaine is a nonselective reuptake inhibitor and thus could enhance synaptic norepinephrine and serotonin, these monoaminergic mechanisms are not likely to be involved in up-regulation of DA transporters for the following reasons. First, nomifensine, which effectively inhibits both DA and norepinephrine uptake (Hyttel, 1982), did not up-regulate DA transporters, which suggests that a noradrenergic mechanism is not involved. Second, bupropion, which has very low potency at the serotonin uptake site in comparison with DA uptake sites (Ferris and Cooper, 1993), is also able to up-regulate the DA transporter. This suggests that a serotonergic mechanism is also not involved in the up-regulation of DA transporter. Therefore, direct actions of cocaine and bupropion on the DA transporter might underlie their neuroadaptive effects on that protein.
The mechanism by which cocaine (Wilson et al., 1994; Tella et al., 1996) and bupropion (present study) self-administration cause up-regulation of DA transporters is not clear. One possibility is that the up-regulation might be a compensatory response to regulate the repeated increases in synaptic DA produced by repeated daily exposure to cocaine and bupropion (Pettit and Justice, 1989; Nomikos et al., 1989; Weiss et al., 1992; Wise et al., 1995). This explanation is not sufficient because nomifensine and GBR-12909, which also increase synaptic dopamine (Church et al., 1987; Carboni et al., 1989; Rothman et al., 1991; Baumann et al., 1994), do not cause up-regulation of DA transporters under identical experimental conditions. Moreover, because bupropion challenge appears to cause enhanced extracellular DA in Acb, but not in CPu (Nomikos et al., 1992), our findings that bupropion causes up-regulation DA transporters in both CPu and Acb regions provide further support for our argument that these observations are not compensatory responses to enhanced synaptic DA. Another mechanism that may underlie the difference between these two classes of drugs is the difference in the rate of occupancy of DA transporter molecule by these drugs. However, available evidence suggests that nomifensine, like cocaine and bupropion, has a fast occupancy rate (Stathis et al., 1995).

Another factor that might underlie the differential regulation of DA transporters by reuptake inhibitors may be the difference in their binding domains on DA transporters. For example, the accumulated evidence supports the existence of multiple binding sites for DA reuptake inhibitors (see the introduction). Nomifensine and GBR-12909, which did not up-regulate DA transporters, differ from cocaine in their binding to DA transporters (Wilson et al., 1994; Saadouni et al., 1994; Refahi-Lyamani et al., 1995; Jones et al., 1995). It is, thus, possible that certain binding domains (cocaine and bupropion binding sites) on DA transporter may be linked to the cascade of adaptive biochemical changes leading to up-regulation of DA transporters that occur after chronic perturbation of DA transporter function, whereas other domains (GBR-12909 and nomifensine binding sites) are not tied to these mechanisms. Interestingly, these two subclasses of DA reuptake inhibitors also appear to differ in their physiologic effects (Tella, 1996; Tella and Goldberg, unpublished).

For example, bupropion is similar to cocaine in producing rapid increases in blood pressure through a central mechanism independent of norepinephrine transporters, whereas nomifensine and GBR-12909 lack these rapid pressor effects in conscious rats (Tella and Goldberg, unpublished). Alternatively, there may be different subtypes or states of the DA transporters with different drug binding profiles (Wilson et al., 1994).

Although the nature and extent of the involvement of DA transporter up-regulation in the addictive process and withdrawal states produced by these drugs remains to be understood, the importance of the present findings has been emphasized by clinical data which support the view that not all DA reuptake inhibitors possess abuse liability (Rothman and Glowa, 1995). This statement raises the possibility of the existence of differential reinforcing efficacies among various classes of reuptake inhibitors. Our previous study with a fixed-ratio schedule of drug self-administration has indeed shown that GBR-12909, which does not up-regulate the DA transporter, does have limited reinforcing effects (Tella et al., 1996). The idea, however, is not supported by the observation that nomifensine, which also does not up-regulate DA transporters, is self-administered (present study). Nevertheless, because fixed-ratio schedules of self-administration testing might not necessarily reveal the differences in the magnitude of reinforcing efficacies of drugs (Johanson and Fischman, 1989), more behavioral in conjunction with biochemical and molecular studies will be needed to further clarify these issues.

In summary, the present data provide further evidence that neuroadaptive changes in DA transporter molecules after chronic exposure to reinforcing doses of DA reuptake inhibitors are divergent, with some DA reuptake blockers being able to up-regulate DA transporters, whereas others do not. In addition, increase in DA levels secondary to DA reuptake blockade does not appear to be responsible for their ability to regulate DA transporters, because they all share that property. Studies focusing on the elucidation of the molecular mechanisms involved in the mediation of the differential neuroadaptive effects of DA reuptake inhibitors on DA transporter molecules promise to clarify the developmental process of cocaine addiction.

References


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