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Behavioral Sensitization and Tolerance to Cocaine and the Occupation of Dopamine Receptors by Dopamine

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Abstract

Data from the authors' laboratory on the neural substrates of Pavlovian conditioning and behavioral sensitization to psychomotor stimulants are reviewed. The findings of a recent experiment on the role of occupation of dopamine receptors by dopamine and its association to behavioral sensitization are reported. Daily intermittent injections of cocaine produced behavioral sensitization to the locomotor response in rats, whereas continuous cocaine infusions produced behavioral tolerance. Behavioral sensitization to cocaine was blocked by coadministration of nimodipine, an L-type calcium channel blocker. The increase in locomotion produced by cocaine was associated with an increase in the occupation of striatal dopamine D_1 and D_2 receptors, measured as the density of receptors protected from denaturation by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). This association was not observed when rats were given a challenge injection of cocaine 10 d after withdrawal from similar treatment regimens. Rats given a cocaine challenge after withdrawal from either intermittent or continuous cocaine treatment regimens exhibited increased occupation of striatal D_1 and D_2 receptors. This increase was similar in magnitude to that observed in rats without a history of cocaine treatments after a challenge injection of cocaine. This suggests tnat the differences in occupancy of striatal dopamine receptors by dopamine observed in the prewithdrawal condition are likely the result of differences in brain levels of cocaine achieved by the two treatment regimens. Occupancy of striatal dopamine D_1 and D_2 receptors does not appear to be related to the development of sensitization to the motor-stimulating effects of cocaine.

Index Entries: Cocaine; sensitization; tolerance; dopamine receptor occupation; treatment regimen; nimodipine; D_1; D_2; dopamine release.

Introduction

Repeated treatment of rats with psychomotor stimulants, including cocaine and amphetamine, can produce an augmentation of certain behavioral responses, termed sensitization. Interest in this phenomenon has been spurred by the suggestion that sensitization may pro-

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vide an animal model of stimulant-induced psychosis (cf Angrist, 1983; Robinson and Becker, 1986), and by the failure to observe clear changes in receptor density that might provide a physiological mechanism for the behavioral changes (*see* for reviews: Robinson and Becker, 1986; Johanson and Fischman, 1989). In addition, a variety of researchers have found that sensitization is largely context-specific. Context specificity has been shown for amphetamine (Tilson and Rech, 1973; Drew and Glick, 1988; Stewart and Vezina, 1991), cocaine (Hinson and Poulos, 1981; Post et al., 1981; Barr et al., 1983; Beninger and Herz, 1986), and apomorphine (Mattingly and Gotsick, 1989). This phenomenon has traditionally been explained as a function of classical conditioning of drug effects to the contextual stimuli, but this interpretation has been challenged (Barr et al., 1983; Drew and Glick, 1988; Gold et al., 1988; Damianopoulos and Carey, 1992). Clinical research has implicated stimuli classical conditioned to drug effects as initiators of "cocaine craving" (O'Brien et al., 1988; Muntaner et al., 1989). Thus, there is an interest in the classical conditioning of psychomotor stimulant effects in addition to its contribution to sensitization.

A major question arises from the classical conditioning of psychomotor stimulant effects: What is conditioned? Behaviorists have discussed classical conditioning in the past in terms of associations between a stimulus (S) and a response (R). Within that paradigm, a clear description of these two elements and their relationship to each other provided a sufficient account of the phenomenon. However, in the case of conditioned drug responses, neither the unconditioned stimulus (US) nor the unconditioned response (UR) is clearly defined. For example, conditioned morphine responses are antagonistic to the direct effects of morphine, leading to an opponent-process theory of drug conditioning (Eikelboom and Stewart, 1982; Poulos and Cappel, 1991). In this case, the unconditioned response that stimuli associate with may be homeostatic mechanisms that compensate for the direct drug effects, rather than the direct pharmacological actions of the drug. Another case occurs with psychomotor stimulants: The environmental stimuli associated with prior drug treatments augment the behavioral effects of a challenge dose. This augmentation can take the form of behaviors that do not occur with a single treatment or in unpaired controls. Stereotyped licking and biting of the cage floor with a reduction in locomotion can be observed with an increase in dose or after conditioning, even if increased locomotion is the behavior associated with the environment. These examples indicate that the response that becomes associated with the stimuli may not be the response that is directly elicited by the drug at the dose used. Defining the unconditioned stimulus can also be difficult. Which of the multitude of pharmacological actions of the drug are relevant as the stimulus?

The research reviewed in the following sections was an attempt to test the hypothesis that the response that is conditioned to psychomotor stimulants is some effect of the drugs on the dopamine system, rather than any specific behavior. We also investigated the possibility that context-specific sensitization is a function of the same process. Much of this research has already been published elsewhere. However, some of the results regarding the occupation of dopamine receptors and the effects of nimodipine on sensitization and tolerance to cocaine are presented for the first time.

Role of Postsynaptic Receptors in Conditioning of Stimulant-Induced Behaviors

It has previously been reported that pimozide, a relatively selective antagonist for dopamine D_2 receptors, can block the establishment (but not expression) of conditioning with amphetamine (Beninger and Hahn, 1983) and cocaine (Beninger and Herz, 1986). However, we failed to replicate this finding using haloperidol, another relatively selective antagonist for the D_2 receptor (Martin-Iverson and McManus, 1990; Reimer and Marlin-Iverson,

1994). Neither haloperidol nor SCH 23390, a selective antagonist for D_1 receptors, blocked the establishment of conditioning to amphetamine, even when the antagonists were combined. Either antagonist alone was effective at blocking the direct effects of amphetamine. Haloperidol also failed to block the conditioning of cocaine's locomotor effects. Examining the literature on the different pharmacological profiles of pimozide and haloperidol, we found that, unlike haloperidol, pimozide was equipotent at blocking L-type calcium channels and D_2 receptors. We therefore investigated whether the effects of pimozide could be mimicked by combining haloperidol with an L-type calcium channel blocker, nimodipine. Indeed, this combination of drugs did attenuate the conditioning of amphetamine's effects to contextual stimuli (DiLullo and Martin-Iverson, 1992b). Thus, the D_2 receptor antagonism by pimozide is confounded with its blockade of L-type calcium channels. The latter action can attenuate dopamine release induced by stimulants (Pani et al., 1990). Interestingly, nimodipine given alone is effective at blocking conditioning of cocaine (Reimer and Martin-Iverson, 1994), whereas it must be combined with haloperidol to attenuate amphetamine conditioning (DiLullo and Martin-Iverson, 1992b).

Role of Presynaptic Release of Dopamine in Conditioning

The above observations led to the speculation that the fundamental neurochemical event that is conditioned to contextual stimuli on repeated administration of stimulant drugs is an increased release of dopamine (Martin-Iverson et al., 1993). In the case of amphetamine, the drug itself directly induces release via a calcium-independent mechanism, and we hypothesized that this mechanism may become conditioned such that the contextual stimuli come to increase release of dopamine. The conditioned release must be at least partially related to a calcium-dependent mechanism, since nimodipine has a synergistic effect with haloperidol at blocking amphetamine conditioning. However, a calcium-dependent mechanism is not sufficient to account for amphetamine conditioning, since nimodipine is not able to block the conditioning on its own. This idea was further examined by investigating the effect of α-methylparatyrosine on the conditioning of amphetamine's motor stimulant effects to contextual stimuli. α-Methylparatyrosine inhibits the enzyme tyrosine hydroxylase, blocking the synthesis of monoamines, including dopamine. This action blocks the direct locomotor stimulant effects of amphetamine (DiLullo and Martin-Iverson, 1991). Indeed, the ability of α-methylparatyrosine to block the direct behavioral effects of amphetamine, along with the inability of reserpine, which disrupts vesicle storage of monoamines, to block amphetamine's effects have led to the concept that amphetamine induces the release of dopamine from a compartment containing newly synthesized dopamine through an impulse- and calcium-independent mechanism (Scheel-Kruger, 1971; Arnold et al., 1977; Kuczenski, 1978; Fischer and Cho, 1979; Raiteri et al., 1979; Miller and Shore, 1982; Westerink et al., 1989; Arbuthnott et al., 1990). However, treating rats with α-methylparatyrosine daily for 10 d prior to conditioning with amphetamine failed to block the establishment of the conditioned locomotor response of amphetamine, even though the direct locomotor stimulant effects of amphetamine were blocked (DiLullo and Martin-Iverson, 1991). Similarly, daily reserpine injections failed to block the establishment of amphetamine conditioning (DiLullo and Martin-Iverson, 1992a). Destruction of noradrenergic terminals with DSP-4 also failed to affect either direct or conditioned amphetamine-induced locomotion (DiLullo and Martin-Iverson, 1991). However, the combination of α-methylparatyrosine with reserpine did block amphetamine conditioning (DiLullo and Martin-Iverson, 1992a). This evidence suggests that the conditioning of amphetamine's behavioral effects to contextual stimuli is associated with two independent processes, either of which is capable of

sustaining a conditioned response. One of these is the calcium- and impulse-independent release of newly synthesized dopamine, and the second is the calcium- and impulse-dependent release of dopamine from vesicles. Both of these processes must be blocked in order to block amphetamine conditioning.

Cocaine is a different case. Since this drug blocks the uptake of dopamine, its effects at increasing dopamine release and locomotor activity require the calcium- and impulse-dependent release of dopamine from reserpine-sensitive vesicles (Ross, 1977). Dihydropyridine L-type calcium channel blockers, such as nimodipine, block the increased extracellular dopamine levels produced by cocaine (Pani et al., 1990). In line with this, we have found that injecting rats with nimodipine before cocaine treatments during conditioning blocks the acquisition of cocaine-conditioned locomotion (Reimer and Martin-Iverson, 1994) and conditioned place preferences (Reimer and Martin-Iverson, in preparation). However, nimodipine is not effective at blocking the expression of conditioning once established (Martin-Iverson and Reimer, 1994). Thus, the effect of pimozide on blocking the establishment but not the expression of cocaine conditioning (Beninger and Herz, 1986) is exactly matched with the effects of nimodipine, suggesting that it is the L-type calcium channel blocking actions of pimozide, and not its dopamine receptor blocking actions that are responsible for its effects on cocaine conditioning.

The above evidence suggests that the critical element in the establishment of context conditioning of psychomotor stimulant motor activation is the release of dopamine, through the calcium-dependent, reserpine-sensitive mechanism in the case of cocaine, or both this mechanism and the calcium-independent, α-methylparatyrosine-sensitive process in the case of amphetamine. This hypothesis is consistent with a previous finding that 6-hydroxydopamine lesions of dopamine terminals in the nucleus accumbens abolish the acquisition of amphetamine conditioning (Gold et al., 1988). Note, however, that a recent microdialysis study failed to observe an increased interstitial concentration of dopamine after exposure of rats to a context previously associated with cocaine (Brown and Fibiger, 1992), although an increase in *fos* expression was observed in limbic areas other than the nucleus accumbens in rats exposed to the cocaine-associated context (Brown et al., 1992).

Relationship of Conditioning to Sensitization

The degree to which conditioned drug effects can account for sensitization has been controversial. There is no doubt that when a specific context is associated with stimulant administration, the context controls the expression of sensitization. It is well established that pseudoconditioned controls (i.e., rats that receive the stimulant drug in a context different from the testing environment) do not show sensitization to amphetamine and cocaine, and display only partial sensitization to apomorphine (*see* references in the first paragraph of the Introduction). It remains possible that the conditioning procedure obscures a nonassociative sensitization process. For example, pseudoconditioned rats display sensitization to amphetamine for one of two behaviors measured after extinction of the conditioning (Stewart and Vezina, 1991). If an explicit random drug-context pairing procedure is followed, sensitization develops to amphetamine (Stewart and Druhan, 1993). However, it should be remembered that in random drug-context pairing, the test context continues to have a certain degree of predictability of drug effects as compared to the home cage environment, and therefore, some conditioning may be expected to occur.

The strongest evidence that sensitization can occur in the absence of conditioning comes from studies that infuse stimulants continuously using osmotic minipumps. If rats receive continuous infusions of a direct D_2 receptor agonist, (+)-4-propyl-9-hydroxynaphthoxazine (PHNO), or amphetamine in their home cages, the behavioral effects develop tolerance during

the day, but sensitization of certain behaviors occurs at night (Martin-Iverson, 1991a; Martin-Iverson et al., 1988a,b). In the case of PHNO, this diurnal tolerance/nocturnal sensitization can be reversed by reversing the light/dark cycle (Martin-Iverson et al., 1988a), and follows circadian rhythms in motor activity in rats maintained under constant lighting conditions (Martin-Iverson and Yamada, 1992). Thus, for these drugs, it is clear that sensitization can occur in the absence of specific contextual cues associated with drug administration. On the other hand, we have not observed sensitization in rats given continuous administration of cocaine at night; only tolerance develops to cocaine when it is given continuously (Burger and Martin-Iverson, in preparation). This has led us to the opinion that sensitization to cocaine, unlike amphetamine or direct dopamine receptor agonists, is exclusively dependent on environmental context.

Recently, we have found evidence for a pharmacological dissociation of cocaine conditioning and sensitization. Specifically, nimodipine completely blocks the establishment of conditioning to cocaine, but only partially blocks the acquisition of sensitization (Reimer and Martin-Iverson, 1994). On the other hand, nimodipine given only on the test days and not on the conditioning days blocks the expression of cocaine sensitization, but not the conditioning of cocaine, unless it is combined with haloperidol (Martin-Iverson and Reimer, 1994). It appears that cocaine may also produce sensitization via a nonassociative process. However, this nonassociative process is normally completely masked by context specificity (Reimer and Martin-Iverson, 1994) as often, but not always (Martin-Iverson, 1991a), happens with amphetamine (Stewart and Vezina, 1991; Stewart and Druhan, 1993).

We hypothesize that both sensitization and conditioned stimulant effects are related to an increase in stimulated dopamine release. The evidence for this hypothesis in conditioning has already been discussed. There is also evidence for an increase in extracellular dopamine concentrations in vivo or in amphetamine-stimulated dopamine efflux in vitro in rats exhibiting sensitization to amphetamine (Castenada et al., 1988; Robinson et al., 1988; Pettit et al., 1990; Patrick et al., 1991; Kalivas and Duffy, 1993). Of particular interest is the comparison of the effects of a challenge dose of cocaine given to rats with a previous history of intermittent cocaine treatments, continuous infusions of cocaine, or treatment with saline (King et al., 1993). Only the intermittent cocaine treatments produced sensitization, with the continuous infusions producing tolerance after a challenge dose of cocaine (King et al., 1992). Similarly, previous intermittent cocaine treatments increased the cocaine-induced dopamine efflux in striatal slices in vitro, whereas a history of continuous cocaine infusions was associated with a decreased dopamine efflux after a cocaine challenge (King et al., 1993).

The latter finding raises the possibility that tolerance to psychomotor stimulant effects on motor activity may be the result of a decrease in dopamine release, rather than, or in addition to, decreases in the density of dopamine receptors. Although receptor density decreases (receptor subsensitivity) is the classical mechanism invoked to explain drug tolerance, it may not be important in tolerance to psychomotor stimulants. Using the D_2 selective agonist PHNO as an example, it can be seen that receptor subsensitivity does not provide an adequate explanation for the behavioral tolerance that develops to this drug on continuous subcutaneous infusions with osmotic minipumps. First, as mentioned previously, tolerance to this drug occurs only during the day; sensitization develops at night. The switch from daytime tolerance to nocturnal sensitization (and vice versa) occurs within a 1-h period, which is too rapid to be a function exclusively of receptor density (Martin-Iverson et al., 1988a). Furthermore, daytime tolerance can be transiently reversed into sensitization in the presence of an arousing stimulus, an effect that requires only minutes to occur (Martin-Iverson et al.,

1988a). This stress-induced reversal of tolerance can be blocked by a D_1 antagonist. Finally, the effect of stress can be mimicked by injection with a D_1 agonist, suggesting that it is a function of stress-induced dopamine release at sites containing D_1 receptors (Martin-Iverson et al., 1988b). These data indicate that tolerance results from a decrease in dopamine release at D_1 receptor sites, rather than from changes in receptor density. It remains possible that the decrease in dopamine release is a function of supersensitivity of dopamine presynaptic autoreceptors that normally inhibit dopamine release. However, such a mechanism would require that the chronic treatment with an agonist would produce an increased density of autoreceptors, an effect that has not been reported.

Another mechanism that has been invoked to explain behavioral tolerance to continuous infusions of psychomotor stimulants is long-term depletion of dopamine as a consequence of neurotoxic effects (Robinson and Becker, 1986). However, chronic administration of PHNO actually increases dopamine content in brain tissue (Martin-Iverson, 1991b). Furthermore, cocaine does not produce similar neurotoxic effects in the striatum (Kleven et al., 1988; Ryan et al., 1988), although it does in the lateral habenula and fasciculus retroflexus (Ellison, 1992). Most damaging to the hypothesis that long-term depletion of dopamine produced by high doses of psychomotor stimulants can be responsible for behavioral tolerance is the finding that both resting and methamphetamine-evoked levels of extracellular dopamine measured in vivo with microdialysis are not reduced in rats with long-term dopamine depletions produced by neurotoxic doses of methamphetamine (Robinson et al., 1990). If tolerance to psychomotor stimulants is a function of a general phenomenon common to all of these drugs, then it appears most likely that it is a result of decreases in dopamine release, possibly related to decreases in impulse activity.

We have examined the role of dopamine release intrasynaptically after continuous cocaine infusions with osmotic minipumps that produce behavioral tolerance or intermittent cocaine injections of an equivalent daily dose that produces behavioral sensitization (Burger and Martin-Iverson, 1994). The effects of nimodipine at a dose that can block behavioral sensitization to cocaine (Reimer and Martin-Iverson, 1994) were also investigated. Rats were given 14 d of treatment, with vehicle or nimodipine and vehicle, intermittent cocaine, or continuous cocaine. On the 14th d of treatment, 30 min after cocaine or cocaine vehicle injections, all rats received an injection of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), which acts by binding to DA receptors and irreversibly denaturing them via alkylation. Because it has a relatively low affinity for dopamine receptors, EEDQ at the dose used does not displace dopamine from receptors and therefore denatures only unoccupied receptors. The density of receptors in the striatum occupied by dopamine at the time of the EEDQ treatment can therefore be measured by in vitro binding assays using [^{3}H]-spiperone as a ligand for D_2 receptors and [^{3}H]-SCH 23390 as a ligand for D_1 receptors. We found that rats receiving intermittent daily injections of cocaine displayed sensitization in their locomotor response to cocaine, and also exhibited a large increase in the occupation of both dopamine D_1 and D_2 receptors in both the striatum (measured using Scatchard analyses) and nucleus accumbens (single-point binding), presumably by dopamine relative to vehicle controls. Rats that received continuous infusions of cocaine developed tolerance to cocaine's behavioral effects and did not exhibit any increase in occupation of dopamine receptors relative to vehicle, although they had received an equivalent daily dose of cocaine. Nimodipine blocked sensitization in rats receiving intermittent cocaine and also blocked the increase in occupation of dopamine receptors.

These data provide evidence that sensitization is associated with an increased interaction of dopamine with both D_1 and D_2 receptors in both the striatum and nucleus accumbens, and that tolerance is associated with a loss of this

increased receptor occupation. However, direct comparisons between rats with a history of intermittent or continuous cocaine treatment and rats with no previous history of cocaine given a single challenge dose of cocaine were not made. Furthermore, it is possible that the two different treatment regimens provide different cocaine concentrations in the brain at the time following the EEDQ injection. We now report on the effects of continuous vs intermittent cocaine treatments on dopamine receptor occupation in the striatum both on the last day of treatment and after a challenge of a single injection of cocaine 10 d after withdrawal from a 10-d treatment regimen.

Methods

Animals

Male Sprague-Dawley rats (Charles River, Canada) weighing 250–350 g were individually housed in colony rooms maintained on a 12-h light–dark cycle (lights on at 8:00 AM) at 23°C. Food and water were available ad libitum. All subjects were weighed every 2nd d throughout the course of the experiment.

Drug Administration Procedure

After 10 d of habituation following arrival, the rats were randomly assigned to one of six drug groups consisting of treatments of vehicle, nimodipine and vehicle, or cocaine (VEH + VEH, VEH + COCi, VEH + COCc, NIM + VEH, NIM + COCi, and NIM + COCc, where VEH is vehicle, COCi is intermittent cocaine, COCc is continuous cocaine, and NIM is nimodipine). Cocaine hydrochloride (BDH [Toronto, Canada], 10 mg/kg/d) and vehicle (double-distilled water) were delivered either continuously via Alzet osmotic minipumps (Alza, model 2ML2) or intermittently with daily intraperitoneal (ip) injections to half of the rats for 14 d. Surgical implantation of the minipumps (method described in Burger and Martin-Iverson, 1993) was performed on d 0. Nimodipine (Miles Pharmaceuticals Ltd. [New Haven, CT], 10 mg/kg) or its vehicle, polyethylene glycol 400 (100%), was injected ip 70 min prior to locomotor testing each day, beginning on d 1. Immediately following the daily cocaine injection (vehicle was injected for those animals with minipumps and for the vehicle controls), each rat was placed in its own box equipped with photocell beams (as described previously by Martin-Iverson and McManus, 1990) to assess activity levels for 1 h. On d 14, 30 min postcocaine or vehicle, eight subjects in each group were administered EEDQ (5 mg/kg, ip) dissolved in ethanol, propylene glycol, and distilled water (2:1:2). The remaining 72 rats (*N*: VEH + VEH = 20, VEH + COCi = 14, VEH + COCc = 14, NIM + VEH = 8, NIM + COCi = 8, NIM + COCc = 8) remained in their home cages for an additional 10 d. All but six rats in the VEH + VEH group were then injected with cocaine (10 mg/kg, ip), placed in the locomotor testing cages, and 30 min later were given an injection of EEDQ as above. Six of the rats from the VEH + VEH group were given a challenge injection of vehicle to serve as controls. Rats were observed periodically to determine whether or not they engaged in high levels of stereotyped behaviors that might confound the locomotor measurements. Twenty-four hours after the EEDQ injections, the rats were decapitated and their brains removed for dissection on ice. Left and right sides of the striatum were removed and stored at –80°C for further neurochemical analysis.

Dopamine Receptor Binding

Unilateral striatal tissue from each rat (left and right sides randomized across assays) was subjected to radioligand binding assays for both D_1 and D_2 receptor labeling as previously described (Burger and Martin-Iverson, 1993). [^{3}H]SCH23390 and [^{3}H]spiperone were used to label D_1 and D_2 receptors, respectively, with the addition of ketanserin to control for 5-HT_2 receptor binding.

Statistics

The 5-min blocks of photobeam interruptions were added together to produce 30-min

totals for the last day of treatment before EEDQ injection (prewithdrawal) or on the challenge test day (postwithdrawal). These data as percent of the vehicle control groups were then subjected to ANOVA with three factors. These factors were:

1. Withdrawal having two levels (0 or 10 d);
2. Nimodipine treatment with two levels (vehicle or 10 mg/kg); and
3. Cocaine treatment with three levels (vehicle, 10 mg/kg/d intermittent injections, or 10 mg/kg/d continuous infusions).

Receptor densities (B_{max}) and affinities (K_d) were calculated from the data from the receptor binding assays using LIGAND and were subjected to ANOVA with the three factors listed above after calculating the percentage of vehicle controls (the vehicle challenge group was the control in the case of the postwithdrawal groups). The D_1 and D_2 receptor subtypes were included as an additional within-subject factor. Multiple F tests (Kiess, 1989) were applied for individual comparisons where appropriate.

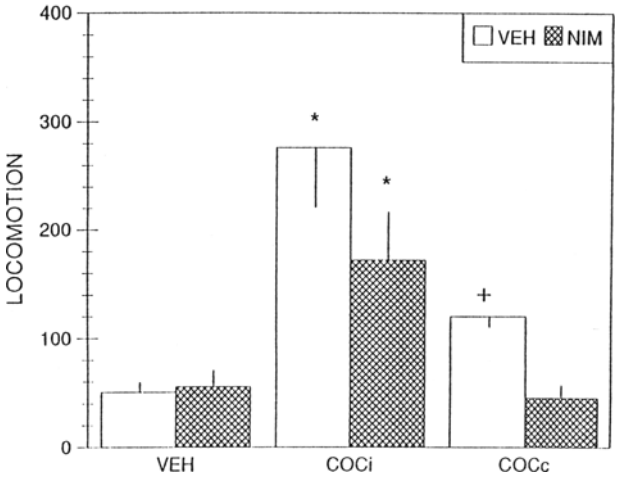

Fig. 1. Locomotor activity (± SEM) in rats ($N = 8$ in each group) for 30 min on the last day of treatment prior to EEDQ injection before withdrawal from treatment. Rats were treated with vehicle (VEH) or nimodipine (NIM, 10 mg/kg/d) and vehicle (VEH), intermittent daily injections of cocaine (COCi), or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg. Pairwise comparisons between groups were made with the Multiple F test for $p < 0.05$. *Significantly different from vehicle control. + Significantly different from the COCi group.

Results

Prewithdrawal

Prewithdrawal locomotor activity is displayed in Fig. 1. Intermittent daily injections of cocaine substantially increased locomotor response on the last day of treatment before the day of EEDQ injections (Fig. 1). Continuous infusions of cocaine produced locomotor activity that was not significantly different from the vehicle control group, but was significantly lower than that of the intermittent cocaine group, indicating the development of tolerance in the continuous infusion group. Nimodipine attenuated the increase in locomotor activity produced by intermittent cocaine, such that this group was intermediate between the intermittent cocaine and the vehicle groups. ANOVA revealed that there were main effects of cocaine treatment regimen (F [2,102] = 25.6, $p < 0.001$), of withdrawal (F [1,102] = 6.5, $p < 0.02$), and of nimodipine (F [1,102] = 5.39, $p < 0.05$). The withdrawal by cocaine treatment interaction was also significant (F [2,102] = 6.31, $p < 0.005$).

The K_ds observed in each group were assessed by ANOVA. There were no significant effects of any of the treatments. The K_d for D_1 receptors was 0.32 ± 0.01 (nM ± SEM) and for D_2 receptors was 0.053 + 0.0017 (nM ± SEM). However, the densities of receptors remaining after denaturation by EEDQ differed markedly between groups depending on treatment. The average densities of dopamine receptors remaining in the vehicle control group after denaturation of receptors by EEDQ were 384 fmol/mg protein (D_1) and 123 fmol/mg protein (D_2). There were significant main effects of withdrawal (F [1,102] = 6.74, $p < 0.02$), and nimodipine (F [1 ,102] = 3.22, $p < 0.02$). There was also a significant interaction

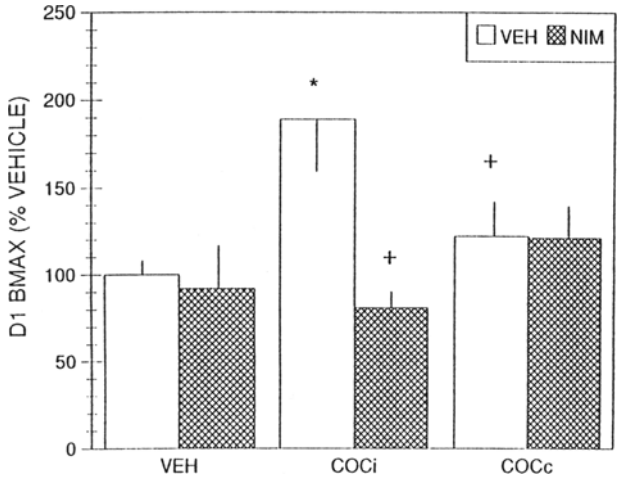

Fig. 2. The density of dopamine D_1 receptors (± SEM) protected from EEDQ denaturation on the last day of treatment as a percent of the vehicle control group. Rats were treated with vehicle (VEH) or nimodipine (NIM, 10 mg/kg/d) and vehicle (VEH), intermittent daily injections of cocaine (COCi), or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg and $N = 8$ for each group. Pairwise comparisons between groups were made with the Multiple F test for $p < 0.05$. *Significantly different from vehicle control. + Significantly different from the COCi group.

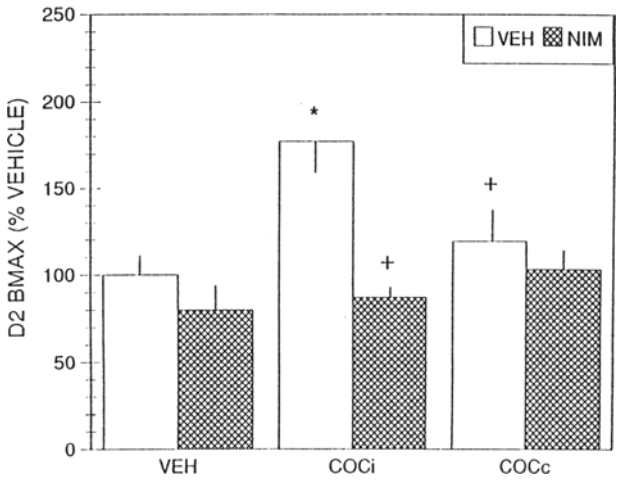

Fig. 3. The density of dopamine D_2 receptors (± SEM) protected from EEDQ denaturation on the last day of treatment as a percent of the vehicle control group. Rats were treated with vehicle (VEH) or nimodipine (NIM, 10 mg/kg/d) and vehicle, intermittent daily injections of cocaine (COCi), or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg, and $N = 8$ for each group. Pairwise comparisons between groups were made with the Multiple F test for $p < 0.05$. *Significantly different from vehicle control. + Significantly different from the COCi group.

between nimodipine and cocaine treatment regimen (F [2,102] = 3.89, $p < 0.025$) and between withdrawal and receptor subtype (F [1,102] = 7.80, $p < 0.01$). In the prewithdrawal groups, only the group receiving intermittent cocaine injections exhibited an increased occupation of D_1 (Fig. 2) and D_2 receptors (Fig. 3). The groups receiving continuous infusions of cocaine did not show significant elevations in the protection of dopamine receptors from EEDQ-induced denaturation. Nimodipine given prior to intermittent cocaine injections blocked the effects of cocaine on striatal receptor occupancy.

Postwithdrawal

After 10 d of withdrawal (Fig. 4), a challenge dose of cocaine given to rats given intermittent injections of cocaine exhibited locomotion levels after the challenge injection significantly greater than that observed in the vehicle group receiving a cocaine challenge. This effect was not affected by nimodipine and was not observed in the groups previously given continuous infusions with cocaine.

The group that received previous treatment with vehicle and was challenged with cocaine exhibited an increase in the occupation of both D_1 (Fig. 5) and D_2 (Fig. 6) receptors. This increase in the occupation of receptors induced by an acute cocaine injection was blocked in those rats with a history of treatment with nimodipine. Intermittent treatment with cocaine increased the occupation of D_1 and D_2 receptors over that observed in the vehicle challenged rats, but not over that observed in the acute cocaine group. Again, previous treatments with nimodipine blocked the effects of the cocaine challenge in this group. Continuous infusions of cocaine slightly, but not significantly, attenuated the increase in both D_1

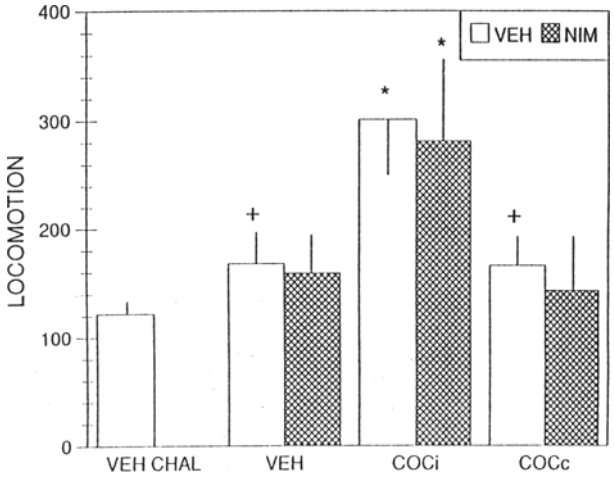

Fig. 4. Locomotor activity (± SEM) in rats for 30 min after a cocaine (10 mg/kg) challenge 10 d after withdrawal from a chronic treatment regimen. A control group was given a vehicle challenge 10 d after withdrawal from vehicle treatment (VEH CHAL, $N = 6$). Rats were chronically treated with vehicle or nimodipine (NIM, 10 mg/kg/d) and vehicle, intermittent daily injections of cocaine (COCi) or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg, $N = 14$ for all cocaine-challenged groups that did not receive nimodipine, and $N = 8$ for all groups receiving nimodipine. Pairwise comparisons between groups were made with the Multiple F test for $p < 0.05$. *Significantly different from vehicle control. + Significantly different from the COCi group given a cocaine challenge.

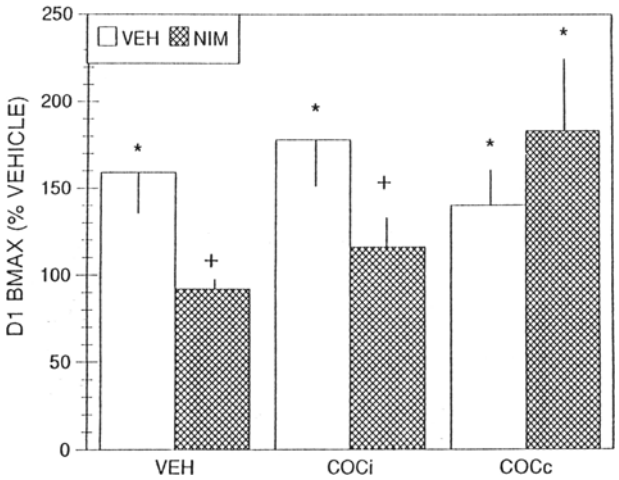

Fig. 5. The density of dopamine D_1 receptors (± SEM) following a cocaine (10 mg/kg) challenge 10 d after withdrawal from a chronic treatment regimen as a percent of the vehicle control group. Rats were chronically treated with vehicle (VEH) or nimodipine (NIM, 10 mg/kg/d) and vehicle, intermittent daily injections of cocaine (COCi), or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg, $N = 14$ for all open bar groups and $N = 8$ for all groups represented by cross-hatched bars. Pairwise comparisons between groups were made with the Multiple F test for $p < 0.05$. *Significantly different from vehicle control. + Significantly different from the COCi group given a cocaine challenge.

and D_2 receptor occupation after a challenge injection of cocaine as compared to the intermittent treatment group. The occupation of receptors in the continuous group tended to be less than the vehicle group challenged with a cocaine injection, but not significantly so. However, prior coadministration of nimodipine had no significant effect on altering the response to cocaine challenge in the continuous cocaine group and, in fact, had a tendency to increase occupation.

Discussion

Intermittent daily treatment of rats with cocaine increased locomotion significantly greater than that observed in rats given continuous infusions of cocaine or vehicle injections. In addition, cotreatment of rats during the 14 d of intermittent cocaine treatment with nimodipine (an L-type calcium channel antagonist) attenuated cocaine-induced locomotion. Both of these effects have been reported previously (Post, 1980; Burger and Martin-Iverson, 1994; Reimer and Martin-Iverson, 1994). The present experiment also determined whether these differences in the stimulant effects of cocaine are associated with differences in the occupation of dopamine D_1 and D_2 receptors by dopamine in the striatum. The density of receptors occupied by dopamine was assessed by measuring the density of receptors after denaturation by the alkylating agent, EEDQ. The rationale is that dopamine bound to receptors during exposure of the receptors to EEDQ would protect those receptors from denatur-

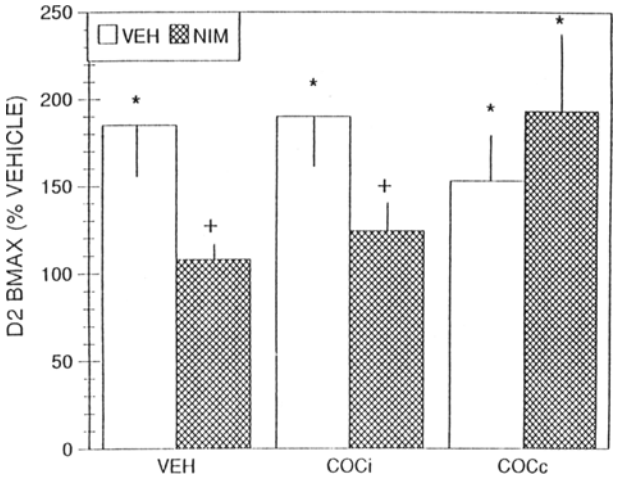

Fig. 6. The density of dopamine D_2 receptors (± SEM) following a cocaine (10 mg/kg) challenge 10 d after withdrawal from a chronic treatment regimen as a percent of the vehicle control group. Rats were chronically treated with vehicle (VEH) or nimodipine (NIM, 10 mg/kg/d) and vehicle, intermittent daily injections of cocaine (COCi), or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg, $N = 14$ in all groups represented by open bars, and $N = 8$ in all groups represented by cross-hatched bars. Pairwise comparisons between groups were made with the Multiple F test for $p < 0.05$. *Significantly different from vehicle control. + Significantly different from the COCi group given a cocaine challenge.

ation by preventing EEDQ from having access to the receptor binding site. Rats receiving intermittent injections of cocaine and exhibiting high levels of locomotion also have an increase in the occupation of both D_1 and D_2 receptor sites (Figs. 2 and 3 and Burger and Martin-Iverson, 1994). However, rats that received the same dosage of cocaine on a daily basis, but via continuous infusions with osmotic minipumps, did not exhibit changes in the occupation of dopamine receptors. Thus, rats exhibiting tolerance to cocaine had no more dopamine occupying receptor sites than those given vehicle.

Nimodipine not only attenuated the behavioral effects of intermittent cocaine, but it also blocked the cocaine-induced increase in occupation of striatal dopamine D_1 and D_2 receptors (Figs. 2 and 3, respectively, and Burger and Martin-Iverson, 1994). This is consistent with a previous report that nimodipine blocks the increase in extracellular dopamine produced by cocaine (Pani et al., 1990). That the decrease in cocaine-induced locomotion produced by either continuous infusions of cocaine or treatment with nimodipine is associated with decreases in the occupation of dopamine receptors increases confidence that this association may be of a causal nature.

Rats that were treated chronically with vehicle, nimodipine and vehicle, or intermittent or continuous cocaine infusions were withdrawn from treatment for 10 d. They then received a challenge injection of cocaine. The intermittent cocaine group exhibited sensitization: higher levels of locomotion than the vehicle group given a challenge. The continuous infusion group did not exhibit differences from the acute cocaine-challenged vehicle group or from a vehicle-challenged control group. Prior treatment with nimodipine had no effect on locomotion in rats that had been withdrawn from nimodipine treatment for 10 d.

The cocaine challenge increased the occupation of both D_1 and D_2 receptors in the vehicle, intermittent cocaine, and continuous cocaine groups (*see* Figs. 5 and 6). The previous history of different treatment regimens did not affect the occupation of D_1 and D_2 receptors. Nimodipine blocked the increase in occupancy in both the vehicle and the intermittent groups. However, it had no significant effects on receptor occupancy when given to rats treated with continuous cocaine, and in fact exhibited a nonsignificant trend toward increasing protection of the receptors from denaturation by EEDQ.

The two different patterns of effects observed pre- and postwithdrawal indicate that the effects on receptor occupancy observed prewithdrawal are likely a function of differences in brain levels of cocaine at the time of EEDQ treatment between the two treatment regimens. If the receptor occupancy was related to behavioral sensitization then a similar pattern of receptor occupancy effects should have been observed pre- and postwithdrawal, since sensitization occurred in the intermittent treated groups under the two con-

ditions. Thus, we observed a dissociation between the behavioral and the receptor occupancy effects of withdrawal from the intermittent treatment regimen.

A second dissociation between the behavior and the receptor occupancy was observed in the nimodipine-treated groups. Prior treatment with nimodipine had no effects on locomotion produced by a challenge dose of cocaine 10 d after cessation of treatment. However, previous nimodipine treatment still reduced the occupation of dopamine receptors in the group with no previous cocaine history and in the group that had a history of intermittent injections of cocaine.

These results indicate that behavioral sensitization is not a function of increased dopamine release in the striatum. This result is similar to some microdialysis studies of amphetamine sensitization (Kuczenski and Segal, 1990; Segal and Kuczenski, 1992a) and for cocaine (Hurd et al., 1989; Segal and Kuczenski, 1992b). There are a number of microdialysis studies that have found increases in extracellular dopamine in the nucleus accumbens (ventral striatum) of rats exhibiting behavioral sensitization to cocaine (Akimoto et al., 1989, 1990; Kalivas and Duffy, 1990; Pettit et al., 1990) and amphetamine or methamphetamine (Robinson et al., 1988; Kazahaya et al., 1989; Akimoto et al., 1990; Patrick et al., 1991). Kalivas and Duffy (1993) may have resolved the reasons for these differences. They found that there was no relation between extracellular dopamine levels in the accumbens and sensitization after a challenge dose of cocaine after short withdrawal periods, but an apparent relation at longer withdrawal periods. Especially relevant to the present experiment is the fact that they did not find significant increases in the area under the curve for dopamine determinations over 120 min after a cocaine challenge 7 or 10 d postwithdrawal. It is clear that Kalivas and Duffy dissociated behavioral sensitization from accumbens dopamine levels in time-course, as we have dissociated sensitization from occupation of striatal dopamine receptors. It remains possible that alterations of dopamine release in other regions, such as the substantia nigra, ventral tegmental area, or extra-accumbens limbic areas, are the critical changes underlying sensitization and tolerance in the locomotor effects of cocaine. This possibility is consistent with previous studies that suggest that behavioral sensitization is associated with dopamine D_1 receptor effects in the dopamine cell body regions (Stewart and Vezina, 1989; Vezina and Stewart, 1990; Vezina, 1993), and with those that indicate that conditioned effects of cocaine alter fos expression in limbic areas other than the nucleus accumbens (Brown et al., 1992).

Summary

In summary, the locomotor stimulant effects of cocaine were found to be associated with changes in the occupancy of dopamine D_1 and D_2 receptors, with a treatment regimen that produces behavioral sensitization increasing occupancy and a regimen that produces tolerance decreasing receptor occupancy. Nimodipine, an L-type calcium channel blocker, decreases both the behavioral and receptor occupancy effects of cocaine. However, a cocaine challenge after a 10-d withdrawal period dissociated the behavioral effects from those on receptor occupancy in the striatum. Behavioral sensitization and tolerance to cocaine were both associated with an increase in the occupation of striatal D_1 and D_2 receptors of a similar magnitude as after a single acute injection of cocaine. If the amount of dopamine interacting with its receptors is important in determining sensitization and tolerance, then it must occur in brain regions other than the striatum.

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