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Presynaptic stimulation and development of locomotor sensitization to the dopamine agonist quinpirole

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Abstract

To assess whether locomotor sensitization induced by repeated injections of the dopamine agonist quinpirole reflects tolerance of the drug's presynaptic depressive effects on locomotion, independent groups of rats were treated chronically (every 3 days × 10) with either a presynaptic dose of quinpirole (0.025 mg/kg; $n=27$), a postsynaptic dose (0.5 mg/kg; $n=27$), or saline ($n=26$). Following chronic treatment, a full dose–response profile was determined to assess the presence of sensitization. Results indicated that treatment with the postsynaptic, but not the presynaptic, dose of quinpirole induced locomotor sensitization. Moreover, chronic treatment with low-dose quinpirole did not yield tolerance of the drug's depressive effects. It is suggested that presynaptic dopamine receptors may require extensive spatial and/or temporal summation to become tolerant.

Keywords: Presynaptic; Locomotor sensitization; Quinpirole

1. Introduction

Chronic administration of dopamine (DA) stimulant drugs, such as the D2/D3 receptor agonist quinpirole, results in behavioral sensitization (Szechtman et al., 1994a,b), characterized by a progressive augmentation in the motor response to the drug (Robinson and Becker, 1986). Behavioral sensitization to a variety of dopaminergic stimulants has been the focus of much study, given the potential relevance of its underlying mechanisms to several psychopathological states in humans, ranging from drug addiction to schizophrenia (Kalivas and Stewart, 1991; Koob and Le Moal, 1997; Robinson and Becker, 1986; Segal and Schuckit, 1983) and obsessive–compulsive disorder (Szechtman et al., 1998, 1999, 2001).

The acute effects of dopamine agonists on behavior are generally biphasic (Harkin et al., 2000; Kelsey and Carlezon, 2002; Van Hartesveldt, 1997; Van Hartesveldt et al., 1992). For instance, the effects of quinpirole on locomotion are biphasic across dose and time (Eilam and Szechtman, 1989). Across dose, doses less than 0.1 mg/kg of quinpirole produce locomotor inhibition while higher doses induce locomotor excitation. Similarly, across time, low doses of quinpirole have a depressive effect on locomotion but with higher doses, the initial inhibitory effects are followed by locomotor excitation in the second hour after drug administration (Eilam and Szechtman, 1989). The depressive effects of quinpirole, as of other dopamine agonists, probably reflect stimulation of presynaptic dopamine receptors (Eilam and Szechtman, 1989; Richtand et al., 2001), although such effects likely depend on the state of the animal's habituation to the test environment (Van Hartesveldt, 1997). Because dopamine agonists have both such depressive and excitatory effects, it has been hypothesized that sensitization reflects tolerance of the drug depressive effects (Baker and Tiffany, 1985; Hinson and Siegel, 1983), possibly due to tolerance or desensitization of presynaptic dopamine receptors induced by chronic exposure to the agonist (Antelman and Chiodo, 1983; Castro et al., 1985; Muller and Seeman, 1979; Richtand et al., 2001).

The present study uses a pharmacological approach to examine the hypothesis that chronic stimulation with the dopamine agonist quinpirole induces tolerance of the drug's presynaptic depressive effects and thereby yields locomotor

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sensitization. We reasoned that if the hypothesis is valid, chronic treatment with a presynaptic dose of quinpirole would induce tolerance of the drug's depressive effects and yield a dose–response profile characteristic of rats sensitized to a high dose of quinpirole. Contrary to such predictions, chronic treatment with low-dose quinpirole did not yield tolerance of the drug's depressive effects on locomotion nor did it induce a sensitized dose–response profile.

2. Materials and methods

2.1. Subjects

Eighty experimentally naive male Long–Evans rats (Charles River, Canada), weighing 250–300 g at the start of the experiment, were used. Rats were housed individually in polyethylene cages (35 × 30 × 16 cm) lined with beta-chip bedding in a temperature controlled (22 °C) colony room, maintained on a 12-h light–dark cycle (lights on at 0700 h), and with free access to food and water. Rats were allowed to acclimatize to the colony room for 1 week following arrival and were handled for 2 min daily for 5 days in the week before the start of the experiment. All treatments were performed during the light phase of the day–night cycle. Animals were housed and tested in compliance with the guidelines described in the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

2.2. Drugs

Quinpirole hydrochloride (Sigma Aldrich) was dissolved in physiological saline. All doses of the drug were administered subcutaneously under the nape of the neck at a volume of 1.0 ml/kg. Equivalent volumes of saline were used for nondrug injections. Animals received all injections in the testing environment.

2.3. Apparatus

The testing environment was a noncolony room containing 10 empty Plexiglas activity chambers (40 × 40 × 35 cm). These were interfaced to a Digiscan 16 monitor and a computer that provided automated recording of locomotor activity using VersaMax software (AccuScan Instruments, Columbus, OH). Ventilated Plexiglas lids were used to cover the activity chambers to prevent animals from escaping.

2.4. Design and procedure

To compare the effects of chronic treatment with pre- and postsynaptic doses of quinpirole on locomotor sensitization, three independent groups of rats were tested in a repeated measure design. The independent factor was chronic drug treatment, where one group of rats ($n = 27$) was treated chronically with a presynaptic dose of quinpirole (0.025 mg/kg), another group ($n = 27$) with a postsynaptic dose (0.5 mg/kg), and the control group ($n = 26$) received chronic injections of saline. The repeated measures factor was test dose of quinpirole and had nine levels (0, 0.01, 0.04, 0.05, 0.06, 0.07, 0.08, 0.2 and 1 mg/kg). Each subject received three of these doses in random order. The doses were selected as to sample the sensitized dose–response curve described previously (Szumlinski et al., 1997), taking into account the steep slope of it in the dose range from 0.04 to 0.08 mg/kg of quinpirole.

The experiment consisted of two phases: a chronic drug treatment phase to induce locomotor sensitization, and a test phase in which the effects of chronic treatment on the dose–response profile to quinpirole was examined. In Phase 1, rats received chronic injections of either the presynaptic (0.025 mg/kg) or the postsynaptic (0.5 mg/kg) dose of quinpirole or injections of saline, every 3 days for a total of 10 injections. The 0.5-mg/kg dose of quinpirole was selected because it is considered to exert a postsynaptic effect and is representative of the behavioral activation induced by quinpirole in doses ranging from 0.25 to 2.5 mg/kg; the 0.025-mg/kg dose was chosen because it is considered to stimulate predominantly presynaptic dopamine receptors as evidenced by the induction of behavioral inhibition (Eilam and Szechtman, 1989; Szechtman et al., 1994b). The injection regimen was chosen based on the findings that the effects of chronic treatment with quinpirole reach a plateau after 8 to 10 drug injections administered 2 to 8 days apart (Szechtman et al., 1994a,b). In Phase 2, rats received three additional injections according to the same schedule as in Phase 1 except that now they were administered one of the nine test doses of quinpirole. Doses were distributed according to a randomized block design where animals from each of the three chronic treatment groups received an equivalent number of the same doses over each test injection.

Prior to each injection, animals were removed from their home cages and weighed on a scale located in the colony room. Following the weighing, they were placed back in the home cages and moved on a cart to the noncolony experiment room located next door, where each rat was taken out of its cage, placed on a towel resting flat on a cart, and injected. Immediately following injection, each rat was placed inside the activity chamber, and locomotor distance was measured for 90 min. Each animal was always tested at the same time of day and in the same activity chamber. After each use, activity chambers were thoroughly cleaned with Windex diluted with water.

2.5. Data analysis

The dependent variable analyzed in this study was distance travelled by the rat in the activity chamber. The

Table 1
Estimate of parameters for the curve of locomotor response versus QNP dose

Parameter[a]	Chronic treatment group		
	Saline	Presynaptic QNP dose	Postsynaptic QNP dose
ED_{50}	0.077 ± 0.006	0.082 ± 0.10	0.084 ± 0.003
R_{max}	161.0 ± 10.6	121.0 ± 9.2[b]	474.3 ± 10.3[c]
n	5.2 ± .2.1	4.7 ± 2.6	4.2 ± 0.6
r^2	.945	.927	.996

[a] Equation (see Materials and methods) fitted to data shown in Fig. 3. ED_{50} is the QNP dose (in mg/kg) with the half-maximal response, R_{max} is the maximal response (in meters travelled), n is a parameter describing the sigmoidicity of the curve, and r^2 indicates the square of the correlation coefficient between raw and fitted data. Standard error refers to the standard error of the estimate of the parameter; the estimate of each parameter is statistically significant except for n in the presynaptic QNP dose group, where $P<.10$.

[b] Different from: saline, $t(10)=2.018$, $P<.05$.

[c] Different from: saline, $t(10)=14.9$, $P<.001$; and, presynaptic QNP dose, $t(10)=18.1$, $P<.001$.

statistical significance of Phase 1 data was evaluated using a two-way ANOVA, with one between-subjects factor (chronic drug treatment group: saline, presynaptic quinpirole dose, postsynaptic quinpirole dose) and one within-subjects injection factor with 10 levels (injection 1–10), and Duncan multiple range post hoc tests as appropriate. Computations were performed using the SPSS/PC+ statistical package. Statistical criteria for significant differences were set at $P<.05$. Data are plotted as means ± S.E.M.

To describe the dose–effect function obtained in the test for sensitization (Phase 2), the parameters providing the best fit for the following asymmetric sigmoid equation were estimated using a nonlinear curve-fitting algorithm (Fig. P Version 2.98, Fig. P Software Corporation, Hamilton, ON):

$$R = \frac{R_{max} \times D^n}{D^n + ED_{50}^n}$$

where R is the locomotor response at quinpirole dose D, and the estimated parameters are the maximal response at an infinite quinpirole dose (R_{max}), the quinpirole dose yielding the half-maximum response (ED_{50}) and a coefficient (n) representing sigmoidicity. In computing R, the lowest response was set to zero. The equation is a function describing linear dose versus effect relationship. ED_{50} and R_{max} are taken as estimates of drug's potency and efficacy, respectively (Szumlinski et al., 1997). Parameter comparisons were performed using two-tailed t tests uncorrected for multiple comparisons (Table 1).

3. Results

3.1. Change across injections

As expected, repeated injections with the 0.5-mg/kg dose of quinpirole induced a progressive increase in the locomotor response to the drug (Fig. 1). However, the depressive effect on locomotion induced by a low dose of quinpirole did not tolerate but persisted during the course of 10 quinpirole injections (Fig. 1), as evidenced by a significant difference across injections between the low dose and saline groups [$F(1,51)=31.4$, $P<.001$] and the absence of a main effect of injection in the low-dose group.

Fig. 2 shows that during the course of drug action the acute effect of 0.5 mg/kg of quinpirole on locomotor activity was biphasic, with the initial depression of locomotion followed by locomotor excitation (Eilam and Szechtman, 1989). However, as reported previously (Szechtman et al., 1994b), with repeated injections of this dose, the excitatory phase increased in magnitude and advanced forward towards the onset of drug action to replace the inhibitory effects of the drug, except for the inhibition still apparent in the first 5 min. In contrast, the time course of the depressive effects of low-dose quinpirole did not appear to change across injections, but a floor effect may have obscured the apparent trend towards even quicker inhibition.

3.2. Dose–response profile

As shown in Fig. 3, the maximal locomotor response to quinpirole was 3- to 4-fold higher in rats treated chronically with the postsynaptic dose of quinpirole than in saline controls or animals treated chronically with the low dose of the drug, respectively. This elevation in the maximal response was statistically significant (Table 1), indicating the presence of locomotor sensitization. However, the maximal response in rats treated chronically with the low dose of quinpirole was significantly lower than the response to acute doses of quinpirole (Table 1). This suggests that chronic low-dose treatment did not result in locomotor

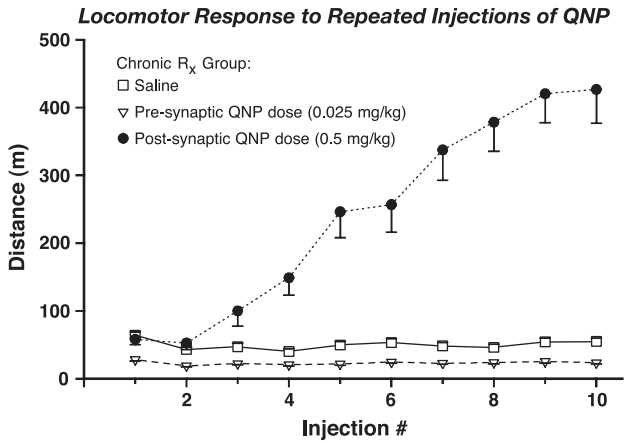

Fig. 1. The effect of chronic treatment with pre- and postsynaptic doses of quinpirole on the development of locomotor sensitization. Symbols represent the mean value (and S.E.M.) of the distance travelled in 90 min following the indicated number of quinpirole or saline injections. If error bars are not visible, they are smaller than the graph symbol. $n=26–27$/group.

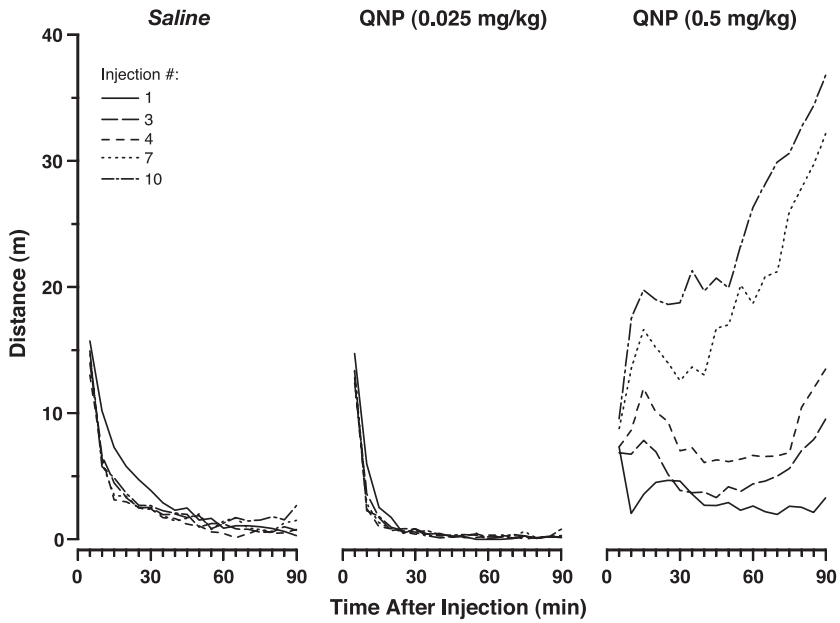

Fig. 2. Time course of locomotor response to presynaptic (0.025 mg/kg) and postsynaptic (0.5 mg/kg) doses of quinpriole as a function of repeated injections of the drug. Same animals as in Fig. 1. For clarity, only selected injections are shown. Each point represents the mean distance travelled in a 5-min interval ending at the indicated time.

sensitization to the excitatory doses of quinpirole. Moreover, it suggests that rather than the expected tolerance of the depressive effects of quinpirole, there may have been a potentiation of the drug's inhibitory effects (Fig. 3).

Chronic treatment with either the high or the low dose of quinpirole did not change the potency of quinpirole to induce locomotor activity (see Fig. 3 and Table 1), suggesting that sensitization does not require a change in drug potency.

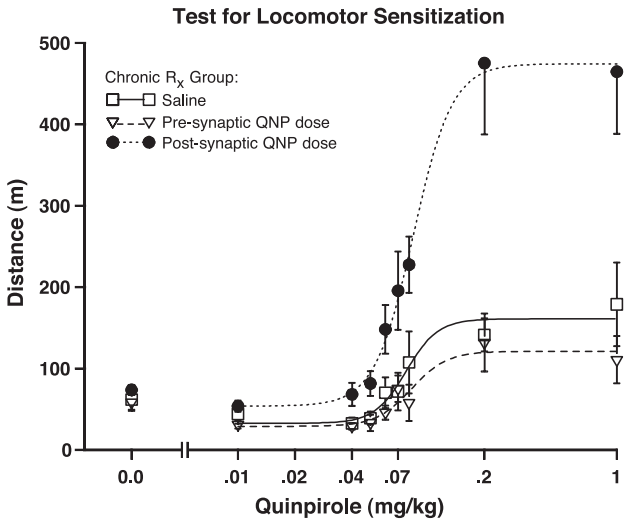

Fig. 3. Locomotor response to various test doses of quinpirole in rats treated chronically (every 3 days × 10) with either a presynaptic (0.025 mg/kg) or a postsynaptic (0.5 mg/kg) dose of quinpirole or saline. Symbols represent the mean value (and S.E.M.) of the distance travelled in 90 min. For each dose, $n = 8–9$; every rat received in random order three of the nine test doses. Lines show the best fit estimate of the equation indicated in the Materials and methods section, with parameters given in Table 1.

4. Discussion

The present study shows that while chronic treatment with a postsynaptic dose of quinpirole induces locomotor sensitization, a similar regimen of treatment with a presynaptic dose of the drug does not lead to sensitization of the locomotor response. At first glance, this finding would seem to contradict the hypothesis that development of sensitization to dopamine agonists reflects a tolerance of the depressive effects induced by those drugs, an effect thought to be mediated by stimulation of presynaptic dopamine receptors. However, as elaborated below, the results of the present findings do not necessarily invalidate the hypothesis but instead suggest that it may need to be modified.

The major shortcoming of the present results in terms of testing the hypothesis that sensitization reflects tolerance of the presynaptic depressive effects of dopamine agonists is the failure to observe such a tolerance with chronic administration of low doses of quinpirole. Thus, the absence of locomotor sensitization to quinpirole in the context of an absence of tolerance of the drug's depressive effects is not revealing in terms of a cause–effect relationship between tolerance and sensitization. Similarly, the fact that chronic treatment with the postsynaptic dose of quinpirole induced locomotor sensitization and provided evidence suggestive of tolerance to the drug's depressive effects is consistent with the hypothesis, but does not constitute a test of it. Thus, the test of the sensitization hypothesis by the present study

rested on the assumption that chronic treatment with low-dose quinpirole would result in tolerance, but surprisingly, no tolerance developed.

The expectation that tolerance to low-dose quinpirole should develop was reasonable. Although we are not aware of studies examining the effects of chronic treatment with low doses of quinpirole, several reports indicate tolerance to repeated injections of presynaptic doses of other dopamine agonists (Allikmets and Vasar, 1982; Dourish and Cooper, 1981). For instance, the effects of low-dose apomorphine on body temperature show tolerance (Szechtman et al., 1988). Thus, the failure to observe tolerance of the depressive effects of quinpirole is not likely due to the use of an inadequate dose of the drug, a suggestion consistent with the observed persistence of locomotor inhibition during the course of chronic low-dose treatment.

Conceivably, presynaptic dopamine receptors may be particularly resistant to develop tolerance considering their vital role in regulating dopamine release in the presence of continuous exposure to dopamine and their high sensitivity to dopamine compared with postsynaptic receptors (Chiodo, 1988). However, such a resistance (if it exists) is not likely to be absolute because rats treated chronically with the higher dose of quinpirole do seem to develop tolerance to the depressive effects of quinpirole. Two pieces of evidence are consistent with the development of such a tolerance: (1) the gradual reduction in the inhibitory phase during the course of drug action (see Fig. 2); and, (2) the switch in the locomotor response to low doses of quinpirole from inhibition in naive rats to excitation in sensitized animals (Fig. 3). To the extent that tolerance of presynaptic receptors requires high-dose stimulation, this would suggest that for tolerance to develop, these receptors depend on extensive temporal and/or spatial summation. That is to say, these receptors may require integration of drug kinetics across a wide time interval and throughout the synapse to reach the threshold for desensitization. Processes of such kind were recently suggested to be involved in the molecular mechanism of tolerance of thymocytes to ligands of differing affinities (Werlen et al., 2003).

Interestingly, unlike with low doses of quinpirole, chronic treatment with low doses of apomorphine may result in locomotor sensitization even in the absence of tolerance to the depressive effects of the drug (Mattingly et al., 1988). Considering that apomorphine stimulates both the D1 and the D2 family of dopamine receptors, and quinpirole stimulates only the D2-type receptors, it may be that the sensitization to apomorphine is more dependent on tolerance of D1 receptors than is the sensitization to the D2/D3 agonist quinpirole. Such a suggestion is consistent with the observation that D1-type, but not D2-type, dopamine antagonists blocked the development of behavioral sensitization to apomorphine (Mattingly et al., 1991), but is made less compelling by findings of a similar D1 blockade of sensitization to quinpirole (Mattingly et al., 1993; Rowlett et al., 1995). In a similar vein, our data are equally ambiguous regarding the hypothesis that sensitization involves tolerance of the locomotor depressive effects induced by stimulation of D3 receptors (Richtand et al., 2001) as low (but not high) doses of quinpirole may be insufficient to induce D3 receptor desensitization.

In summary, the hypothesis that development of locomotor sensitization to dopamine agonists reflects tolerance of the depressive effects mediated by presynaptic dopamine receptors is likely in need of refinement. The present study shows that tolerance to presynaptic doses of quinpirole does not develop, and neither does locomotor sensitization as indexed by excitatory doses of quinpirole. However, because chronic treatment with postsynaptic doses of quinpirole induces locomotor sensitization, which may be accompanied by a tolerance of the drug's presynaptic effects, it is suggested that presynaptic dopamine receptors may require extensive spatial and/or temporal summation to become tolerant.

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