Insulin Replacement Restores the Behavioral Effects of Quinpirole and Raclopride in Streptozotocin-Treated Rats

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ABSTRACT

Streptozotocin (STZ)-induced diabetes can modulate dopamine (DA) neurotransmission and thereby modify the behavioral effects of drugs acting on DA systems. Insulin replacement, and in some conditions repeated treatment with amphetamine, can partially restore sensitivity of STZ-treated rats to dopaminergic drugs. The present study sought to characterize the role of insulin and amphetamine in modulating the behavioral effects of drugs that selectively act on D2/D3 receptors. In control rats, quinpirole and quinelorane produced yawning, whereas raclopride and γ-hydroxybutyric acid (GHB) produced catalepsy. Raclopride antagonized quinpirole- and quinelorane-induced yawning with similar potency. STZ treatment increased blood glucose concentration, decreased body weight, and markedly reduced sensitivity to quinpirole-induced yawning, quinelorane-induced yawning as well as to raclopride-induced catalepsy, while enhancing sensitivity to GHB-induced catalepsy. Repeated treatment with amphetamine partially restored sensitivity of STZ-treated rats to amphetamine-stimulated locomotion and also produced conditioned place preference, without affecting blood glucose and body weight changes. However, amphetamine treatment did not restore sensitivity to the behavioral effects of quinpirole, raclopride, or GHB, suggesting differential regulation of dopamine transporter activity and sensitivity of D2 receptors in hypoinsulinemic rats. Insulin replacement in STZ-treated rats normalized blood glucose and body weight changes and fully restored sensitivity to quinpirole-induced yawning, as well as to raclopride-induced catalepsy, while reducing sensitivity to GHB-induced catalepsy. Overall, these data indicate that changes in insulin status markedly affect sensitivity to the behavioral effects of dopaminergic drugs. The results underscore the importance of insulin in modulating DA neurotransmission; these effects might be especially relevant to understanding the co-morbidity of eating disorders and substance abuse.

Several drugs of abuse (e.g., amphetamine and cocaine) and some drugs that are used in the clinic (e.g., haloperidol and bromocriptine) are thought to act predominantly on dopamine (DA) systems. Activity at DA D2 receptors can modulate DA neurotransmission by affecting DA synthesis, release, uptake, or neuronal activity (Zahniser and Doolen, 2001). Importantly, insulin has been shown to regulate DA signaling in the brain (Figlewicz et al., 1994, 1996). Insulin can cross the blood-brain barrier and act on receptors (i.e., insulin receptors, insulin-like growth factor-1 receptors) that are densely concentrated in the basal ganglia, a region richly expressing D2/D3 receptors and DA transporters (DATs) (Ciliax et al., 1995; Larson and Ariano, 1995; Schulingkamp et al., 2000; Figlewicz et al., 2003). The proximity of insulin and DA systems seems to have functional consequences. For example, rats with decreased circulating insulin showed decreased coupling of DA D2 receptors to G_iα proteins (Abbracchio et al., 1989) and reduced DAT activity (Owens et al., 2005) in the striatum. Food-deprived (i.e., hypoinsulinemic) rats also showed reduced DAT mRNA in the ventral tegmental area/substantia nigra and decreased DAT activity in the striatum (Patterson et al., 1998). Moreover, drug [alloxan or streptozotocin (STZ)]-induced hypoinsulinemia can alter [increase (Lozovsky et al., 1981; Trulson and Himmel, 1983; Serri et al., 1985) or decrease (Rowland et al., 1985)] the density of striatal D2 receptors and impair D2 receptor-coupled signal transduction (Abbracchio et al., 1989). Finally, hypoinsulinemic rats showed decreased synthesis (Kono and Takada, 1994), uptake (Owens et al., 2005), and turnover (Kwok and Juorio, 1986; Lim et al., 1994) of DA in the striatum. Thus, it is clear that changes in plasma insulin and glucose can have profound effects on DA neurotransmission. Changes in insulin status also can modify the behavioral

ABBREVIATIONS: DA, dopamine; DAT, dopamine transporter; STZ, streptozotocin; CPP, conditioned place preference; GHB, γ-hydroxybutyrate; ANOVA, analysis of variance.
effects of dopaminergic drugs. For example, STZ-treated rats were less sensitive to the effects of apomorphine (a direct-acting DA agonist) and amphetamine (an indirect-acting DA agonist) on locomotor activity and also to the positive reinforcing effects of amphetamine (Marshall, 1978; Rowland et al., 1985; Galici et al., 2003). Moreover, the cataleptic effects of the DA receptor antagonist haloperidol were markedly reduced in STZ-treated rats (Sevak et al., 2005). Food restriction, a condition that can alter insulin and glucose status, can enhance oral as well as i.v. drug intake (Carroll et al., 1981; Carroll and Stotz, 1983) and potentiate amphetamine-induced hyperactivity (Campbell and Fibiger, 1971). Thus, changes in plasma insulin that modulate DA neurotransmission can also modify the behavioral effects of drugs acting on DA systems.

Despite a growing literature on the role of insulin signaling in regulating DA neurotransmission, little is known regarding the effects of altered insulin status on the behavioral effects of direct-acting DA D2/D3 receptor agonists and antagonists. It is well established that direct-acting DA receptor agonists can produce yawning (Kurashima et al., 1995; Collins et al., 2005) and that direct-acting DA receptor antagonists can produce catalepsy (Kanes et al., 1993; Sevak et al., 2004). These two behavioral endpoints were used to examine changes in sensitivity to the behavioral actions of direct-acting DA drugs and also in drug combination studies with raclopride to confirm the role of DA receptors in the yawning produced by quinpirole and quinolnolane. The pharmacological selectivity of changes in sensitivity to the behavioral effects of direct-acting DA drugs was examined by comparing those effects to the effects obtained with an indirect-acting DA agonist (amphetamine on locomotion and conditioned place preference) and to the effects obtained with a drug [3-hydroxybutyric acid (GHB)] that exerts cataleptic effects through a non-DA (GABAA receptors) mechanism (Carter et al., 2005). It was hypothesized that decreased circulating insulin (after STZ) would decrease sensitivity to the behavioral effects of drugs acting directly on DA receptors and that insulin replacement would restore sensitivity to those drugs.

DAT activity can affect DA D2 receptor function (Jones et al., 1999). For example, activation of D2/D3 receptors by quinpirole can reduce DA synthesis and release neuronal firing, and mice lacking DAT are less sensitive to the effects of quinpirole (Jones et al., 1999). Because repeated treatment with amphetamine can normalize DAT activity in STZ-treated rats (Owens et al., 2005), the present study also examined whether repeated treatment with amphetamine restores sensitivity of STZ-treated rats to the behavioral effects of drugs acting directly at DA receptors.

**Materials and Methods**

**Animals**

One hundred and twenty-one male rats (Harlan, Indianapolis, IN), weighing 300 to 350 g, were individually housed in an environmentally controlled room (24 ± 1°C, 50 ± 10% relative humidity) under a 12-h light/dark cycle with food and water available continuously. Thirty-six rats were rendered diabetic by an i.p. injection of 50 mg/kg STZ. Behavioral experiments began 1 week after the administration of STZ (i.e., on day 1), 1 week after the administration of STZ (on day 8), immediately after the conditioned place preference (CPP) test (on day 17), immediately before insertion of the insulin pellets (Linplant; LinShin Canada Inc., Toronto, Canada; day 28), and every other day thereafter until completion of the study. Animals were maintained and experiments were conducted in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio, and with the 1996 Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, the National Research Council, and the National Academy of Sciences).

**Procedures**

**Yawning.** Yawning was defined as opening of the mouth such that the lower incisors were completely visible followed by closure, in not more than 3 s. A time course for yawnning produced by the D2/D3 receptor agonists quinpirole and quinelorane was obtained in two separate groups of 10 rats each. All 10 rats received each dose of either quinpirole or quinelorane according to a Latin-square design. Yawning was examined for 60 min, in 10-min bins, immediately after an i.p. injection of quinpirole or quinelorane.

Based on the data obtained from the time-course study, subsequently, yawnning was observed for 10 min, beginning 20 min after the i.p. administration of quinpirole or quinelorane. Forty-eight rats were divided into two groups of 24 rats each. One group of 24 rats was used to establish dose-response curves for quinpirole-induced yawnning (0.0178, 0.056, 0.178, 0.56, 1.78, and 5.6 mg/kg i.p.) in the presence and absence of raclopride. The other group of 24 rats was used to establish dose-response curves for quinelorane-induced yawnning (0.001, 0.0032, 0.01, 0.032, 0.1, 0.32, and 1.0 mg/kg i.p.) in the presence and absence of raclopride. For each test, eight rats were randomly selected from the pool of 24 rats. Raclopride (0.056, 0.1, and 0.178 mg/kg s.c.) was administered 30 min before the injection of quinpirole or quinelorane.

Twenty-four experimentally naive rats were treated with STZ and divided into two groups of 12 rats each. One group of 12 rats was used to assess quinpirole-induced yawnning (0.0178, 0.056, and 0.178 mg/kg), and the other 12 rats were used to assess quinelorane-induced yawnning (0.001, 0.0032, and 0.01 mg/kg). For each dose of an agonist, six rats were randomly selected from the pool of 12 rats. All rats were tested two to three times per week with at least 48 h between tests. The order in which the doses were tested was randomized among rats.

**Catalepsy.** Catalepsy was studied using a bar test, whereby the forelimbs were placed on a horizontal, cylindrical metal bar (diameter, 1.0 cm; height, 10 cm), and the time until both forelimbs touched the table surface was recorded, up to a maximum of 120 s (Sevak et al., 2004). Raclopride-induced catalepsy was studied for six consecutive 25-min cycles, whereas GHB-induced catalepsy was studied for seven consecutive 25-min cycles. At the beginning of the first cycle, 1 ml/kg saline was administered, forelimbs were placed on the bar, and the time until both forelimbs touched the table surface was recorded. At the beginning of the second and each of the remaining cycles, cumulative doses of raclopride (0.0178, 0.056, 0.178, 0.56, and 1.78 mg/kg) or GHB (56, 100, 178, 320, 560, and 1000 mg/kg i.p.) were administered with catalepsy assessed 25 min after each injection. A group of eight otherwise untreated rats was used to study catalepsy produced by raclopride as well as GHB. Finally, the same 12 STZ-treated rats that were used for studying quinpirole-induced yawning were also used to assess catalepsy produced by raclopride as well as GHB.

**Locomotion and CPP.** Separate groups of experimentally naive rats were given a single injection of 50 mg/kg i.p. STZ (n = 12) or saline (n = 9) and used to study locomotion and CPP, according to the methods described previously (Bormann and Cunningham, 1998). Locomotor activity and CPP were studied using Plexiglas chambers measuring 26 × 61 × 23 cm in height and located within sound-attenuating cubicles. The floors of the chambers were removable and
varied in texture across conditions. For half of the rats, the drug was paired with the hole floor-texture (6 mm in diameter, 9 mm center-to-center) and the vehicle with the grid floor-texture (6 × 6-mm wire mesh supported by 5-mm-diameter metal rods spaced 16 mm apart); pairings were opposite for the remaining rats. On test days, half (26 × 30.5 cm) of the floor was the hole texture and half was the grid texture. Horizontal activity and location in the chamber were measured with four pairs of infrared photobeams.

Conditioning sessions were preceded by a single 30-min habituation session (during which the floor was covered with a sheet of paper). For the next eight daily 30-min sessions, rats received either amphetamine (1.78 mg/kg i.p.) or saline (1 ml/kg i.p.) and were placed in a chamber equipped with a floor made up of only one texture (hole or grid, alternating across days and specifically paired with either drug or vehicle). A dose of 1.78 mg/kg amphetamine was chosen because it has been shown that four every-other-day injections of 1.78 mg/kg amphetamine can normalize DA clearance in STZ-treated rats (Owens et al., 2005). The CPP test was performed on the day after the last (8th) conditioning trial, when all rats received an i.p. saline injection and were placed in the chamber with a floor of both textures, and the time spent on the floor areas with the amphetamine- and the saline-paired textures was recorded for 30 min.

**Effects of Repeated Treatment with Amphetamine and of Insulin Replacement on Quinpirole-Induced Yawning and Raclopride- and GHB-Induced Catalepsy.** The same 12 STZ-treatment rats that were used for studying amphetamine-induced locomotion and CPP were used to evaluate the behavioral effects of quinpirole, raclopride, and GHB. The day after the CPP test (i.e., 18 days after the administration of STZ) as well as 2 and 4 days thereafter (i.e., 20 and 22 days after STZ), quinpirole-induced (0.0178, 0.056, and 0.178 mg/kg) yawning was studied in the 12 STZ-treated rats (four different STZ-treated rats for each dose of quinpirole). These rats also were tested for raclopride- and GHB-induced catalepsy 2 and 4 days thereafter (i.e., 24 and 26 days after STZ).

On day 28, two insulin pellets (i.e., Linplant) were surgically implanted (s.c.) in each rat under ketamine (36 mg/kg i.m.) and xylazine (4.8 mg/kg i.m.) anesthesia. Ten days after the insertion of Linplant (i.e., 38 days after STZ administration) as well as 2 and 4 days thereafter (i.e., 40 and 42 days after STZ), quinpirole-induced (0.0178, 0.056, and 0.178 mg/kg) yawning was studied in the 12 STZ-treated rats (four different STZ-treated rats for each dose of quinpirole). These rats were further tested for raclopride- and GHB-induced catalepsy 2 and 4 days thereafter (i.e., 44 and 46 days after STZ).

**Drugs**

d-Amphetamine hydrochloride was provided by the Research Technology Branch, National Institute of Drug Abuse (Rockville, MD). STZ, raclopride tartrate, quinpirole dihydrochloride, quinelorane dihydrochloride, and GHB sodium were purchased from Sigma-Aldrich (St. Louis, MO). Linplant pellets (i.e., sustained-release insulin implants) were purchased from LinShin Canada Inc. According to the manufacturer, each Linplant releases 2 U of insulin over 24 h for at least 40 days (Wang, 1991). Ketamine hydrochloride and xylazine hydrochloride were purchased from Vetus Animal Health, Burns Veterinary Supply Inc. (Westbury, NY). Amphetamine, STZ, raclopride, quinpirole, quinelorane, and GHB were dissolved in sterile 0.9% saline. STZ solutions were prepared immediately before administration. Amphetamine, STZ, quinpirole, quinelorane, and GHB were administered i.p. in a volume of 1 ml/kg. Raclopride was administered s.c. in a volume of 1 ml/kg. The combined solution of ketamine and xylazine was administered i.m. in a volume of 0.6 ml/kg.

**Data Analyses**

The blood glucose concentrations and body weights of 12 STZ-treated rats (which were subsequently treated with amphetamine and insulin) were analyzed separately using a one-factor analyses of variance (ANOVA) with days as the factor, followed by Tukey-Kramer tests. A dose of quinpirole and quinelorane that produced half of the maximum number of yawns (i.e., ED$_{50}$) was calculated for each of six time-bins using linear regression. The time-bin in which quinpirole and quinelorane were the most potent (i.e., in which the ED$_{50}$ values were the smallest) was used for subsequent experiments.

Differences among the dose-response curves for yawning produced by quinpirole and quinelorane, administered alone and in combination with raclopride, were analyzed by simultaneously fitting straight lines to the linear portion of the dose-response curves (GraphPad Prism version 4.02 for Windows; GraphPad Software Inc., San Diego, CA), using the following equation: $y = 	ext{slope} \times \log(x) + y_0$ + intercept. Likewise, differences among the dose-response curves for catalepsy produced by GHB in control rats, in STZ-treated rats, and in STZ-treated rats that received amphetamine and insulin, were analyzed by simultaneously fitting straight lines to the linear portion of the dose-response curves using the equation. Simpler models were compared with more complex models by means of an F-ratio test using the parameters of common slope and intercept. If the calculated F for two models was statistically significant, the more complex model was used to fit the data; otherwise, the simpler model was used.

A common slope, a common maximum, and different intercepts were used to estimate ED$_{50}$ values for quinpirole and quinelorane in best-fitting models. Ratios of ED$_{50}$ values of quinpirole or quinelorane in the presence of three different doses of raclopride to the ED$_{50}$ values in the absence of antagonist were calculated and used to construct Schild regression plots according to methods described by Arunlakshana and Schild (1959). To test whether raclopride antagonized the ascending and descending portions of the dose-response curves for quinpirole and quinelorane with similar potency, it was determined whether the Schild regressions for these data could be fitted with a single line with a common slope and a common $y_0$ value.

Locomotor activity was analyzed by a three-factor ANOVA with group (STZ-treated and saline-treated), treatment (amphetamine and saline), and conditioning trial (1–4) as factors. Place preference was considered significant when the 95% confidence limits around the mean difference (minutes) between the time spent on the amphetamine-paired floor-texture and the time spent on the saline-paired floor-texture did not include zero.

Dose-response curves for yawning produced by quinpirole or quinelorane were separately analyzed using a two-factor ANOVA, with group (control and STZ-treated) and dose (0.0178, 0.056, and 0.178 mg/kg quinpirole or 0.001, 0.0032, and 0.01 mg/kg quinelorane) as factors. Effects of repeated treatment with amphetamine on quinpirole-induced yawning were analyzed using a two-factor ANOVA, with group (control, STZ-treated, and STZ-treated rats that received amphetamine) and dose as factors. Data for quinpirole-induced yawning on days 20, 22, 40, and 42 after the administration of STZ in amphetamine-treated rats were not included in the analyses. To determine any effects of insulin replacement on quinpirole-induced yawning, dose-response curves for quinpirole were analyzed using a two-factor ANOVA, with group (control, STZ-treated, and STZ-treated rats with insulin replacement) and dose as factors.

Dose-response curves for raclopride-induced catalepsy were analyzed using a two-factor ANOVA, with group (control and STZ-treated) and dose (0.0178, 0.056, 0.178, 0.56, and 1.78 mg/kg) as factors. Effects of repeated treatment with amphetamine on raclopride-induced catalepsy were analyzed using a two-factor ANOVA, with group (control, STZ-treated, and STZ-treated rats that received amphetamine) and dose as factors. To determine any effects of insu-
Results

Insulin Normalized Blood Glucose and Body Weight Changes in STZ-Treated Rats. Statistical analyses of the data for blood glucose concentration and body weight in 12 STZ-treated rats that were subsequently treated with amphetamine and insulin showed a significant main effect of days (F_{13,143} = 87.7; p < 0.001 and F_{13,143} = 221.5; p < 0.001, respectively). In these 12 rats, blood glucose concentration remained significantly elevated on days 8, 17, and 28 after the administration of STZ, compared with that measured before STZ treatment on day 1 (Fig. 1, top). There was no significant difference among blood glucose concentrations measured 8, 17, and 28 days after STZ treatment, indicating that treatment with amphetamine (days 9–16) and other drugs (quinpirole, raclopride, GHB; days 18–28) did not affect blood glucose concentration. Body weight was significantly decreased 8 days after STZ treatment and further decreased over 28 days (Fig. 1, bottom) regardless of drug treatment (i.e., amphetamine, quinpirole, raclopride, and GHB). After insulin replacement on day 28, a progressive decrease in blood glucose and an increase in body weight were evident over days, such that 10 days after the Linplant insertion (i.e., 38 days after the injection of STZ), mean blood glucose concentration (139.6 ± 19.8 mg/dl) and body weight (348.9 ± 5.9 g) were no longer different from those measured before STZ administration on day 1 (101.6 ± 7.4 mg/dl; 346 ± 4.2 g). For at least 20 days after the Linplant insertion (i.e., until the completion of study), blood glucose concentrations remained at control levels, and continued weight gain was evident.

Raclopride Antagonized Quinpirole- and Quinlorane-Induced Yawning. Quinpirole and quinlorane produced yawning in a time- and dose-dependent manner (Fig. 2). Little or no yawning was evident for the first 10 min after the injection of quinpirole or quinlorane. The maximum number of yawns was observed from 21 to 30 min after administration of intermediate doses, for both drugs. ED_{50} values for quinpirole and quinlorane to induce yawning were the smallest (0.017 and 0.001 mg/kg, respectively) for data obtained 21 to 30 min after drug administration; therefore, data from a 10-min time period, beginning 20 min after drug administration, were used in subsequent studies to generate dose-response curves for quinpirole and quinlorane alone and in combination with raclopride. The dose-response curves for quinpirole and quinlorane were inverted U-shaped (Fig. 3). Raclopride dose-dependently shifted the ascending and descending portions of the quinpirole and quinlorane dose-response curves to the right, in a parallel manner (Fig. 3, left and right, respectively). Thus, doses of 0.056, 0.1, and 0.178 mg/kg raclopride shifted the ascending and descending portions of the dose-response curves for quinpirole and quinlorane to the right 3.0- to 4.0-fold, 8.3- to 13.5-fold, and 22.3- to 31.2-fold, respectively. The simplest model that adequately fitted the Schild regressions for the ascending and descending portions of the dose-response curves for quinpirole and quinlorane was a single line with a common slope (−2.1) and a common pA2 value of 4.13 mmol/kg (i.e., 0.037 mg/kg).

Amphetamine Increased Locomotor Activity and Produced CPP in Control and STZ-Treated Rats. Statistical analyses of locomotor activity data showed significant main effects of group (F_{1,19} = 28.3; p < 0.001) and treatment (F_{1,19} = 175.8; p < 0.001). Locomotion after saline treatment (i.e., spontaneous locomotion) was less in STZ-treated rats than in control rats (Fig. 4, left, closed and open circles; F_{1,19} = 47.6; p < 0.001). The first injection of amphetamine (1.78 mg/kg) increased locomotor activity similarly in both groups of rats, although the absolute number of activity counts was significantly lower in STZ-treated rats than in control rats (Fig. 4, triangles above 1; F_{1,19} = 12.5; p < 0.01). Over four, every-other-day injections of amphetamine, the locomotor-stimulating effect of amphetamine did not change significantly in control rats (Fig. 4, open triangles); however, in STZ-treated rats, amphetamine-stimulated locomotion increased over successive injections (F_{1,3,33} = 6.25; p < 0.01), such that there was no difference between groups after the fourth injection of amphetamine (Fig. 4, open and closed triangles above 4). Thus, with repeated amphetamine treatment, differences in amphetamine-stimulated locomotion between control and STZ-treated rats became less apparent. All nine control rats spent more time on the amphetamine-paired floor-texture, whereas only nine of 12 STZ-treated rats spent more time on the amphetamine-paired floor-texture.
ture. However, 95% confidence limits around the mean differences (Fig. 4, right) did not include zero for either group, indicating amphetamine-induced CPP in both groups of rats.

**STZ-Treated Rats Were Less Sensitive to Quinpirole- and Quinelorane-Induced Yawning and to Raclopride-Induced Catalepsy.** Statistical analyses of quinpirole-induced yawning in control and STZ-treated rats showed significant main effects of group \( F_{(1,36)} = 17.7; p < 0.001 \) and dose \( F_{(2,36)} = 3.5; p < 0.05 \), and no interaction \( F_{(2,36)} = 3.1; p > 0.05 \). Thus, STZ markedly reduced quinpirole-induced yawning (Fig. 5, left, compare open and closed circles). Likewise, statistical analyses of quinelorane-induced yawning showed significant main effects of group \( F_{(1,36)} = 19.5; p < 0.001 \), dose \( F_{(2,36)} = 5.6; p < 0.01 \), and a significant inter-

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**Fig. 2.** Time course of yawning produced by quinpirole (left) and quinelorane (right) in control rats. Each panel shows data obtained from separate groups of rats with each symbol representing the mean ± S.E.M. of 10 rats. Yawning was examined for 60 min, in 10-min bins, immediately after an i.p. injection of quinpirole or quinelorane.

**Fig. 3.** Dose-response curves for quinpirole (left) and quinelorane (right) in the absence and presence of raclopride. Yawning was observed for 10 min, beginning 20 min after the administration of quinpirole, quinelorane, or saline (S). Raclopride was administered 30 min before quinpirole or quinelorane. Each symbol represents the mean ± S.E.M. of eight rats.

**Fig. 4.** Left, locomotor activity (mean ± S.E.M. counts per 30-min session) during each of four every-other-day amphetamine (1.78 mg/kg) conditioning trials in control (open symbols; \( n = 9 \)) and STZ-treated (closed symbols; \( n = 12 \)) rats that received saline on intervening days. The point above S shows locomotor activity counts averaged across the four saline conditioning trials. Right, CPP produced by amphetamine (1.78 mg/kg) in saline-treated (open) and STZ-treated (closed) rats. Data in the right panel represent the mean difference (minutes) between the time spent on the amphetamine-paired floor and the time spent on the saline-paired floor. Error bars in the right panel indicate 95% confidence limits around the mean. *, \( p < 0.05 \) compared with control (saline-stimulated) rats in saline-conditioning sessions. #, \( p < 0.05 \) compared with amphetamine-stimulated locomotion of control rats in the corresponding trial (i.e., compare open and closed triangles).
action \( F_{(2,36)} = 5.2; p < 0.05 \). A dose of quinpirole (i.e., 0.0032 mg/kg) that produced the highest number of yawns (6.6 ± 1.2 yawns/10 min) in normal rats produced significantly less yawning in STZ-treated rats [0.4 ± 0.3 yawns/10 min; \( F_{(2,36)} = 5.2; p < 0.05 \) (data not shown). Yawning produced by 0.001 and 0.01 mg/kg quinpirole was not different between control rats (1.75 ± 1.0 and 1.80 ± 0.84 yawns/10 min, respectively) and STZ-treated rats (0.17 ± 0.14 and 0.33 ± 0.18 yawns/10 min, respectively) (data not shown).

Statistical analyses of raclopride-induced catalepsy in control and STZ-treated rats showed significant main effects of group \( F_{(3,124)} = 7.04; p < 0.05 \), dose \( F_{(5,90)} = 35.98; p < 0.001 \), and a significant interaction \( F_{(15,90)} = 7.56; p < 0.001 \). Catalepsy produced by 0.56 and 1.78 mg/kg raclopride was significantly less in STZ-treated rats than in control rats. Conversely, STZ-treated rats were more sensitive than control rats to GHB-induced catalepsy (Fig. 5, right, open and closed circles). In contrast with raclopride, GHB produced near maximal catalepsy under all conditions. The simplest model that could be simultaneously fitted to the linear portions of the GHB dose-response curves in control rats, in STZ-treated rats, and in STZ-treated rats that received amphetamine and insulin was one with a common slope [193.7; \( F_{(3,122)} = 0.553; p > 0.20 \) and different intercepts \( F_{(3,127)} = 22.78; p < 0.001 \), indicating a leftward shift in the GHB dose-response curve after STZ treatment. Thus, STZ reduced sensitivity to raclopride-induced catalepsy, but significantly enhanced sensitivity to GHB-induced catalepsy.

Repeated Treatment with Amphetamine Did Not Restore Quinpirole-Induced Yawning or Raclopride-Induced Catalepsy in STZ-Treated Rats. Statistical analyses of quinpirole-induced yawning in control, STZ-treated, and STZ-treated rats that received amphetamine showed significant main effects of group \( F_{(2,45)} = 11.85; p < 0.001 \) and dose \( F_{(2,45)} = 3.41; p < 0.05 \), and no interaction \( F_{(4,45)} = 1.74; p > 0.10 \) (Fig. 5, left). Repeated treatment with amphetamine did not restore sensitivity to the behavioral effect of quinpirole in STZ-treated rats; the dose of quinpirole (i.e., 0.056 mg/kg) that produced 10.8 ± 2.9 yawns/10 min in control rats produced an average of less than 2 yawns/10 min on days 18, 20, and 22 after STZ treatment. Likewise, sensitivity to the cataleptic effects of raclopride was not restored by repeated treatment with amphetamine in STZ-treated rats (Fig. 5, middle, closed circles and diamonds). Statistical analyses of raclopride-induced catalepsy in control, STZ-treated, and STZ-treated rats that received amphetamine showed significant main effects of group \( F_{(2,29)} = 4.5; p < 0.05 \), dose \( F_{(5,145)} = 54.37; p < 0.001 \), and a significant interaction \( F_{(10,145)} = 4.74; p < 0.001 \). However, no significant difference was detected between the dose-response curves for raclopride-induced catalepsy in STZ-treated rats and in STZ-treated rats treated with amphetamine. GHB-induced catalepsy was not modified by amphetamine treatment in STZ-treated rats as indicated by the fact that the intercepts of the GHB dose-response curves in STZ-treated rats and in STZ-treated rats repeatedly treated with amphetamine were not significantly different \( F_{(1,70)} = 1.08; p > 0.20 \).

Insulin Replacement Restored Sensitivity to Quinpirole-Induced Yawning and to Raclopride-Induced Catalepsy in STZ-Treated Rats. Ten days after insulin replacement (i.e., 38 days after STZ), when blood glucose concentrations were restored to control levels (Fig. 1), sensitivity to quinpirole-induced yawning was not different from control rats. Analyses of quinpirole-induced yawning in control, STZ-treated, and STZ-treated rats with insulin replacement showed significant main effects of group \( F_{(2,45)} = 14.38; p < 0.001 \), dose \( F_{(2,45)} = 10.84; p < 0.001 \), and a significant interaction \( F_{(4,45)} = 2.73; p < 0.05 \). There was no significant difference between the dose-response curves for quinpirole in control rats and in STZ-treated rats that also received insulin (on day 38; Fig. 5, left, compare open circles and open diamonds). Moreover, the dose of quinpirole (i.e., 0.056 mg/kg) that produced 10.8 ± 2.9 yawns/10 min in control rats produced 16 ± 2.1 and 13.8 ± 2.1 yawns/10 min on days 40 and 42, respectively. Sensitivity to raclopride-induced catalepsy also was restored to control levels after insulin replacement in STZ-treated rats (Fig. 5, middle, compare open circles and open diamonds). After insulin replacement, a leftward shift in the GHB dose-response curve was no longer evident in STZ-treated rats (Fig. 5, right, open circles and open diamonds); however, the GHB dose-response curve \( p < 0.001 \) was shifted significantly rightward from the control dose-response curve.
Discussion

Data from this study show that changes in circulating insulin and glucose can markedly affect the behavioral effects of drugs acting on DA receptors. The data also suggest that activity of DAT and D2/D3 receptors is differentially affected by hypoinsulinemia (hyperglycemia) in rats, because amphetamine treatment that restored DAT activity in STZ-treated rats (Owens et al., 2005) did not restore sensitivity to the behavioral effects of drugs acting on D2/D3 receptors.

Quinpirole and quinelorane are agonists at D2-like (i.e., D2, D3, and D4) receptors, and each agonist shows similar affinity for D2 and D3 receptors (Keabian et al., 1997). In agreement with others (Kurashima et al., 1995; Collins et al., 2005), the present results show that quinpirole and quinelorane produced yawning, with dose-response curves being inverted U-shaped. Collins et al. (2005) suggested that the biphasic nature of dose-response curves for quinpirole and quinelorane involve two distinct DA receptor mechanisms: D3 receptors mediating the emergence of yawning (i.e., the ascending portion of the dose-response curve) and D2 receptors mediating the disappearance of yawning (i.e., the descending portion of the dose-response curve). Results of the current study showed that raclopride shifted both the ascending and the descending limbs of the quinpirole and quinelorane dose-response curves to the right in a parallel manner. Moreover, the Schild regressions for the ascending and descending portions of the dose-response curves for quinpirole and quinelorane could be fitted adequately with a single line with a common slope and a common pA2 value, indicating that raclopride antagonized the effects of quinpirole and quinelorane with similar potency, consistent with the involvement of the same D2/D3 receptors in their effects.

Increased blood glucose concentration and decreased body weight in STZ-treated rats confirm that STZ eliminates insulin-secreting pancreatic β-islet cells (Galici et al., 2003), resulting in hypoinsulinemia (Carr 1996). An important finding of this study is that STZ decreased quinpirole- and quinelorane-induced yawning as well as raclopride-induced catalepsy. Reduced sensitivity of STZ-treated rats to the behavioral effects of drugs acting on D2/D3 receptors parallels changes that can occur in DA receptors (e.g., receptor density and signaling) in hypoinsulinemic rats (Lovozsky et al., 1981, Rowland et al., 1985; Abbracchio et al., 1989). To the extent that activity at different DA receptors accounts for the ascending (D3) and descending (D2) portions of the dose-response curve for yawning (Collins et al., 2005), the near absence of yawning observed in STZ-treated rats could indicate a decreased sensitivity of D3 receptors to quinpirole and quinelorane, an increased sensitivity of D2 receptors to quinpirole and quinelorane, or to changes in sensitivity at both receptor types. Regardless of the underlying mechanism(s), insulin replacement restored quinpirole-induced yawning, raclopride-induced catalepsy, blood glucose concentration, and body weight in STZ-treated rats. Thus, attenuation of the behavioral effects of drugs acting on D2/D3 receptors in STZ-treated rats seems to be a confirmation of marked changes in dopaminergic systems that can occur under conditions where insulin and glucose concentrations are perturbed.

The pharmacological selectivity of decreased sensitivity to the behavioral effects of drugs acting on D2/D3 receptors was evident by the finding that STZ-treated rats were more sensitive to the cataleptic effect of GHB, a drug that does not act at DA receptors. The mechanism by which GHB induces catalepsy is not known, although emerging evidence suggests that this effect involves agonist activity at GABA_B receptors (e.g., Carter et al., 2005). Evidence that different mechanisms contribute to the cataleptic effects of raclopride and GHB was provided by an earlier study in which the same dose of the N-methyl-D-aspartate (glutamate) receptor antagonist dizocilpine attenuated catalepsy produced by the D2 receptor antagonist haloperidol, while enhancing catalepsy produced by GHB (Sevak et al., 2004). Thus, changes in insulin and glucose status do not affect all drugs in a similar manner, although it remains to be determined whether the effects of drugs acting on other receptors and neurochemical systems (e.g., other monoamines) also change as a function of insulin and glucose status.

Repeated treatment with amphetamine can normalize DAT activity in STZ-treated rats (Owens et al., 2005) and under some conditions DAT activity co-varies with changes in D2 receptor function (Jones et al., 1999; Fauchey et al., 2000). Whereas amphetamine increased locomotion and produced CPP in control and in STZ-treated rats, the same treatment that restored DAT activity (Owens et al., 2005) failed to restore sensitivity to quinpirole-induced yawning or raclopride-induced catalepsy in STZ-treated rats. That amphetamine treatment did not restore sensitivity of STZ-treated rats to drugs acting directly at DA receptors indicates that DAT activity and sensitivity of DA receptors are differentially affected by altered insulin status and by amphetamine treatment.

In summary, this study shows that changes in insulin and glucose status affect sensitivity of rats to the behavioral effects of drugs acting directly at D2/D3 receptors. It remains to be seen whether these changes reflect altered sensitivity at D2, D3, or both D2 and D3 receptors. STZ treatment can also affect norepinephrine neurotransmission (e.g., Figlewicz et al., 1996), and amphetamine can modulate norepinephrine uptake (e.g., Kuczenski and Segal, 2001); thus, mechanisms in addition to DA might underlie these differences in sensitivity to amphetamine and other drugs observed in STZ-treated rats. Ongoing studies are evaluating whether less dramatic changes in glucose and insulin status (e.g., produced by modest food restriction) also modify sensitivity to indirect-acting and direct-acting DA receptor agonists. Several reports indicate that eating disorders, where plasma insulin levels markedly fluctuate, show high co-morbidity with substance abuse (Krahn, 1991; Holderness et al., 1994). Because dopaminergic mechanisms are presumed to account for the positive reinforcing effects of many drugs of abuse and insulin can regulate DA neurotransmission, understanding the functional relationships among insulin status, glucose status, and the behavioral effects of dopaminergic drugs could facilitate the development of treatments for substance abuse and eating disorders.

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