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Streptozotocin-induced diabetes differentially modifies haloperidol- and γ-hydroxybutyric acid (GHB)-induced catalepsy

Rajkumar J. Sevak[a], Wouter Koek[a,b], Charles P. France[a,b,*]

[a]Department of Pharmacology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900 USA
[b]Department of Psychiatry, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900 USA

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Abstract

To examine whether dopamine-mediated behavioral effects are altered in diabetes, this study compared the cataleptic effects of the dopamine receptor antagonist haloperidol (0.032–0.56 mg/kg) and γ-hydroxybutyric acid (GHB; 56–1000 mg/kg) in control and streptozotocin (STZ)-treated rats. Haloperidol and GHB produced catalepsy in control and diabetic rats; haloperidol was less potent in diabetic rats (D_{50}=0.44 mg/kg) than in controls (D_{50}=0.19 mg/kg), while GHB was more potent in diabetic rats (D_{50}=392 mg/kg) than in controls (D_{50}=550 mg/kg). In diabetic rats, the non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine (0.32 mg/kg) further attenuated haloperidol-induced catalepsy (D_{50}=1.2 mg/kg) and further enhanced GHB-induced catalepsy (D_{50}=248 mg/kg). That haloperidol is less potent to produce catalepsy in diabetic rats is consistent with reports of altered dopamine receptor binding in diabetes.

Keywords: D2 receptors; Haloperidol; Streptozotocin; γ-hydroxybutyric acid; Dizocilpine

1. Introduction

One important mechanism of action of many drugs that are used in the clinic as well as many drugs that are abused is modulation of dopaminergic neurotransmission and it is known that dopamine systems can be altered by experimentally induced diabetes (Lim et al., 1994; Lozovsky et al., 1981; Sumiyoshi et al., 1997; Trulson and Himmel, 1983). Catalepsy is a convenient method for evaluating changes in dopamine receptor systems and in non-human species, most commonly in rodents, catalepsy is characterized by the extent to which an animal remains in an unnatural position while maintaining muscular tone. Classically, catalepsy is produced by dopamine receptor antagonists such as haloperidol (Kanes et al., 1993; Marchese et al., 2003), although drugs from other classes produce catalepsy as well.

The current study used sensitivity to the cataleptic effects of the dopamine receptor antagonist haloperidol as an index of changes in dopamine receptor function that occur after the induction of diabetes by administration of streptozotocin (STZ). In a previous study (Sevak et al., 2004) the *N*-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine enhanced the cataleptic effects of γ-hydroxybutyric acid (GHB) and attenuated the cataleptic effects of haloperidol. In the current study, GHB was used to examine the pharmacologic specificity of changes in sensitivity to haloperidol in STZ-treated rats that might reflect changes in dopamine receptor function. Moreover, the ability of dizocilpine to modify the cataleptic effects of haloperidol and GHB in diabetic rats was examined to see whether glutamatergic modulation of catalepsy was altered in diabetes.

2. Materials and methods

2.1. Animals

Sixteen male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN), weighing 250–350 g, were individually housed

* Corresponding author. Department of Pharmacology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, Mail Code 7764, San Antonio, TX 78229-3900 USA. Tel.: +1 210 567 6969; fax: +1 210 567 0104.
E-mail address: france@uthscsa.edu (C.P. France).

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in an environmentally controlled room (23±2 °C; relative humidity, 45±1%), under a 12/12 h light/dark cycle (lights on at 0800 h), with food (Rodent Diet, Harlan Teklad, Madison, WI) and water available continuously. Eight rats were rendered diabetic by a single i.p. injection of 50 mg/kg of STZ. Behavioral experiments began one month after administration of either STZ or saline. It is well-established that this treatment with STZ induces a diabetic condition within 7 days. One week after administration of STZ a blood specimen was obtained from the tail vein for measuring blood glucose concentration using a Prestige LX™ monitoring device (Home Diagnostics Inc., Ft. Lauderdale, USA). 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, National Research Council, National Academy of Sciences).

2.2. Catalepsy

Catalepsy was studied using two procedures, the bar test and the hind limb test. In the bar test, 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. In the hind limb test, the right hind limb was placed on a metal platform (height, 5 cm) and the time until the limb touched the table surface was recorded, up to a maximum of 30 s. Cumulative doses of GHB (56, 100, 178, 320, 560, 1000 mg/kg) or haloperidol (0.032, 0.056, 0.1, 0.178, 0.32, 0.56 mg/kg) were administered at the beginning of each of six 25-min (bar test, hind limb test) cycles in separate groups of control or STZ-treated rats. Each of the two indices of catalepsy was assessed once per cycle. On separate occasions and in the same STZ-treated rats, a single injection of dizocilpine (0.32 mg/kg) or vehicle was administered immediately before the first dose of GHB, haloperidol or vehicle.

2.3. Drugs

γ-Hydroxybutyric acid (GHB) sodium salt, haloperidol and streptozotocin (STZ) were purchased from Sigma-Aldrich (St. Louis, MO). Dizocilpine maleate was purchased from Research Biochemicals International (Natick, MA). GHB, dizocilpine and STZ were dissolved in 0.9% saline. Haloperidol was dissolved in 0.9% saline with lactic acid. All drugs were administered intraperitoneally (i.p.).

2.4. Data analyses

Blood glucose concentrations in STZ- and vehicle-treated rats were compared with a Student's t test for independent samples. Catalepsy was measured as the mean time until both forelimbs (bar test) or the right hind limb (hind limb test) touched the surface of the table. Dose–response functions were determined from the mean duration of catalepsy after each cumulative dose. For each catalepsy-inducing drug (i.e., haloperidol, GHB) and for each catalepsy test (i.e., bar test, hind limb test), the dose–response data obtained in non-diabetic rats pretreated with vehicle, in diabetic rats pretreated with vehicle, and in diabetic rats pretreated with dizocilpine, were analyzed by simultaneously fitting logistic curves to the data points by means of the non-linear regression program AllFit (De Lean et al., 1978). This program uses the following equation:

$$Y = (A - D)/\left(1 + (X/C)^B\right) + D$$

where X and Y are the dose and the response, respectively. The four fitted parameters represent the expected maximal response (A), slope factor (B), dose needed to produce 50% of the maximal response (D_{50}) (C), and minimal response (D). An important feature of the AllFit program is the possibility to fit models of varying complexity to the data. AllFit allows one to simplify a model by selecting common and/or constant parameters (e.g., common slope, constant maximum), and to compare simpler models with more complex models by means of an F-ratio test: if the calculated F for two models is statistically significant, this indicates that the more complex model is required to fit the data; if, however, the value of F is not significant, the simple model should be used (for detailed examples of this approach see Kenakin, 1997). For D_{50} values and their ratios, and for slope values, 95% confidence intervals were calculated from the asymptotic standard errors (S.E.M.) provided by AllFit using the following equation:

$$parameter\,value\ \pm S.E.M.*t$$

where t is obtained from the Student's t distribution with $\alpha = 0.05$ and the degrees of freedom (df) are equal to the number of data points minus the number of parameters fitted by non-linear regression.

3. Results

The blood glucose concentration of STZ-treated rats (463.9±25.1 [mean±S.E.M.] mg/dl) was significantly higher ($t = 15.6$, $df = 14$, $P < 0.0001$) than the blood glucose concentration of vehicle-treated rats (64.1±5.7 mg/dl). Haloperidol and GHB dose-dependently produced catalepsy in the bar test and in the hind limb test in non-diabetic rats (Fig. 1, closed circles). In diabetic rats (open circles), the effects of haloperidol and GHB appeared to be different from those in non-diabetic controls, with haloperidol being less potent and GHB more potent. These differences were further enhanced by pretreatment with dizocilpine (0.32 mg/kg,); in diabetic rats (solid triangles) haloperidol became even less potent and GHB more potent.

Simultaneously fitting logistic functions to the three sets of dose–response data in each of the four panels of Fig. 1 showed the three curves in each panel to be significantly different from each other. The simplest models that adequately fitted ($R^2 >= 0.99$) the haloperidol data obtained in the bar and in the hind limb tests were those where the haloperidol dose–response curves shared a common minimum of 0, a common maximum (i.e., 120 in the bar test, 30 in the hind limb test), but had different slopes (i.e., non-diabetic controls = 2.9 [95% CL = 2.4–3.4]; vehicle- or dizocilpine-treated rats = 1.8 [1.4–2.1]) and different D_{50} values (Table 1). The simplest models that adequately fitted ($R^2 >= 0.99$) the GHB data were those where the GHB dose–response curves shared a common minimum of 0, a common maximum (i.e., 120 in the bar test, 30 in the hind limb test), a common slope (i.e., bar test = 6.2 [5.2–7.1]); hind limb test = 5.3 [4.2–6.3]), and different D_{50} values (Table 1).

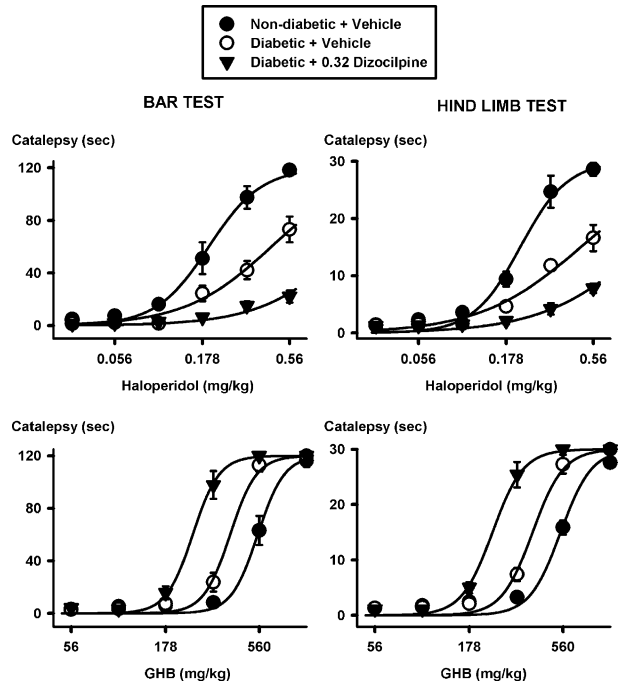

Fig. 1. Haloperidol-(upper) and GHB-(lower) induced catalepsy in the bar test (left) and in the hind limb test (right) in non-diabetic rats (closed circles) and in diabetic rats that also received saline (open circles) or 0.32 mg/kg dizocilpine (triangles). Data points represent the mean ± S.E.M ($n = 8$ per dose). Each of the dose–response curves could be fitted adequately ($R^2 >= 0.98$) by logistic functions with the same minimum and maximum, with the same (upper panels) or different (lower panels) slope, and, within each panel, with different D_{50} values.

Using these simplest, best-fitting regression models, haloperidol was found to be 2.2 to 2.3-fold less potent to produce catalepsy in diabetic than in non-diabetic rats, and was a further 2.3 to 2.7-fold less potent in the presence of dizocilpine (Table 1). In contrast, GHB was 1.4-fold more potent to produce catalepsy in diabetic than in non-diabetic rats, and was a further 1.6-fold more potent in the presence of dizocilpine.

4. Discussion

GHB and haloperidol produced catalepsy as measured by the bar test and the hind limb test, consistent with prior reports on the cataleptic effects of these compounds under similar conditions (Kanes et al., 1993; Marchese et al., 2003; Navarro et al., 1998; Sevak et al., 2004). However, GHB and haloperidol appear to exert their cataleptic effects through different mechanisms since STZ treatment or acute administration of dizocilpine differentially modified the cataleptic effects of these two drugs: both treatments attenuated the effects of haloperidol and both treatments enhanced the effects of GHB. Haloperidol is thought to exert cataleptic effects by antagonism at dopamine D2 receptors; the mechanism by which GHB exerts cataleptic effects is not established, although presumably it is through non-dopaminergic mechanisms. NMDA receptor antagonists also enhance morphine-induced catalepsy at the same doses that attenuate catalepsy induced by dopamine receptor antagonists (Schmidt and Bubser, 1989; Tzschentke and Schmidt, 1996; Yoshida et al., 1991).

The potency of haloperidol to produce catalepsy was markedly reduced in rats made diabetic by a single injection of STZ. Converging lines of evidence indicate that dopaminergic systems are changed in diabetes. For example, diabetic rats are less sensitive to the effects of apomorphine (direct dopamine agonist) and amphetamine (indirect dopamine agonist) on locomotor activity and to the positive reinforcing effects of amphetamine (Marshall, 1978; Galici et al., 2003; Rowland et al., 1985). Experimentally induced diabetes also has profound effects on neurochemistry, including altered dopamine synthesis (Trulson and Himmel, 1983), release (Saller, 1984), uptake (Patterson et al., 1998), turnover (Kwok and Juorio, 1986) and changes in dopamine D2 receptor binding (Lozovsky et al., 1981; Trulson and Himmel, 1983; Lim et al., 1994; Rowland et al., 1985; Sumiyoshi et al., 1997). Thus, attenuation of haloperidol-induced catalepsy in diabetic rats appears to be a confirmation of marked changes in dopaminergic systems that can occur under conditions where insulin and glucose concentrations are perturbed.

In contrast to attenuation of haloperidol-induced catalepsy in diabetic rats, rats treated with STZ were more sensitive than normal rats to the cataleptic effects of GHB, demonstrating that diabetes does not alter the cataleptic effects of all drugs in a similar manner. The mechanism by which GHB induces catalepsy is unknown although emerging evidence suggests that this effect involves

Table 1
D_{50} values and potency ratios (95% CL) for GHB and haloperidol, administered alone and after 0.32 mg/kg dizocilpine or vehicle, to produce catalepsy in diabetic and non-diabetic rats

Group	Pretreatment	Treatment	D_{50} (mg/kg), i.p.			
			Bar test	Potency ratio	Hind limb test	Potency ratio
Control	Vehicle	Haloperidol	0.19 (0.18–0.21)		0.21 (0.20–0.23)	
Diabetic	Vehicle	Haloperidol	0.44 (0.40–0.49)	2.3 (2.0–2.6)[a]	0.47 (0.40–0.55)	2.2 (1.8–2.6)[a]
Diabetic	Dizocilpine	Haloperidol	1.2 (0.9–1.5)	2.7 (2.1–3.4)[a]	1.1 (0.74–1.4)	2.3 (1.6–3.0)[a]
Control	Vehicle	GHB	550 (526–575)		545 (506–583)	
Diabetic	Vehicle	GHB	392 (369–416)	1.4 (1.3–1.5)[a]	386 (355–418)	1.4 (1.3–1.6)[a]
Diabetic	Dizocilpine	GHB	248 (232–264)	1.6 (1.4–1.7)[a]	236 (215–257)	1.6 (1.4–1.8)[a]

[a] Significant difference as 95% confidence limit does not include 1.

$GABA_B$ agonist activity (Carter et al., 2005). Evidence for the role of different mechanisms in the cataleptic effects of haloperidol and GHB was provided by an earlier study in which the same dose of the NMDA receptor antagonist dizocilpine attenuated haloperidol-induced catalepsy and enhanced GHB-induced catalepsy (Sevak et al., 2004).

Similar to results obtain in normal rats (Sevak et al., 2004), dizocilpine had opposite effects in modifying the cataleptic effects of haloperidol and GHB in diabetic rats. That the interactions between dizocilpine and both haloperidol and GHB were qualitatively and quantitatively similar in diabetic and in normal rats suggest that glutamatergic modulation of catalepsy is not altered in diabetes. Collectively these data are consistent with reported changes in dopamine receptor systems in diabetes (e.g., D2 receptor density) and suggest that altered insulin or glucose status can have marked effects on the behavioral actions of drugs acting through dopaminergic systems.

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