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Prazosin inhibits spontaneous locomotor activity in diabetic mice

Akiyoshi Saitoh[a], Kenji Onodera[b], Kayo Morita[a], Midori Sodeyama[a], Junzo Kamei[a,*]

[a]*Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-chome, Shinagawa-ku, Tokyo 142-8501, Japan*

[b]*Department of Pharmacology, School of Dentistry, Okayama University, Sikada-Cho, Okayama 700-8525, Japan*

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Abstract

We examined the effect of prazosin, a selective α_1-adrenergic receptor antagonist, on the enhanced spontaneous locomotor activity in streptozotocin-induced diabetic mice. Spontaneous locomotor activity in diabetic mice was significantly greater than that in nondiabetic mice. Pretreatment with either intracerebroventricular (5, 10 nmol) or intraperitoneal (0.5, 1.0 mg/kg) injection of prazosin dose-dependently reduced the spontaneous locomotor activity in diabetic mice, but not in nondiabetic mice. Furthermore, the enhanced dopamine turnover ratio in the limbic forebrain in diabetic mice was reduced to the same level as that in nondiabetic mice after the administration of prazosin. Thus, these results suggest that α_1-adrenergic receptors might play an important role in the enhanced spontaneous locomotor activity in diabetic mice. Furthermore, α_1-adrenoceptor antagonism might have an inhibitory effect on presynaptic dopaminergic neurotransmission in the limbic forebrain in diabetic mice.

Keywords: α_1-Adrenoceptor antagonist; Diabetic mice; Spontaneous locomotor activity; Prazosin

1. Introduction

We previously reported that spontaneous locomotor activity in diabetic mice was significantly greater than that in nondiabetic mice (Kamei and Saitoh, 1996a,b,c, 1997; Kamei et al., 1994; Saitoh et al., 1998). This enhanced spontaneous locomotor activity in diabetic mice was significantly reduced by treatment with haloperidol or SCH23390, a selective dopamine (DA) D_1 receptor antagonist (Kamei et al., 1994). Furthermore, the rate of DA turnover in the limbic forebrain in diabetic mice was significantly higher than that in nondiabetic mice (Kamei and Saitoh, 1997; Kamei et al., 1994). Based on these results, we proposed that the enhanced spontaneous locomotor activity in diabetic mice may result from increased DA neurotransmission, which might be due to an increase in DA release from presynaptic nerve terminals in diabetic mice. It is well known that some psychiatric disorders are associated with dopaminergic dysfunction in the central nervous system (e.g., Jentsch et al., 1997). Diabetes mellitus is associated with an increased incidence of psychiatric disorders, which has been suggested to be due to alteration of the central monoaminergic neuronal system (Lozovsky et al., 1981; Popkin et al., 1988; Frederick et al., 1999; Horrobin and Bennett 1999). However, little information is available concerning the mechanisms that underlie these abnormalities. Thus, understanding the effects of diabetes on dopaminergic function may help to clarify the mechanisms of the development of psychiatric disorders in the diabetic state.

Several lines of evidence have indicated that noradrenergic neuron may interact with the mesolimbic dopaminergic system, particularly with regard to locomotor activity and DA release from presynaptic neurons (Dickinson et al., 1988; Blanc et al., 1994; Sommermeyer et al., 1995; Mathè et al., 1996; Darracq et al., 1998). For example, Mathè et al. (1996) reported that prazosin, a selective α_1-adrenergic receptor antagonist, effectively blocked the psychotomimetic, noncompetitive NMDA receptor antagonist, MK-801 [(+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo-(*a*,*d*)cyclohepten-5,10-imine]-induced locomotor stimulation and DA release in the nucleus accumbens, without affecting basal DA activity. Based on these results, they suggested that α_1-adrenoceptor antagonism exerts a preferential effect on evoked DA

* Corresponding author. Tel.: +81-3-5498-5030; fax: +81-3-5498-5029.
E-mail address: kamei@hoshi.ac.jp (J. Kamei).

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release, but not on basal release in the mesolimbic DA system. On the other hand, it has been reported that diabetes mellitus causes functional changes in the central noradrenergic system (Bitar et al., 1986, 1999; Weber and MacLeod, 1997; Kamei and Ohsawa, 1997). Weber and MacLeod (1997) reported an increase in the maximum response to α_1-adrenergic receptor stimulation in diabetic arteries. Bitar et al. (1999) also showed that the density of [^{3}H]prazosin binding in the spinal cord was increased in diabetic rats. Thus, these functional abnormalities in the noradrenergic system of diabetic animals may alter their behavioral activities and DA transmission. To test this hypothesis, we examined the effect of prazosin, a selective α_1-adrenergic receptor antagonist, on the increased spontaneous locomotor activity and enhanced DA turnover in the limbic forebrain in diabetic mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Tokyo Animal Laboratory, Tokyo, Japan), aged 4 weeks and weighing about 20 g at the beginning of the experiments, were used. They had free access to food and water in an animal room that was maintained at 24 ± 1 °C with a 12-h light–dark cycle. Animals were rendered diabetic by an injection of streptozotocin (200 mg/kg iv) prepared in 0.1 N citrate buffer at pH 4.5. Age-matched naive mice were injected with the vehicle alone. The experiments were conducted 2 weeks after injection of streptozotocin or vehicle. Mice with serum glucose levels above 400 mg/dl were considered diabetic. This study was carried out in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Locomotor activity

The locomotor activity of mice was measured by an ambulometer (ANB-M20; O'Hara, Tokyo, Japan). The principle of the device and the measurement method have been described by Hirabayashi and Alam (1981). Briefly, a mouse was placed in a tilting-type round activity cage 20 cm in diameter and 19 cm high. Any slight tilt of the activity cage, which was caused by horizontal movement of the animal, was detected by microswitches. Total activity counts during each 10-min period were automatically recorded for 30 min prior to injection, and for 180 min following the administration of saline or drugs. Mice were placed in the tilting cages for a habituation period of 30 min, and then injected with saline or drugs. Locomotor activity was assessed between 1000 and 1500 h each day.

2.3. Biochemical analysis

The concentrations of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined by high-performance liquid chromatography (HPLC). The brain was quickly removed and the limbic forebrain (containing the nucleus accumbens and olfactory tubercles) was dissected on an ice-cold glass plate. The tissues were homogenized in 500 ml of 0.2 M perchloric acid containing 100 mM EDTA (2Na) and 100 ng of isoproterenol, as an internal standard. To remove the proteins completely, the homogenates were placed in cold water for 60 min. The homogenates were then centrifuged at 20,000 × g for 20 min at 0 °C, and the supernatants were maintained at pH 3.0 using 1 M sodium acetate. Solution samples of 20 ml were analyzed by HPLC with electrochemical detection. The HPLC system consisted of a delivery pump (EP-10; Eicom, Kyoto, Japan), an analytical column (EICOMPAK, MA-5ODS; Eicom) and a guard column (Eicom). The electrochemical detector (EC-300; Eicom) included a graphite electrode (WE-3G; Eicom), which was used at a voltage setting of 0.7 V vs. an Ag/AgCl reference electrode. The mobile phase consisted of a 0.1 M sodium acetate/0.1 M citric acid buffer, pH 3.5, containing 13–15% methanol, sodium L-octanesulfonate and EDTA (2Na). The flow rate was set to 1.0 ml/min with a column temperature of 25 °C.

2.4. Drugs

Streptozotocin and prazosin were purchased from Sigma (St. Louis, MO). Streptozotocin was dissolved in 0.1 N citrate buffer. Prazosin was dissolved in saline. The doses for prazosin, which had no effect on the spontaneous locomotor activity by itself, in this study were determined as described previously (Darracq et al., 1998; Mathè et al., 1996).

2.5. Statistics

Data are expressed as the mean ± S.E.M. Behavioral data (total activity counts) were statistically evaluated with a one-way repeated measures analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons.

3. Results

3.1. Effects of prazosin, a selective α_1-adrenergic receptor antagonist, on spontaneous locomotor activity in diabetic and nondiabetic mice

Fig. 1 shows the time courses of the effect of prazosin (1 mg/kg ip) on the spontaneous locomotor activity in both diabetic and nondiabetic mice. As shown in Fig. 1, spontaneous locomotor activity in diabetic mice was clearly greater than that in nondiabetic mice. The mean total

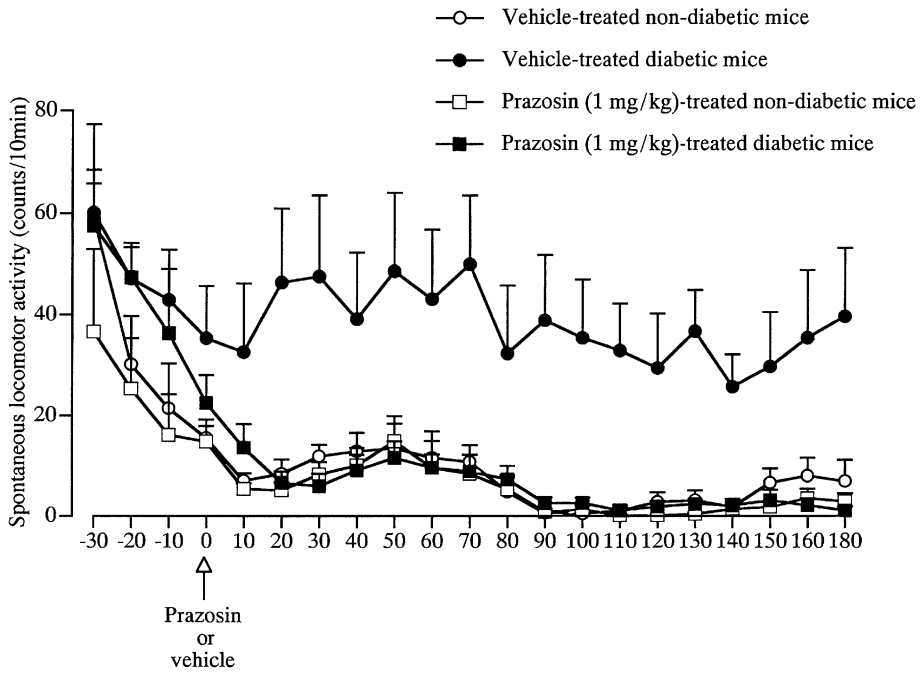

Fig. 1. Effect of prazosin, a selective α_1-adrenergic receptor antagonist, on spontaneous locomotor activity in nondiabetic and diabetic mice. Each point represents the mean locomotor activity counts with S.E.M. of 8–12 animals for 10 min.

activity count over 3 h of subcutaneous saline-treated diabetic mice was significantly greater than that of nondiabetic mice (Fig. 2A: diabetic mice, 677.9 ± 164.1, $n = 10$; nondiabetic mice, 127.4 ± 12.1, $n = 12$). As shown in Fig. 2A, pretreatment with prazosin, an α_1-adrenergic receptor antagonist, at doses of 0.5 and 1.0 mg/kg ip, significantly and dose-dependently reduced the spontaneous locomotor activity in diabetic mice. A higher dose of prazosin (1 mg/kg ip) reduced the mean total activity count to the level observed in nondiabetic mice. On the other hand, Fig. 2B shows the effects of intracerebroventricular prazosin on spontaneous locomotor activity in diabetic and nondiabetic mice. The mean total activity count over 3 h of intracerebroventricular saline-treated diabetic mice was also significantly greater than that of nondiabetic mice (Fig. 2B: diabetic mice, 807.3 ± 74.2, $n = 10$; nondiabetic mice, 143.8 ± 23.2, $n = 10$). Furthermore, intracerebroventricular administration of prazosin, at doses of 5 and 10 nmol, also significantly reduced the spontaneous locomotor activity of diabetic mice in a dose-dependent manner (Fig. 2B). Prazosin had no significant effects on spontaneous locomotor activity in nondiabetic mice.

3.2. Effects of prazosin on the DA turnover ratio in the mouse limbic forebrain in nondiabetic and diabetic mice

The effects of prazosin on the DA turnover ratio in the mouse limbic forebrain in nondiabetic and diabetic mice are shown in Table 1. The DA turnover ratio (DOPAC + HVA/DA) in the limbic forebrain in vehicle-treated diabetic mice was significantly ($P < .05$, about 1.3-fold) greater than that in nondiabetic mice. Prazosin (1 mg/kg ip) alone did not affect the DA turnover ratio in nondiabetic mice. However, the enhanced DA turnover ratio in diabetic mice was

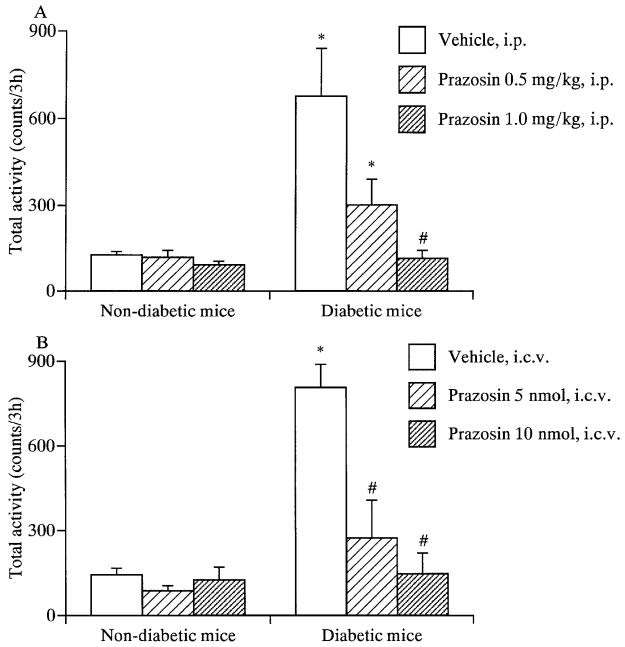

Fig. 2. Effects of prazosin, a selective α_1-adrenergic receptor antagonist, on spontaneous locomotor activity in diabetic and nondiabetic mice. Prazosin was administered subcutaneously (A) or intracerebroventricularly (B). Each column represents the mean locomotor activity count with S.E.M. of 8–12 animals for 3 h after treatment. $^{*}P < .05$ vs. respective nondiabetic value. $^{\#}P < .05$ vs. vehicle-treated value.

Table 1
Effects of prazosin on the DA turnover (DA ratio) in the mouse limbic forebrain in nondiabetic and diabetic mice

	DOPAC (ng/g wet tissue)	HVA (ng/g wet tissue)	DA (ng/g wet tissue)	DA ratio
Vehicle (intraperitoneal) treatment group (n = 8)				
Nondiabetic mice	2760.3 ± 591.3	1018.5 ± 141.9	18,528.1 ± 2784.5	0.208 ± 0.009
Diabetic mice	2629.5 ± 211.5	1407.2 ± 134.3	15,580.0 ± 1460.8	0.265 ± 0.018*
Prazosin (1 mg/kg ip) treatment group (n = 6)				
Nondiabetic mice	1885.6 ± 240.0	868.5 ± 38.6	12,492.3 ± 405.6	0.219 ± 0.016
Diabetic mice	2054.4 ± 272.2	915.0 ± 51.3	14,678.6 ± 797.0	0.200 ± 0.012

The data were shown as mean ± S.E.M. The DA ratio was calculated as follows: (DOPAC + HVA/DA).
* $P < .05$ vs. value in respective nondiabetic mice.

significantly reduced by pretreatment with prazosin. Indeed, there was no significant difference in the DA turnover ratio in the limbic forebrain between prazosin-treated nondiabetic and prazosin-treated diabetic mice.

4. Discussion

In this study, spontaneous locomotor activity in diabetic mice was significantly greater than that in nondiabetic mice. Furthermore, the rate of DA turnover in the limbic forebrain in diabetic mice was significantly higher than that in nondiabetic mice. These results confirmed our previous conclusion that the enhanced spontaneous locomotor activity in diabetic mice may result from increased DA neurotransmission, which might be due to an increase in DA release from presynaptic nerve terminals in diabetic mice (Kamei and Saitoh, 1997; Kamei et al., 1994, 1995). In the present study, pretreatment with prazosin, a selective α_1-adrenoceptor antagonist, dose-dependently reduced the spontaneous locomotor activity in diabetic mice, but not in nondiabetic mice. Furthermore, the enhanced DA turnover in the limbic forebrain in diabetic mice was reduced to the same level as that in nondiabetic mice by pretreatment with prazosin. In addition, prazosin had no significant effect on the spontaneous locomotor activity in nondiabetic mice. This result suggests that the effect of prazosin on the spontaneous locomotor activity in diabetic mice may not be due to an indirect effect of this drug on the motor system, such as drowsiness or catalepsy. Thus, these results suggested that α_1-adrenergic receptor-mediated mechanisms may play an important role in the enhanced spontaneous locomotor activity in diabetic mice.

Many investigators have reported that cortical noradrenergic systems may interact with locomotor activity and DA release from presynaptic neurons in the mesolimbic dopaminergic system (Dickinson et al., 1988; Lategan et al., 1990; Blanc et al., 1994; Sommermeyer et al., 1995; Mathè et al., 1996; Darracq et al., 1998). For example, Lategan et al. (1990) demonstrated using a microdialysis assay that D-amphetamine-induced DA release in the nucleus accumbens was reduced following the destruction of ascending noradrenergic pathways. Similarly, it has been reported that the locomotor hyperactivity induced by D-amphetamine is reduced by pretreatment with prazosin (Dickinson et al., 1988; Blanc et al., 1994). Recently, Mathè et al. (1996) indicated that the increased release of DA in the nucleus accumbens and the hyperlocomotion evoked by MK-801 were effectively reduced by pretreatment with prazosin. In this study, we observed that the blockade of α_1-adrenergic receptors by prazosin reduced the enhanced spontaneous locomotor activity in diabetic mice, but not nondiabetic mice. Furthermore, we found that the enhanced DA turnover in the limbic forebrain in diabetic mice was reduced to the same level as that in nondiabetic mice by prazosin. Thus, it is possible that α_1-adrenoceptor antagonism might have a presynaptic inhibitory effect on dopaminergic neurotransmission in the limbic forebrain in diabetic mice. Furthermore, it seems likely that the decrease in spontaneous locomotor activity in diabetic mice may be due to suppression of the enhanced DA transmission in the limbic forebrain in diabetic mice, as the result of impaired α_1-adrenergic transmission by pretreatment with prazosin.

Recently, we reported that the levels of noradrenaline turnover in the frontal cortex were significantly lower in diabetic mice than in nondiabetic mice (Kamei and Ohsawa, 1997). Bitar et al. (1999) also reported that streptozotocin-treated diabetic rats showed reduced noradrenaline turnover in the spinal cord. However, they found an increase in the density of [^{3}H]prazosin binding to spinal synaptosomal membranes in diabetic rats, but not in nondiabetic rats. Based on these results, they suggested that the decrease in noradrenaline turnover in the diabetic state could initiate a compensatory increase in postsynaptic α_1-adrenergic receptors. In the present study, prazosin reduced the spontaneous locomotor activity in diabetic mice, but had no effects in nondiabetic mice. Thus, these findings suggest that the modification of mesolimbic DA neurotransmission mediated by cortical α_1-adrenergic receptor may be altered in diabetic mice; as a result, diabetic mice, but not nondiabetic mice, may show enhanced spontaneous locomotor activity. However, further studies are necessary before these possibilities can be established with greater certainty.

In conclusion, the present results suggested that α_1-adrenergic receptors might play an important role in the enhanced spontaneous locomotor activity in diabetic mice.

Furthermore, α_1-adrenoceptor antagonism might have a presynaptic inhibitory effect on dopaminergic neurotransmission in the limbic forebrain in diabetic mice.

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References

Bitar M, Koulu M, Rapoport SI, Linnoila M. Diabetes-induced alteration in brain monoamine metabolism in rats. J Pharmacol Exp Ther 1986;236: 432–7.

Bitar MS, Bajic KT, Farook T, Thomas MI, Pilcher CWT. Spinal cord noradrenergic dynamics in diabetic and hypercortisolaemic states. Brain Res 1999;830:1–9.

Blanc G, Trovero P, Vezina P, Hervö D, Godeheu AM, Glowinski J, Tassin JP. Blockade of prefronto-cortical adrenaline α_1-adrenergic receptors prevents locomotor hyperactivity induced by subcortical D-amphetamine injection. Eur J Neurosci 1994;6:293–8.

Darracq L, Blanc G, Glowinski J, Tassin JP. Importance of the noradrenaline–dopamine coupling in the locomotor activation effects of D-amphetamine. J Neurosci 1998;7:2729–39.

Dickinson SL, Gadie B, Tulloch IF. α_1- and α_2-Adrenoreceptor antagonists differentially influence locomotor and stereotyped behavior induced by D-amphetamine and apomorphine in the rat. Psychopharmacology 1988; 96:521–7.

Frederick C, Eileen A, Bernard JC. Elevated frequency of diabetes mellitus in hospitalized manic–depressive patients. Am J Psychiatry 1999;156: 1417–20.

Hirabayashi M, Alam MR. Enhancing effect of methamphetamine on ambulatory activity produced by repeated administration in mice. Pharmacol Biochem Behav 1981;15:925–32.

Horrobin DF, Bennett CN. Depression and bipolar disorder: relationships to impaired fatty acid and phospholipid metabolism and to diabetes, cardiovascular disease, immunological abnormalities, cancer, aging and osteoporosis. Prostaglandins, Leukotrienes Essent Fatty Acids 1999; 60:217–34.

Jentsch JD, Redmond DE, Elsworth JD, Taylor JR, Youngren KD, Roth RH. Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. Science 1997;277:953–5.

Kamei J, Ohsawa M. Role of noradrenergic function in the modification of naloxone-precipitated withdrawal jumping in morphine-dependent mice by diabetes. Life Sci 1997;60:PL223–8.

Kamei J, Saitoh A. Involvement of dopamine D2 receptor-mediated function in the modulation of morphine-induced antinociception in diabetic mouse. Neouropharmacology 1996a;35:273–8.

Kamei J, Saitoh A. Modification of effects of 7-OH-DPAT, a dopamine D3-receptor agonist, on morphine-induced hyperlocomotion by diabetes. Life Sci 1996b;59:773–80.

Kamei J, Saitoh A. Role of spleen or spleen products in the reduced locomotor-enhancing effect of morphine in diabetic mice. Neurosci Lett 1996c;210:57–60.

Kamei J, Saitoh A. Evidence for the modulation of spontaneous locomotor activity by higher serum glucose levels and/or spleen-derived factor(s) in diabetic mice. Life Sci 1997;60:1699–708.

Kamei J, Saitoh A, Iwamoto Y, Funada M, Suzuki T, Misawa M, Nagase H, Kasuya Y. Effects of diabetes on spontaneous locomotor activity in mice. Neurosci Lett 1994;178:69–72.

Kamei J, Ohsawa M, Saitoh A, Iwamoto Y, Suzuki T, Misawa M, Nagase N, Kasuya Y. Modification of m-opioid agonist-induced locomotor activity and development of morphine dependence by diabetes. J Pharmacol Exp Ther 1995;274:700–6.

Lategan AJ, Marien MR, Colpaert FC. Effects of locus coeruleus lesions on the release of endogenous dopamine in the rat nucleus accumbens and caudate nucleus as determined by intracerebral microdialysis. Brain Res 1990;523:134–8.

Lozovsky D, Saller C, Kopin IJ. Dopamine receptor binding is increased in diabetic rats. Science 1981;214:1031–3.

Mathè JM, Nomikos GN, Hildebrand BE, Hertel P, Svensson TH. Prazosin inhibits MK-801-induced hyperlocomotion and dopamine release in the nucleus accumbens. Eur J Pharmacol 1996;309:1–11.

Popkin MK, Callies AL, Lentz RD, Colon EA, Sutherland DE. Prevalence of major depression, simple phobia, and other psychiatric disorders in patients with long-standing type I diabetes mellitus. Arch Gen Psychiatry 1988;45:64–8.

Saitoh A, Morita K, Sodeyama M, Kamei J. Effects of the experimental diabetes on dopamine D_1 receptor-mediated locomotor-enhancing activity in mice. Pharmacol Biochem Behav 1998;60:161–6.

Sommermeyer H, Frielingsdorf J, Knorr A. Effects of prazosin on the dopaminergic neurotransmission in rat brain. Eur J Pharmacol 1995; 276:267–70.

Weber LP, MacLeod KM. Influence of streptozotocin diabetes on the alpha-a adrenoceptor and associated G proteins in rat arteries. J Pharmacol Exp Ther 1997;283:1469–78.