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Ethopharmacology of the Antidepressant Effect of Clonazepam in Diabetic Rats

R. GOMEZ AND H. M. T. BARROS

Division of Pharmacology, Fundação Faculdade Federal de Ciências Médicas de Porto Alegre, Sarmento Leite, 245, PC 90050-170, Porto Alegre, RS Brazil

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GOMEZ, R. AND H. M. T. BARROS. *Ethopharmacology of the antidepressant effect of clonazepam in diabetic rats.* PHARMACOL BIOCHEM BEHAV **66**(2) 329–335, 2000.—Diabetes-associated depression may occur due to changes in the quality of life imposed by treatment, or may be a consequence of the biochemical changes accompanying the disease. It was our objective to evaluate the behaviors of diabetic rats through an animal model of depression, and determine if a positive GABA modulator agent, clonazepam, is an effective antidepressant. Wistar male rats were submitted to the forced-swimming test after 26 days of the induction of diabetes with streptozotocin (60 mg/kg). Test and retest days analyzed with an ethological approach. Clonazepam (control, 0.25, 0.5, and 1.0 mg/kg) was administered IP 24, 5, and 1 h before the retest. Diabetic rats presented longer immobility duration during test and retest of forced swimming. Diabetic rats dived significantly less during the test. Clonazepam 0.25 and 0.5 mg/kg decreased immobility of diabetic rats with no consequences on the behaviors of nondiabetic rats. These results demonstrate that diabetic rats present more intense depressive-like behavior, such as immobility and lack of interest in exploring the environment, when exposed to the forced-swimming test. It is possible that decreased GABA function is involved in depression associated with diabetes, because a benzodiazepine partially counteracts these changes without modifying blood glucose and glycogen parameters. © 2000 Elsevier Science Inc.

Antidepressant Clonazepam Forced swimming Diabetes

DIABETES mellitus is accompanied by hormonal and neurochemical changes that can be associated with anxiety and depression (3,20). The prevalence of depression is approximately 18% higher in diabetic patients than in the general population, and only 33% of depression cases among diabetic patients are diagnosed and treated (10,21). The recognition and treatment of diabetes-associated depression is important because treatment of depression is associated with higher patient compliance, resulting in more rigorous adherence to the therapeutic regimen required to achieve ideal plasma glucose levels (10).

Previous animal studies have reproduced these depression-like behavioral changes seen in humans (16,25). The duration of immobility in the forced-swimming test, an accepted animal model of depression, was longer in diabetic mice. Insulin treatment reversed the prolonged immobility, providing additional evidence for an association between behavioral changes and diabetes (16). Learned helplessness, however, was unexpectedly similar in intensity when streptozotocin-diabetic rats were compared to nondiabetics. The only difference in the diabetic rats was a decreased effect of tricyclic antidepressant agents on helpless behavior (24,25). Whether the different results seen in these two studies depend upon the species used or the model chosen has not been determined.

The mechanisms involved in depression are still a matter of extensive debate. Diabetes-associated depression could be related to the changes in the quality of life imposed by the chronic illness and/or its treatment, or may be a consequence of neurochemical changes induced by the disease. The most frequently studied neurochemical alterations related to depression include changes in the neurotransmitters serotonin, dopamine, noradrenaline, and gamma-aminobutyric acid (GABA) (6,22,26,29,43). Reduced brain tryptophan levels (21) and decreased brain turnover of cathecolamines and serotonin in diabetic rats (6,22,43) suggests involvement of decreased monamine activity in the genesis of diabetes-associated depression. Other studies have shown that streptozotocin-induced diabetes unbalances GABA, noradrenaline, serotonin, and monoamine metabolite concentration in the ventromedial hypothalamus (29).

The inhibitory amino acid neurotransmitter GABA is present in pancreatic beta cells in concentration as high as

Requests for reprints should be addressed to Helena M. T. Barros, Division of Pharmacology, FFFCMPA, R. Sarmento Leite, 245, PC 90050-170, Porto Alegre, Brazil.

those found in the brain (31). Pancreatic islet binding sites for GABA and benzodiazepines have been shown to be of functional significance in the secretion of insulin in rodents and humans (15,23,39,46). Furthermore, a decrease in GABA concentration in pancreatic islet cells is associated with the insulin synthesis and release deficiency present in diabetes (30). GABA levels are decreased in cerebrospinal fluid of depressed patients (11,34); however, the concentration of GABA within the brains of diabetic patients and its correlation with diabetes-induced behavioral changes has yet to be determined.

Antidepressant agents such as monoamine oxidase inhibitors, tricyclic antidepressants, and serotonin selective reuptake inhibitors interfere with the regulation of insulin release, precluding appropriate control of blood glucose levels (13). Agents acting at the GABA–benzodiazepine receptor complex, such as valproic acid, alprazolam, and clonazepam, are considered to be effective treatments for depressive and bipolar disorders (5,17–19,27). Because these GABAergic drugs are already in clinical use for several neuropsychiatric disorders, their potential for the control of diabetes-induced depression and their effect on blood glucose should be assessed.

Because a GABAergic imbalance might occur in diabetes, also resulting in the symptoms of clinical depression, it was our goal to evaluate the behavior of diabetic rats during the forced-swimming test and to determine whether a positive $GABA_A$ receptor modulator, clonazepam, is an effective antidepressant. We also evaluated the possible interference of this drug with plasma glucose levels and brain and liver glycogen.

METHOD

Animals

Experiments were carried out with 84 adult male Wistar rats reared in the animal quarters of Fundação Faculdade Federal de Ciências Médicas de Porto Alegre, Brazil. Animals were housed in groups of four in polypropylene cages under standard environmental conditions (room temperature of 22 ± 2°C and light cycle from 0700–1900 h. All rats had free access to food and water except for the night before streptozotocin was administered when they were fasted for 12 h. Rearing, maintenance, and experimental procedures comply with the guidelines of the Society of Neuroscience and of the Brazilian College for Animal Experimentation.

Drugs

Sterptozotocin (Sigma Chemical Co., St. Louis, MO) was dissolved to a concentration of 60 mg/ml in 0.05 M citrate buffer (pH 4.3). Clonazepam (Roche do Brasil S.A., São Paulo, Brazil) was dispersed in saline added with 0.5% (v/v) Tween 80, to concentrations of 0.25, 0.5, and 1.0 mg/mL. The control solution was saline with 0.5% Tween 80. All the solutions were prepared immediately before use and were administered IP in a fixed volume of 1 ml/kg body weight.

Glucose Estimation

The presence of glucose in the urine of the animals 72 h after diabetes induction was confirmed with Glukotest (Boehringer Mannheim, Germany). Plasma glucose levels were assessed by means of a colorimetric assay using the glucose oxidase method (Glucose GOD-Ana-Labtest kit, São Paulo, Brazil). Absorbancy was read with a CELM E210D (Brazil) spectrophotometer at 505 nm.

Glycogen determination was analyzed as proposed by Montgomery (26). Immediately after, the blood collection brains and livers were rapidly removed, weighed, and added to 5 ml of a 30%-NaOH solution for digestion. Hot digestion of this mixture was carried out by putting the vial in boiling water for 5 min, until all chunks of tissue disappeared. Solutions were stored at −18°C until glycogen assays were run. For glycogen measurements, 1 ml of tissue–alkali solution was precipitated with 1.5 ml of alcohol 95%. After centrifugation, the pellet was suspended in 2.6 ml of $CaCl_2$ solution (0.75 M) with iodine (3.9×10^{-5} M). Absorbency of glycogen at 460 nm was read immediately.

Procedure

Diabetes was experimentally induced by a single intraperitoneal injection of streptozotocin 60 mg/kg (STZ) to the rat, which were fasted overnight. The animals were kept in individual cages for 72 h, until the presence of glucose in urine was confirmed. From this moment on animals were group housed once more.

Three weeks after diabetes induction, rats were submitted to the forced-swimming test as described by Porsolt et al. (35,36). In this paradigm, antidepressants decrease the immobility time of rats placed with no chance of escape, into an aquarium (22 × 22 × 35 cm) filled with 27 cm of cold water (25°C). On the first day (test), the diabetic and nondiabetic rats were individually placed in the aquarium for 15 min. After the rescue, animals were dried, and both groups were subdivided in four groups to receive clonazepam 0.25, 0.5, and 1.0 mg/kg or control treatment. The doses were administered three times, 24, 5, and 1 h before the next day (retest). On the retest, all animals were introduced into aquariums for 5 min. Diabetic and control animals were also observed for 5 min in an open-field prior to retest and locomotion was measured. All behavioral experiments were performed between 1300 and 1500 h. Both days forced-swimming (test and retest) were recorded on a VCR. These recordings were analyzed with the aid of a BASIC written software (Kevin Willioma, KD Ware Computer, Boston, MA, modified by Thomas Vatne) by a trained observer. The frequency and duration of acts and postures of climbing, swimming, immobility, diving, head shakes, and total mobility were detailed. The latency for the first immobility posture was measured.

Three days later, treatment was repeated using the same doses of clonazepam as before the forced swimming. The rats were decapitated 30 min after the last dose, and blood was collected to measure plasma glucose levels. Brains and livers were removed for glycogen determination.

Statistical Analysis

Frequency and duration of activities within the 15-min test were analyzed as intervals of 5 min and as total time. Individual parameters of the first day of the forced-swimming test of rats were analyzed using a two-way repeated-measure analysis of variance (ANOVA), considering as factors the diabetic condition and the intervals of observation.

The behavioral results of the retest session, plasma glucose levels and tissue glycogen were compared through a two-way analysis of variance (ANOVA-two ways) to analyze the outcome of clonazepam treatment and of the diabetic condition factors. Further differences between the groups were disclosed with the Student–Newman–Keuls test. All results are expressed as mean ± SEM. A difference of $p < 0.05$ was considered statistically significant.

TABLE 1

ANALYSIS OF THE BEHAVIORAL IN THE FORCED-SWIMMING OF DIABETIC (STZ) AND NONDIABETIC RATS (CTR) DURING THE FIRST EXPERIENCE (15-MIN TEST)

Treatment	Immobility Duration	Total Mobility Duration	Immobility Frequency	Total Mobility Frequency	Dive Frequency	Head-Shake Frequency
CTR	558.3 ± 19.9	319.8 ± 19.4	39.9 ± 2.4	38.4 ± 2.5	4.7 ± 0.4	40.7 ± 3.7
STZ	631.9 ± 13.2	246.9 ± 11.7	38.4 ± 2.8	37.6 ± 2.7	3.4 ± 0.5	39.0 ± 3.3
F (1,83)	9.728	10.597	5.319	5.200	3.080	0.108
p	= 0.003	= 0.002	= 0.022	= 0.023	0.026	>0.05

The values are expressed as mean ± SEM; $n = 42$ in each group.

RESULTS

Test and retest sessions were analyzed to better describe the behavioral changes induced by diabetes in rats during forced swimming. The analysis of behaviors during forced swimming on the first day showed that diabetic rats ($n = 42$) presented significantly longer duration of immobility than nondiabetics ($n = 42$; Table 1). Consequently, these animals spent significantly less time executing mobile acts, such as climbing and swimming, around the aquarium. The diabetic rats also presented significantly lower frequencies of mobile and immobile postures and fewer dives than the nondiabetic rats, corroborating the fewer attempts to search for escape. However, head shakes did not differ from nondiabetics, showing that reactivity to the environment was preserved in the diabetic animals. Many acts observed during the first day test were decreased or not present on retest day.

Temporal analysis of behavior in the test session (Table 2) was performed by dividing the whole session into three intervals (first 5 min, from 5 to 10 min, and last 5 min of observation). In general, diabetic rats were more immobile during the forced-swimming test, $F(1, 248) = 6.962$; $p = 0.01$. In both diabetic and nondiabetic rats there was a incipient initial phase of intense mobile behavior, characterized by swimming in the middle of the water and followed by tentative climbing of walls, during the first 5 min, $F(2, 248) = 221.883$, $p < 0.001$. Overall, immobility occurred during less than 46% of the time in the first interval and gradually increased, being observed for 75% of the time of the third interval. However, there is an interaction between diabetic condition and the time interval, $F(2, 248) = 3.550$, $p = 0.031$, which reflects the propensity to display the immobile posture in diabetic animals. In the second interval, nondiabetic rats spent only 69% of the time in immobility, while diabetic animals were immobile for 82% of the time.

Diving, $F(2, 248) = 133.216$, $p < 0.001$, and head shakes, $F(2, 248) = 118.059$, $p < 0.001$, were also more frequent during the first interval than on subsequent intervals. Also, there was an interaction between the diabetic condition and diving on the different time intervals of the test, $F(2, 248) = 4.892$, $p = 0.009$. Diabetic rats dived significantly less frequently because the beginning of the behavioral observation, showing less interest in searching a way out through the bottom (Table 2).

On the retest session of the forced-swimming test, performed 24 h later (Fig. 1), the diabetic condition continued to be associated with longer immobility, $F(1, 83) = 24.957$, $p < 0.001$. Clonazepam treatment significantly interacted with the diabetic condition, $F(3, 83) = 4.281$, $p = 0.008$, and its antiimmobility effect was detected only in the diabetic rats group. Clonazepam 0.25 and 0.5 mg/kg significantly decreased the immobility of diabetic rats in comparison to controls and clonazepam 1.0 mg/kg (Fig. 1A). However, only the 0.25-mg/kg dose decreased immobility to the same level seen in non-diabetic rats. Diabetic and nondiabetic rats showed similar immobility latencies during retest, $F(1, 83) = 1.415$, $p > 0.05$, and, for both conditions, clonazepam 0.5 mg/kg delayed the first immobile posture as may be seen in Fig. 1B, $F(3, 83) = 4.886$, $p = 0.003$. Mobile behaviors total duration was a mirror image in respect to the immobile behavior [diabetic condition, $F(1, 83) = 25.452$, $p < 0.001$; treatment, $F(3, 83) = 3.051$, $p = 0.034$; diabetes × treatment, $F(3, 83) = 4.445$, $p = 0.006$.] For both diabetic and nondiabetic rats, climbing behavior predominated. Swimming occurred in very short bouts in between climbing tentatives. However, there is a significant decrease in this active behavior in diabetic rats, $F(1, 83) = 21.303$, $p < 0.001$. There is an interaction between the diabetic condition and clonazepam treatment, $F(3, 83) = 3.351$, $p = 0.023$, be-

TABLE 2

TEMPORAL ANALYSIS OF BEHAVIORS IN THE FORCED-SWIMMING CONSIDERING THREE DIFFERENT INTERVALS OF TIME DURING TEST DAY (15-MIN) OF DIABETIC RATS (STZ) AND NONDIABETIC RATS (CTR)

Condition	Time Interval (s)	Immobility Duration (s)	Total Mobility Duration (s)	Dive Frequency	Head-Shake Frequency
CTR	0–300	133.6 ± 5.3	163.4 ± 4.9	4.6 ± 0.4	23.9 ± 2.1
	300–600	207.4 ± 9.2	89.8 ± 8.4*	0.5 ± 0.2*	9.2 ± 1.4
	600–900	234.6 ± 10.3	61.4 ± 10.2*	0.05 ± 0.1*	5.2 ± 0.9
STZ	0–300	**142.4 ± 5.4**	**154.4 ± 5.0**	**3.0 ± 0.4**	25.6 ± 1.9
	300–600	**246.8 ± 5.5**	**48.4 ± 5.0***	**0.3 ± 0.1***	13.6 ± 1.9
	600–900	**257.1 ± 6.5**	**37.9 ± 5.1***	**0.07 ± 0.1***	5.2 ± 0.8

The values are expressed as mean ± SEM; $n = 42$ in each group.
Bold represents differences between diabetic and nondiabetic rats on the same time frame.
*Represents difference from the respective 0–300 s interval.

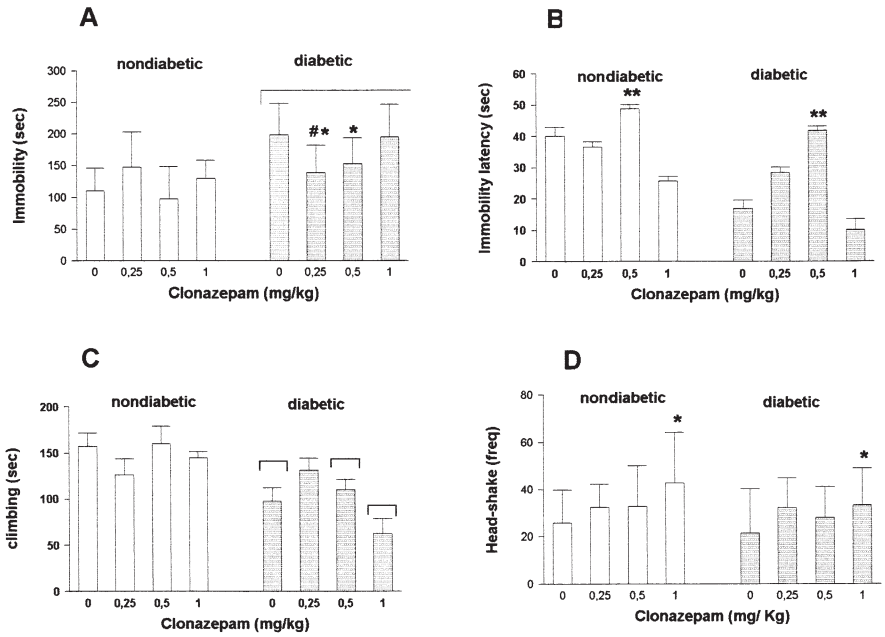

FIG. 1. Duration of immobility (A), immobility latency (B), duration of climbing (C), and frequency of head-shakes (D) of streptozotocin-induced diabetic rats (STZ) and nondiabetic rats (CTR) treated with clonazepam 24, 5, and 1 h before the 5 min forced-swimming retest. Results are means ± SEM. n = 10–12 animals each group. Denotes significant differences between diabetic/nondiabetic rats; *Denotes significant differences from clonazepam 0.0 mg/kg; #Denotes significant differences from clonazepam 1.0 mg/kg; **Denotes differences from all others doses of clonazepam.

cause clonazepam 0.25 mg/kg increased climbing duration to levels similar to those of nondiabetic animals (Fig. 1C).

During the retest, the number of times animals shifted between mobile and immobile behavior was not influenced by diabetes, because immobility frequency was similar for both condition groups (CTR = 15.5 ± 1.8 and STZ = 13.9 ± 1.0). Treatment with clonazepam did not alter the frequency of active and immobile postures. There was no difference for the number of head shakes of diabetic and nondiabetic animals. Head-shake frequency was influenced by clonazepam treatment (Fig. 1D), with increased number after the highest dose of clonazepam, $F(3, 83) = 2.854$, $p = 0.043$, irrespective to the diabetic condition. Swimming frequency (CRT = 18.1 ± 4.7) was not modified by the diabetic condition or by clonazepam treatment. Although the number of dives was very small in the forced-swimming retest session, clonazepam doses decreased this behavior in a significant way, $F(3, 83) = 3.681$, $p = 0.016$ (results not shown).

To eliminate the possibility of a decrease in immobility as consequence of an excitatory effect of clonazepam (33), the rats were observed in an open field. Clonazepam deceased locomotion and rearing behavior in a dose-dependent manner (data not shown).

As expected, plasma glucose levels of the STZ-treated rats were significantly higher than those of the controls, $F(1, 83) = 661.04$, $p < 0.01$. Brian glycogen concentration was also significantly increased, $F(1, 77) = 9.19$, $p = 0.003$, while liver glycogen was significantly decreased in STZ-treated rats, $F(1, 77) = 47.09$, $p < 0.001$. Clonazepam did not change blood glucose values, brain glycogen, and liver glycogen levels, as may be seen in Table 3.

DISCUSSION

The results described herein demonstrate that STZ-induced diabetic rats prematurely and repeatedly present more intense immobility in the forced-swimming test, demonstrating their susceptibility to behavioral alterations in this animal model.

The forced-swimming test is accepted as an animal model of depression due to its very good face and predictive validity. The immobility of the animals is a relatively specific phenomenon

TABLE 3

PLASMA GLUCOSE AND LIVER AND BRAIN GLYCOGEN OF DIABETIC RATS (STZ) AND NONDIABETIC RATS (CTR) TREATED WITH CLONAZEPAM (CNZ)

Condition	CNZ (mg/kg)	n	Glucose (mg/dl)	Glycogen (mg%)	
				Liver	Brain
CTR	0.0	10	84.5 ± 8.4	5.5 ± 1.0	0.040 ± 0.014
	0.25	10	106.1 ± 9.3	5.8 ± 1.8	0.042 ± 0.022
	0.5	10	108.1 ± 15.7	5.8 ± 1.5	0.045 ± 0.021
	1.0	9	114.9 ± 14.8	6.4 ± 1.1	0.051 ± 0.025
STZ	0.0	8	**437.5 ± 67.7**	**2.6 ± 1.5**	**0.054 ± 0.014**
	0.25	7	**385.2 ± 55.0**	**3.1 ± 1.2**	**0.051 ± 0.014**
	0.5	9	**365.9 ± 74.7**	**3.9 ± 2.3**	**0.063 ± 0.017**
	1.0	8	**380.9 ± 77.6**	**3.0 ± 1.4**	**0.055 ± 0.020**

Bold represents differences between diabetic and nondiabetic rats. The values are expressed as mean ± SEM; n = numbers of animals.

and reproduces some aspects of depression in humans (2,35,36,45), although there are conflicting opinions in this regard. The characteristic immobile behavior is interpreted as giving up trying to escape from the unpleasant and stressful situation. Additional support for the face validity of the forced-swimming test is found in the literature. Forced-swimming decreases motivation and perseverance (45), and produces cognitive impairment (1). The immobile posture during the test is higher in more "emotional" strains (33), and when animals are isolated (47). Rats subjected to the forced-swimming test also present anhedonia and slower learning in a Morris water maze (Rates and Barros, in preparation). Our present results add to the face validity of the forced swimming, because they replicate in diabetic animals depression-like behavioral changes similar to those seen in diabetic humans (13,20,21).

In most studies, the behavior of the animals on the initial test day of forced swimming is not analyzed. With the detailed study of both sessions, it is possible to observe intense and premature immobility already on the first forced-swim experience, when rats are diabetic. The search for a way out by tentative climbing of the pool walls and diving was also reduced earlier in diabetics during the test session and replicated during the retest. More immobility and less active behaviors can be interpreted as a lack of interest in exploring the environment and seeking for escape by the diabetic animals. Diabetic rats also presented longer immobility during the second day of the forced-swimming test, in agreement with previous observations of enhanced immobility in streptozotocin-induced diabetic mice (16). It becomes evident that there are no species differences between mice and rats concerning the behavioral changes due to diabetes in the forced-swimming model. These observations, together with the demonstration of the reversal of the immobility after insulin treatment, support the conclusion that diabetes alters the physiological balance, leading to more intense depression-like behaviors. Experiments with other depression models such as the inescapable shock test (24,25), however, show that diabetic rats cope with the stressful stimuli like nondiabetic animals. It is possible that the forced-swimming test is a more sensitive indicator of depression-like behaviors in diabetic rodents than the inescapable shock test.

It is widely accepted that all antidepressant agents, whether of tricyclic or atypical structure, acting as nonselective or serotonin-selective reuptake inhibitor, or through MAO inhibition, reduce immobility (2,7,9,35,36). Other psychoactive agents, such as psychostimulants, hypnotics, and anxiolytics, lacking clinical antidepressant efficacy do not change behaviors in the forced swimming, as recently reviewed (2). This study clearly demonstrates that clonazepam treatment produces an antiimmobility effect in diabetic animals. This antiimmobility property does not seem to be related to any anxiolytic effect, because the same doses of clonazepam did not increase frequency or duration of open-arm entries by diabetic rats tested in the elevated plus-maze (12).

Contradictory results have been published regarding the effects of other benzodiazepines on forced-swimming test behaviors. Chlordiazepoxide and diazepam were seen to decrease immobility (32), not to affect duration of immobility of rats (35) or to increase immobility of mice (28). The present experiment with an agent acting at the "central-type" benzodiazepine receptors (42) demonstrates that clonazepam shows a significant antiimmobility effect only in diabetic animals, which present longer duration of immobile behavior. Differing from the antidepressants, which decrease immobility in comparison to nondiabetic controls (2,7,9,35,36), clonazepam only decreased immobility of the diabetic rats to the levels of control animals. The same pattern of effect was demonstrated when diabetic mice were treated with insulin and had the immobility duration decreased to the same level as those of control nondiabetic mice (16). Nevertheless, the immobility latency, proposed as a novel and suitable parameter to detect antidepressant efficacy (8), was increased in both diabetic and nondiabetic rats after clonazepam dosing. This partial effect might mean that clonazepam has weak antidepressant action. The effect in diabetic rats could also be considered to predict antidepressant effects in specific cases of depressive disorders. Any of these explanations could justify why only now clonazepam is getting acceptance, and is slowly being used as a therapeutic agent for psychiatric disorders (19,27). The antidepressant effect of clonazepam is described as marked to moderate, and to occur within 1 week of treatment for more than 80% of patients (19); however, there are few controlled clinical studies with this drug.

It is likely that the mechanism of clonazepam's antiimmobility effect is related to the GABA system, although this hypothesis has yet to be tested in diabetic rats. Plasma concentration of GABA is 10 to 15% lower in patients with mania and depression (34) and low GABA plasma levels are found in patients with alcoholism and mood disorders (40) and with the menstrual cycle-related mood disorders in women (14). Cerebrospinal fluid GABA levels are inversely correlated with the severity of the symptoms in depressed individuals (11). Antidepressant drug treatment stabilizes the GABA system to its normal levels, correlating with the remission of the symptoms (34). Consistent with these clinical data, GABA is decreased in the nucleus accumbens and cerebral cortex of rats submitted to forced swimming (4), and GABA agonists decrease immobility in the rat or mouse forced-swimming test, which is reversed by $GABA_A$ receptor antagonists (28). In addition, prolonged administration of different antidepressants decreases the density of benzodiazepine–ligand sites at the $GABA_A$ complex (42) and diminishes $GABA_B$ receptors (41). The neurochemical mechanism involved in the decrease of the extracellular GABA in depression is still unknown; however, it must involve the interaction between GABA and norepinephrine (29) or GABA and serotonin (34). Both interactions may have produced changes in different behavioral displays after clonazepam. The shorter immobility by clonazepam was associated with increased duration of climbing, and may be interpreted as due to enhanced catecholaminergic activity (7). An interaction of GABA-acting drugs with brain serotonin could justify the head shakes and antidepressant effect of clonazepam (37,38) and of valproate, another GABA-enhancing agent (44), and deserves further attention.

In conclusion, the forced-swimming test is a reliable model of depression in diabetic rats. In our current preclinical study, low doses of clonazepam were shown to be a moderately effective antidepressant in diabetic rats. It may be foreseen that more knowledge on $GABA_A$-acting agents in diabetes can be an interesting source of new therapeutic approaches in this chronic disease. Additional studies will be necessary to establish the relationship between behavioral outcome and brain and pancreas GABAergic systems. Likewise, the clinical use of clonazepam as an antidepressant agent for insulin-dependent diabetic patients deserves further controlled clinical studies, because it is an useful anxiolytic and does not interfere with blood glucose control.

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