Antidepressant Effects of Tricyclic Antidepressants and Selective Serotonin-Uptake Blockers in Diabetic Rats

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Because it was reported that diabetic rodents were resistant to the effects of several tricyclic antidepressants in various psychopharmacological models, we decided to test the hypothesis that the serotonergic dysfunction seen in diabetes might participate in this phenomenon. The ability of three serotonin-uptake blockers to reverse the performance deficit in learning induced by previous uncontrollable stress (learned-helplessness paradigm) was investigated in streptozocin-induced diabetic rats. Three weeks after induction of diabetes, rats were subjected to a session of 60 inescapable electric foot shocks and, after 48 h, to three daily sessions of two-way shuttle-box training. Three serotonin-uptake blockers were given intraperitoneally over 5 consecutive days. As with nondiabetic rats, citalopram (1 mg · kg^{-1} · day^{-1}), fluoxetine (2 and 4 mg · kg^{-1} · day^{-1}), and fluvoxamine (4 mg · kg^{-1} · day^{-1}) reduced the number of escape failures in diabetic rats. From these data, we suggest that it is unlikely that the impaired response of diabetic rats to tricyclic antidepressants is caused by serotonergic dysfunction. *Diabetes* 38:1161–64, 1989

The biogenic amine theory of depression remains the most widely accepted basis for understanding mood disorders. This theory proposes that insufficient catecholamine and serotonin activity at central nervous system postsynaptic levels are associated with depression and that antidepressants increase monoamine levels by inhibiting amine reuptake. Diabetes has been shown to be associated with alterations in central noradrenergic (1,2) and serotonergic (3,4) systems.

Because tricyclic antidepressants are considered (at least) preferential or selective inhibitors of norepinephrine uptake, previous findings that showed an impaired response of diabetic rats to tricyclic antidepressants in various test conditions suggested possible involvement of noradrenergic dysfunction in the impaired responses (5,6). The possibility that a serotonin deficiency may be involved has not been tested systematically. However, serotonergic mechanisms have been considered critical for understanding the action of antidepressant drugs (7). Indeed, clinical trials have indicated that serotonin-uptake blockers (i.e., citalopram, fluvoxamine, fluoxetine) are effective antidepressants and comparable in efficacy to widely used tricyclic antidepressants (8,9).

Among animal models of depression, learned helplessness is based on the fact that exposure to uncontrollable stress produces performance deficits in subsequent learning (10). Our aim was to investigate the response of diabetic rats to the action of specific serotonin-uptake blockers.

RESEARCH DESIGN AND METHODS

Wistar male Allinghton Farms (AF) rats (R. Janvier, Centre d'Elevage, Le Genest-St.-Jôle, France) weighing 200 ± 5 g at the beginning of the experiments were housed in groups of 10 per cage under standard conditions (room temperature 21 ± 1°C, 12-h light-dark cycle, water and food ad libitum).

Induction of helplessness. Electric shocks were delivered to stainless steel–grid floors (1.5-cm mesh) in chambers (20 × 10 × 10 cm) with Plexiglas walls and covers. A constant-current shocker delivered 60 scrambled, randomized, inescapable 15-s shocks every min ± 15 s. For each rat, the shock intensity was adjusted to be lower than the threshold of vocalization to avoid painful stimulation. Control rats were placed in identical chambers for 1 h, and no shock was administered. Induction of helplessness was performed on day 1 between 0900 and 1100.

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Conditioned avoidance training. To evaluate escape deficits, avoidance training was initiated on day 3, 48 h after inescapable-shock preconditioning in automated two-way shuttle boxes (60 × 21 × 30 cm) with Plexiglas walls and a floor consisting of stainless steel rods spaced 1-cm apart. Each shuttle box was divided into two chambers of equal size by a stainless steel partition with a gate providing access to the adjacent compartment through a 7 × 7-cm opening. Animals were placed individually into the shuttle box and allowed to habituate to the environment for 5 min (for 1st session only) and then were subjected to 30 avoidance trials; between-trial intervals were 30 s. During the first 3 s of each trial, a light signal (used as conditional stimulus [CS]) was presented. Crossing the gate into the other compartment of the box during this CS-only period (referred to as avoidance response) allowed the rats to avoid shocks. A 3-s period with CS plus electric foot shock (0.8 mA) was presented if an avoidance response did not occur within this CS-only period. Crossing the gate into the other compartment during the CS plus shock period constituted an escape response. Absence of an escape response during the 3-s duration of CS plus shock was considered an escape failure. Escape failure is usually defined as failure to escape within a 30- to 60-s period in procedures designed to assess learned helplessness. However, we defined this deficit to exist if an animal did not escape within 3 s. This procedural modification was designed to avoid prolonged noxious stimulation and was justified by previous studies that indicated that the first few seconds after shock is given are critical for detecting escape deficits in animals preexposed to inescapable shocks (11–14). Avoidance sessions were performed for 3 consecutive days (days 3–5) in the morning, and the number of escape failures (no-crossing responses) during shock delivery was recorded.

Drug administration. Four weeks before testing, experimental diabetes was induced by a series of three injections of streptozocin (STZ; 37.5, 37.5, and 50 mg/kg i.p.) at 3-day intervals. Only the diabetic rats (97% of the animals) were used for behavioral study. Diabetes was detected with Ames strips sensitive to glycosuria. The extent of diabetes in all rats was assessed by measurement of fasting blood glucose levels with an Ames Glucocheck and glucose-sensitive reagent sticks. Protamine zinc insulin (10 U/kg s.c., Novo, Bagsvaerd) was injected once a day for 1 wk to imipramine-treated rats from the 30th day after injection of STZ. The insulin doses were subsequently adjusted to maintain nonfasting blood glucose levels of ~5 mM.

On day 30, the rats (10/group) were randomly treated according to one of the following protocols. *1*) Controls (diabetic and nondiabetic rats), which received no shock, were given vehicle (distilled water). *2*) Experimental animals (diabetic and nondiabetic rats), given inescapable shocks, were injected daily with serotonin-uptake blockers (1 mg/kg citalopram, 2 and 4 mg/kg fluoxetine, or 4 mg/kg fluvoxamine), tricyclic antidepressants (24 mg/kg clomipramine, 24 mg/kg desipramine, or 32 mg/kg imipramine), or vehicle. STZ (Upjohn, Kalamazoo, MI); clomipramine, desipramine, imipramine (Ciba-Geigy, Basel); citalopram (Lundbeck, Copenhagen); fluoxetine (Lilly, Indianapolis, IN); and fluvoxamine (Duphar, Southampton, UK) were injected as solutions in volumes of 0.5 ml/100 g body wt i.p. Serotonin-uptake blockers were suspended in acacia gum (5%), and the other drugs were dissolved in distilled water.

Injections were given 6 h after induction of learned helplessness and then twice a day, in the morning and afternoon, in divided doses (so that daily dose for citalopram was 1 mg/kg).

Dosage and treatment schedules of tricyclic antidepressants and serotonin-uptake blockers were chosen according to Martin et al. (13,15) to produce a reversal of escape failures that would be complete and rapid in onset.

Between-group comparisons were made by two-way analysis of variance (ANOVA), the variables being group and session, followed by Dunnett's *t* test.

RESULTS

ANOVA revealed that non-drug-treated rats preexposed to inescapable shocks (experimental helpless rats) exhibited significantly more escape failures than non-drug-treated rats (controls) not preexposed to inescapable shocks ($P < .001$ for each group). This difference was observed whether the rats were diabetic or nondiabetic (Table 1). Because of this finding, the performances of the diabetic and nondiabetic helpless rats were pooled in Fig. 1; statistical comparisons were performed on the performances of the appropriate groups.

The mean ± SD plasma glucose level of diabetic rats on the 30th day after injection of STZ was 26.04 ± 9.3 mM, whereas that of nondiabetic rats was 5.79 ± 1.66 mM. The value for insulin-treated rats was 5.51 ± 2.62 mM.

ANOVAs (session × group) performed on the data obtained from the three consecutive shuttle-box sessions with the helpless rats revealed the following. *1*) The number of escape failures exhibited by diabetic rats treated with tricyclic antidepressants (Fig. 1) was not significantly different from that of the vehicle-treated rats and was significantly higher than that of the corresponding nondiabetic drugged rats (clomipramine, $F[1,58] = 24.14$, $P < .001$; desipramine, $F[1,58] = 37.18$, $P < .001$; imipramine, $F[1,58] = 29.57$, $P < .001$). Furthermore, the insulin regimen of the diabetic rats subgroup permitted the imipramine to act as it usually does to lower the number of escape failures (Fig. 2). *2*) The number of escape failures exhibited by diabetic rats treated

TABLE 1
Number of escape failures during 3 consecutive daily shuttle-box sessions in diabetic or nondiabetic rats not treated with drugs

Rats	*n*	Shuttle-box session 1	2	3
Nondiabetic	20			
Control		7.0 ± 2.2	4.4 ± 1.6	3.8 ± 1.0
Experimental		21.6 ± 1.4	20.0 ± 1.3	18.1 ± 1.9
Diabetic	20			
Control		7.3 ± 1.9	5.7 ± 1.9	5.0 ± 1.5
Experimental		18.1 ± 2.1	16.0 ± 2.4	21.1 ± 2.4

Values are means ± SE of number of escape failures/30 trials in each session. Escape failure refers to rat's failure to change compartments during electric foot shock. Experimental diabetic rats were shock preconditioned by being given 60 inescapable shocks before the shuttle-box sessions. Controls were not shock preconditioned.

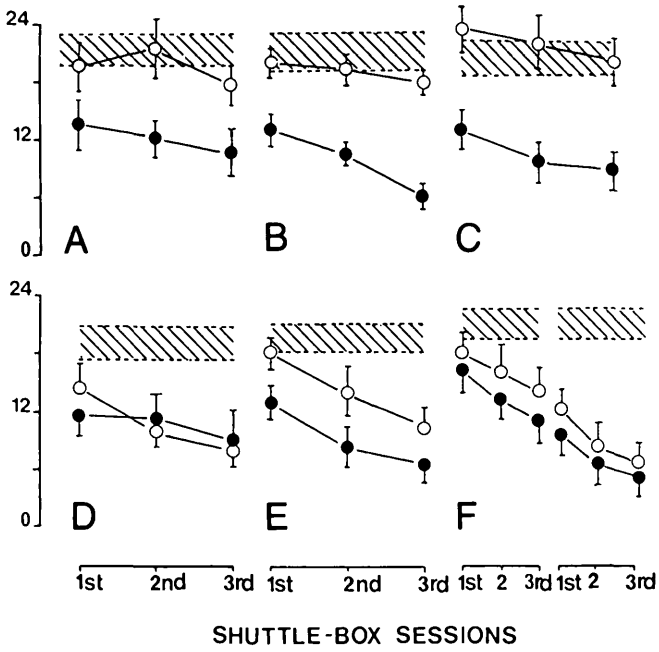

FIG. 1. Reversal by clomipramine (*A*, 24 mg · kg^{-1} · day^{-1}), imipramine (*B*, 32 mg · kg^{-1} · day^{-1}), desipramine (*C*, 24 mg · kg^{-1} · day^{-1}), citalopram (*D*, 2 mg · kg^{-1} · day^{-1}), fluvoxamine (*E*, 4 mg · kg^{-1} · day^{-1}), and fluoxetine (*F*, 2 and 4 mg/day) of helpless behavior (escape failure) in nondiabetic (●) and diabetic (○) rats. Escape failure refers to rat's failure to change compartments during electric foot shock (30 trials/session). Serotonin-uptake blockers and tricyclic antidepressants were injected intraperitoneally twice daily from end of induction of helplessness. Diabetes was induced by 3 injections of streptozocin 4 wk before behavioral testing. *A–C*: $P < .001$ (by analysis of variance) for ability to escape from shock of drugged nondiabetic vs. diabetic rats. *Hatched areas*, averaged mean (± SE) performance and variation of diabetic and nondiabetic helpless rats tested with vehicle and included in separate experiments. Values are means ± SE of escape failures at each of 3 consecutive daily shuttle-box sessions.

with serotonin-uptake blockers significantly differed from that of helpless nontreated rats. The scores of diabetic and nondiabetic rats treated with serotonin-uptake blockers citalopram and fluoxetine were not statistically different at the third shuttle-box session, although they were significantly different for fluvoxamine at the first shuttle-box session. However, all three drugs were able to induce an attenuation of escape failures in diabetic and nondiabetic rats.

DISCUSSION

Most clinically active antidepressant drugs, particularly tricyclics, are known to induce a reversal of depressive behavior in rats tested in the learned-helplessness paradigm (11–14).

It has been shown that repeated injections of citalopram, fluvoxamine, or zimelidine, which are serotonin-uptake inhibitors with antidepressant activity in humans (8,9), prevented escape deficits in rats at low doses if these substances were administered after shuttle-box sessions (15). Our data show that the escape deficit induced by inescapable shocks was not reduced in helpless diabetic rats treated with tricyclic antidepressants as it was in the helpless nondiabetic group.

This impaired response of diabetic rats treated with tricyclics has been previously reported and could not be accounted for by differences between diabetic and nondiabetic rats in concentrations of desipramine in the brain (5). The reversal of the behavioral deficit of diabetic rats by insulin therapy suggests that the resistance may have been a functional consequence of diabetes rather than STZ on the central nervous system. One of the mechanisms possibly underlying this resistance is postsynaptic impairment of the central noradrenergic neurons.

In this regard, numerous data from animal experiments indicate a complex indolamine dysfunction in diabetes. L-Tryptophan levels are reduced, whereas branched-chain amino acid concentrations are increased in the diabetic rat brain (3,16). Moreover, diabetic rats require a higher dose of L-tryptophan than nondiabetic rats to induce the same changes in brain L-tryptophan as nondiabetic rats (3,17). The brain amino acid pattern in diabetes partly results from the effects of insulin insufficiency. Indeed, insulin has been reported to increase brain L-tryptophan indirectly by lowering serum levels of the amino acids that compete with L-tryptophan for brain uptake (18). However, there is a poor correlation between central L-tryptophan levels and serotonin function in diabetic animals. As a matter of fact, brain concentrations of serotonin and 5-hydroxyindoleacetic acid were reported to increase during the early development of diabetes (15 days; 19) or remain normal, whereas the reductions in brain L-tryptophan were large (17,18). This could be attributed to enhancement in biosynthesis and/or decline in the rate of serotonin metabolism. Enhancement of the activity of tryptophan 5-monooxygenase (which could account for compensatory serotonin synthesis) and decreased monoamine oxidase inhibitor activity (which suggests de-

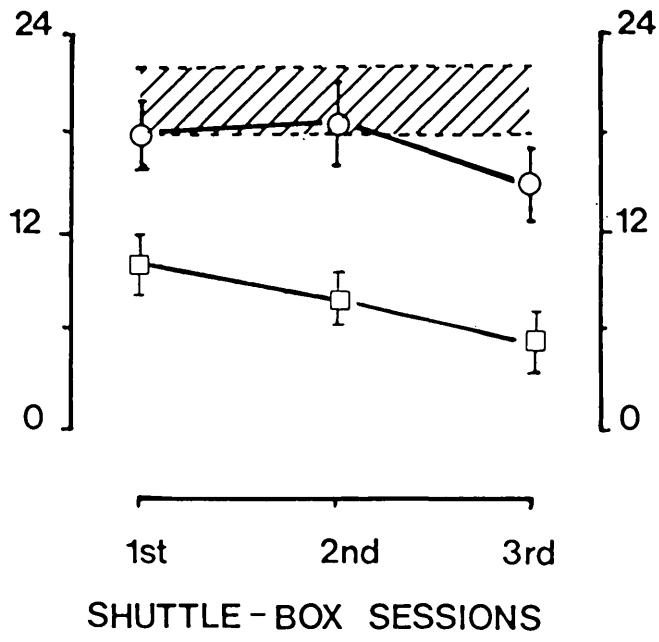

FIG. 2. Effects of insulin (10 U/kg s.c.) therapy on reversal of escape failures by imipramine (32 mg · kg^{-1} · day^{-1}) in diabetic rats on 30th day after injection of streptozocin. Data are means ± SE of escape failures of 30 shocks at each of 3 consecutive daily shuttle-box sessions (Fig. 1). $P < .001$ (by analysis of variance) for ability to escape from shocks of diabetic rats treated with imipramine plus insulin (□) vs. diabetic rats treated with imipramine alone (○). *Hatched area*, averaged mean (± SE) performance and variation of diabetic and nondiabetic helpless rats tested with vehicle.

creased metabolism) have been found soon after the onset of diabetes (20–22).

The in vivo rate of brain serotonin synthesis has been clearly shown to be decreased in untreated diabetic rats (4,21). Of particular interest are the electrochemical studies by Broderick and Jacoby (23), who showed an increase in extracellular serotonin level in the striatum of untreated acutely diabetic (3 days) rats that returned to normal after 3–7 wk (23). The same authors also found that untreated diabetic rats responded to L-tryptophan with a dramatic and significant decrease in striatal serotonin release (24).

However, little is known about the ability of diabetic animals to respond to a serotonin-uptake blocker. It has been previously shown that, although fluoxetine administration increased brain serotonin levels in control and diabetic rats, the level in control rats was higher than that in the alloxan-induced diabetic rats, suggesting that the brain does not respond to fluoxetine, which was found in the control rats (19). Although the selective serotonin-uptake blockers tested were active in reversing depressive behavior in helpless diabetic and helpless nondiabetic rats, our data do not support involvement of a central serotonergic dysfunction as a critical factor of the impaired response of diabetic rats to tricyclic antidepressants.

In addition, the fact that serotonin-reuptake blockers are better than tricyclic antidepressants in diabetic rats for alleviation of learned helplessness could be of practical clinical importance, because humans with diabetes are a population at risk for developing depressive diseases (25).

REFERENCES

1. Bitar M, Koulu M, Rapoport SI, Linnoila M: Diabetes-induced alteration in brain monoamine metabolism in rats. *J Pharmacol Exp Ther* 236:432–37, 1985
2. Trulson ME, Himmel CE: Effects of insulin and streptozotocin-induced diabetes on brain norepinephrine metabolism in rats. *J Neurochem* 44:1873–76, 1985
3. McKenzie RG, Trulson ME: Effects of insulin and streptozotocin-induced diabetes on brain tryptophan and serotonin metabolism in rats. *J Neurochem* 30:205–11, 1978
4. Trulson ME, Jacoby JH, MacKenzie RG: Streptozotocin-induced diabetes reduces brain serotonin synthesis in rats. *J Neurochem* 46:1068–72, 1986
5. Massol J, Martin P, Belon JP, Puech AJ, Soubrié P: Helpless behavior (escape deficits) in streptozotocin-diabetic rats: resistance to antidepressant drugs. *Psychoneuroendocrinology* 14:145–53, 1989
6. Massol J, Martin P, Chatelain F, Soubrié P, Puech AJ: Impaired response of experimental diabetic mice to tricyclics: a possible beta-adrenergic mechanism. *Pharmacol Biochem Behav* 31:807–12, 1988
7. Willner P: Antidepressants and serotonergic neurotransmission in integrative review. *Psychopharmacology* 85:387–404, 1985
8. Lemberger L, Fuller RW, Zerbe RL: Use of specific serotonin uptake inhibitors as antidepressants. *Clin Neuropharmacol* 4:299–317, 1985
9. Asberg M, Eriksson B, Martensson B, Träskman-Bendz L, Wägner A: Therapeutic effects of serotonin uptake inhibitors in depression. *J Clin Psychiatry* 47 (Suppl. 4):23–35, 1986
10. Seligman MEP: *Helplessness: On Development, Depression and Death.* San Francisco, CA, Freeman, 1975
11. Telner JI, Singhal RL, Lapierre YD: Reversal of learned helplessness by nortriptyline. *Prog Neuro-psychopharmacol* 5:587–95, 1981
12. Sherman AD, Sacquitne JL, Petty F: Specificity of the learned helplessness model of depression. *Pharmacol Biochem Behav* 16:449–54, 1982
13. Martin P, Soubrié P, Simon P: Shuttle-box deficits induced by inescapable shocks in rats: reversal by the beta-adrenoceptor stimulants clenbuterol and salbutamol. *Pharmacol Biochem Behav* 24:177–81, 1986
14. Martin P, Soubrié P, Simon P: The effect of mono-amine oxidase inhibitors compared with classical tricyclic antidepressants on learned helplessness paradigm. *Prog Neuro-psychopharmacol Biol Psychiatry* 11:1–7, 1987
15. Martin P, Laporte AM, Soubrié P, El Mestikawy S, Hamon M: Reversal of helpless behavior in rats by serotonin uptake inhibitors. In *Proc Int Conf Behavioural Pharmacology of 5-HT, Amsterdam, 24–27 November 1987*
16. Crandall EA, Fernstrom JD: Acute changes in brain tryptophan and serotonin after carbohydrate or protein ingestion by diabetic rats. *Diabetes* 29:460–66, 1980
17. McKenzie RG, Trulson ME: Regional accumulation of tryptophan and serotonin metabolism following tryptophan loading in diabetic rats. *J Neurochem* 31:157–60, 1978
18. Curzon G, Fernando JCR: Drugs altering insulin secretion: effects on plasma and brain amino acids and on brain 5-hydroxytryptamine turnover. *Br J Pharmacol* 60:401–408, 1977
19. Kolta MG, Soliman KFA, Williams BB: Role of 5-hydroxytryptamine in the regulation of brain neuropeptides in normal and diabetic rat. *Horm Res* 23:112–21, 1986
20. Mayanil CSK, Kazmi SMI, Baquer NZ: Changes in monoamine oxidase activity in rat brain during alloxan diabetes. *J Neurochem* 38:179–83, 1982
21. Crandall EA, Gillis MA, Fernstrom JD: Reduction in brain serotonin synthesis rate in streptozotocin-diabetic rats. *Endocrinology* 109:310–12, 1981
22. Fernstrom JD: Tryptophan availability and serotonin synthesis in rat brain: effects of experimental diabetes. In *Progress in Tryptophan and Serotonin Research.* Schlossberger HG, Kochen W, Linzen B, Steinhart H, Eds. Berlin, de Gruyter, 1986, p. 161–72
23. Broderick PA, Jacoby JH: Serotonergic function in diabetic rats: psychotherapeutic implications. *Biol Psychiatry* 24:234–39, 1988
24. Broderick PA, Jacoby JH: Diabetes-related changes in L-tryptophan-induced release of striatal biogenic amines. *Diabetes* 37:956–60, 1988
25. Murawski BJ, Chazan BI, Balodimos MC, Ryan JR: Personality patterns in patients with diabetes mellitus of long duration. *Diabetes* 19:259–63, 1970