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Subsensitivity to 5-hydroxytryptamine in agonists occurs in streptozocin-diabetic rats with no change in [^{3}H]-5-HT receptor binding

M. E. TRULSON*, R. G. MACKENZIE,† *Department of Psychology, University of Texas at Dallas, P.O. Box 688, Richardson, Texas 75080 and †Department of Biology, Texas A. & M. University College Station, Texas 77843, U.S.A.*

Diabetic rats display large decreases in brain tryptophan concentrations, but no significant changes in the concentrations of 5-hydroxytryptamine (5-HT) or its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (Curzon & Fernando 1977; MacKenzie & Trulson 1978a,b). This outcome may be attributable, at least in part, to a compensatory increase in tryptophan hydroxylase activity in diabetic animals (Trulson & MacKenzie 1980). Diabetes reduces brain tryptophan by producing large increases in plasma branched chain amino acids which compete with tryptophan for entry into the brain and by decreasing the plasma tryptophan concentration (Clark et al 1968; Bloxam 1972; MacKenzie & Trulson 1978c). As a result of the reduced tryptophan uptake, brain 5-HT and 5-HIAA accumulation is attenuated following systemic tryptophan loading in diabetic rats (MacKenzie & Trulson 1978b). This attenuation is manifested in behavioural subsensitivity to tryptophan after monoamine oxidase inhibition, as assessed with a behavioural syndrome that specifically reflects the activity in central 5-HT-mediated synapses (MacKenzie & Trulson 1978d). Diabetic rats were also subsensitive to *p*-chloroamphetamine, a 5-HT-releasing agent (Gallager & Sanders-Bush 1973; Wong et al 1973; Trulson & Jacobs 1976), even though the endogenous stores of 5-HT are not changed in the diabetic state. To more fully explore this phenomenon, we have examined the behavioural responsiveness of diabetic rats to L-5-hydroxytryptophan, which bypasses the rate-limiting tryptophan hydroxylase step (Gal 1975), 5-methoxy-*NN*-dimethyltryptamine, a direct-acting 5-HT receptor agonist (Fuxe et al 1972; Trulson & Jacobs 1976), and amphetamine, a 5-HT releaser (Sloviter et al 1978) for which the uptake into the brains of diabetic rats has been measured. We also measured specific [^{3}H]-5-HT receptor binding in diabetic animals to determine whether the behavioural subsensitivity to 5-HT agonists is attributable to an alteration in 5-HT receptors.

Behavioural observations were made with rats placed in pairs in round plastic buckets (20 cm high × 35 cm in diameter) with metal screen lids and wood shavings covering the floor. After drug administration, the rats were examined for signs of the behavioural syndrome consisting of resting tremor, rigidity, Straub tail, hindlimb abduction, lateral head weaving and reciprocal forepaw treading (Jacobs 1976). If at least four of these six signs were observed the syndrome was rated as present.

Female Sprague-Dawley rats (250–320 g) were made diabetic by injections of streptozocin (75 mg kg^{-1} i.p.) dissolved in citrate buffer pH 4·5 to 75 mg ml^{-1}. Controls received equivolume injections of buffer alone. Diabetes was verified by polydipsia, polyuria, and glucosuria. In addition, blood glucose concentrations (measured on blood from the tail vein) were determined (using a YSI Model 23A glucose analyzer) on a random sample of 6 diabetic and 4 control rats. Four to six weeks after the injections of streptozocin or buffer, the rats were administered L-5 hydroxytryptophan (5-HTP, 50, 100, 150, 200, 250, 300, 350 or 400 mg kg^{-1}, i.p.), 5-methoxy-*NN*-dimethyltryptamine (5-MeODMT, 0·50, 1·0, 1·5, 2·0, 2·5 or 3·0 mg kg^{-1}, i.p.) or (+)-amphetamine sulphate (10, 20, 40, 60, or 80 mg kg^{-1}, i.p.). The rats were observed for signs of the syndrome for 1 h after drug administration. Estimates of the ED50 for each drug were obtained by probit analysis (Bliss 1952). Differences between control and diabetic groups were

* Correspondence.

analysed for statistical significance by two-tailed Student's *t*-test.

Additional groups of control and diabetic rats were assayed for specific 5-HT receptor binding using the method of Bennett & Snyder (1976). Four to six weeks after injection of streptozocin or buffer the rats were decapitated and the brains and spinal cords rapidly dissected into forebrain (all tissue anterior to a coronal cut through the optic chiasm) and brainstem (all tissue posterior to a coronal cut immediately anterior to the superior colliculi minus the cerebellum) plus spinal cord (C_1 through T_6). The tissues from two animals were pooled, weighed and homogenized in 40 volumes of 0·05 M Tris-HCl buffer, centrifuged at 50000 *g* for 10 min, and the pellet washed twice by re-homogenization and centrifugation. The washed pellet was resuspended in 0·05 M Tris-HCl buffer (15 mg original wet tissue weight ml^{-1}) containing 0·1% L-ascorbic acid, 10 μM pargyline and 5 mM $CaCl_2$. Two ml aliquots were incubated in duplicate for 10 min at 37 °C after addition of tritiated and unlabelled ligands. [^{3}H]-5-HT (27·8 Ci $mmol^{-1}$) was added in concentrations of 3,6,9, 18, 27 and 36 nM. Unlabelled 5-HT (10 μM) was included as blanks for non-specific binding. The samples were rapidly filtered through Whatman GF/B filters, and the incubation tubes rinsed with three 5 ml aliquots of Tris buffer. The filters were then immersed in 15 ml of Hydromix, extracted overnight at 4 °C, and the radioactivity quantitated in a Searle model 6868 liquid scintillation counter at 48% counting efficiency. KD and B_{max} values were determined by Scatchard analysis. Blood was collected from the cervical wound at the time of decapitation and assayed for glucose.

Diabetic rats displayed a pronounced subsensitivity to 5-HTP (ED50 for controls 146 mg kg^{-1}, for diabetics 349 mg kg^{-1}), and a much smaller, but statistically significant, subsensitivity to 5-MeODMT (ED50 for controls 1·3 mg kg^{-1}, for diabetics 2·0 mg kg^{-1}) and amphetamine (ED50 for controls 23 mg kg^{-1}, for diabetics 38 mg kg^{-1}) (Table 1). Thus, the ratios of diabetic ED50/control ED50 for the various drugs were: 5-HTP, 2·4; 5-MeODMT, 1·5; and amphetamine, 1·7. There were no significant changes in specific [^{3}H]-5-HT receptor binding in diabetic rats, compared with control values (Table 2).

These data demonstrate that the subsensitivity to 5-HT agonists in streptozocin-diabetic rats is not mediated by an alteration in post-synaptic 5-HT receptors. The behavioural syndrome we used is mediated by brainstem and spinal cord 5-HT receptors (Jacobs & Klemfuss 1975), and these c.n.s. regions showed no changes in [^{3}H]-5-HT receptor binding (Table 2).

The degree of subsensitivity to 5-HTP in the present study is comparable to that observed to L-tryptophan (after monoamine oxidase inhibition) in a previous study (MacKenzie & Trulson 1978d). The reduced sensitivity to L-tryptophan in streptozocin-diabetic rats is apparently due to the large increases in plasma branched chain amino acids which compete with tryptophan for entry into the brain, since the accumulation of brain tryptophan, 5-HT and 5-HIAA in these animals is greatly attenuated after systemic tryptophan loading (MacKenzie & Trulson 1978b). The subsensitivity of diabetic rats to 5-HTP may be attributable to the same process, i.e., reduced accumulation of brain 5-HTP, 5-HT and 5-HIAA, since 5-HTP is transported

Table 1. Dose-response relationships between 5-HTP, 5-MeODMT, and amphetamine, and the syndrome in control and diabetic rats. L-5-hydroxytryptophan (5-HTP), 5-methoxy-*NN*-dimethyltryptamine (5-MeODMT) and (+)-amphetamine sulphate were injected i.p. in 0·9% NaCl in control and diabetic rats. N = 5–7 for each drug at each dose. Diabetics differ significantly from controls using 2-tailed *t*-tests: 5-HTP, $P<0.01$; 5-MeODMT, $P<0.05$; amphetamine, $P<0.05$. Plasma glucose values were: control, 116·4 ± 6·1 mg%; diabetic, 491·8 ± 38·3 mg%.

Drug	Dose (mg kg^{-1})	% displaying syndrome Control	Diabetic
5-HTP	50	0·0	—
	100	20·0	—
	150	57·1	0·0
	200	80·0	0·0
	250	100·0	20·0
	300	100·0	33·3
	350	—	66·7
	400	—	85·7
5-MeODMT	0·5	0·0	—
	1·0	33·0	0·0
	1·5	60·0	20·0
	2·0	100·0	50·0
	2·5	100·0	83·3
	3·0	—	100·0
Amphetamine	10	16·7	0·0
	20	50·0	33·3
	40	83·3	57·1
	60	100·0	80·0
	80	—	100·0

Table 2. [^{3}H]-5-HT binding in control and diabetic rats, Rats were killed for binding assays 4–6 weeks after administration of streptozocin or vehicle. Data are presented as means ± s.e.m., n = 8 per group. No significant differences were found between control and diabetic groups. Plasma glucose values were: control, 120·2 ± 3·4 mg%; diabetics, 468·6 ± 27·6 mg%. There was a linear relationship ($r = 0.96$, $P<0.001$) between tissue weight and B_{max}. That the method used to measure [^{3}H]-5-HT binding is able to detect small changes is indicated by the fact that in a previous study (Trulson & Jacobs 1979) we found significant changes in 5-HT binding after repeated administration of LSD.

Group	Forebrain KD (nM)	Forebrain B_{max} (pmol g^{-1})	Brainstem plus spinal cord KD (nM)	Brainstem plus spinal cord B_{max} (pmol g^{-1})
Control	17·2 ± 1·4	20·3 ± 1·3	23·6 ± 1·0	13·1 ± 0·9
Diabetic	18·0 ± 1·1	18·7 ± 1·2	21·9 ± 1·6	13·8 ± 0·8
% change	(+4·7%)	(−7·8%)	(−7·2%)	(+5·3%)

from blood to brain by the same amino acid carrier as tryptophan (Oldendorf 1971).

Diabetic rats also displayed a subsensitivity to 5-MeODMT, a direct-acting 5-HT agonist, but the magnitude of the effect was much less than that observed with 5-HTP. The carrier mechanism for transporting 5-MeODMT into the brain is not well understood, but it is clearly different from that which transports amino acids. 5-HT itself penetrates the brain poorly from the systemic circulation, while 5-methoxytryptamine enters the brain in significant quantities (Kveder & McIsaac 1961). Similarly, *NN*-dimethyltryptamine is readily accumulated by brain following its systemic administration (Szara 1961). Therefore, both the 5-methoxy and *NN*-dimethyl moieties influence the uptake of 5-MeODMT into the brain, but it is not known whether diabetes would restrict the uptake of 5-MeODMT into the c.n.s.

Diabetic rats in the present study displayed a subsensitivity to amphetamine, which was similar in magnitude to that observed with 5-MeODMT. Marshall et al (1976) reported subsensitivity of alloxan-diabetic rats to the anorectic and locomotor stimulating actions of amphetamine. While they found no statistically significant differences in brain [^{3}H]amphetamine concentrations in diabetic vs control rats after systemic administration of the drug, examination of their data reveals that amphetamine concentrations in the diabetic group were lower at all times and doses tested. This tendency may have contributed to the behavioural subsensitivity to amphetamine observed in their study. Curzon found significant decreases in amphetamine uptake in the brains of streptozocin-diabetic rats. However, when these differences were corrected, by injecting diabetics with larger amphetamine doses, diabetic rats continued to show an impaired amphetamine-induced hyperthermic response (Fernando & Curzon 1977). Therefore, the subsensitivity of diabetic rats to the syndrome-inducing effects of amphetamine in the present study may be partially due to uptake of amphetamine, and partially due to altered c.n.s. mechanisms.

In conclusion, the present study demonstrates that streptozocin-diabetic rats are subsensitive to 5-HT agonists, and this subsensitivity is not mediated by a change in post-synaptic 5-HT receptor binding. The decreased behavioural responsiveness in these animals may be largely attributable to reduced uptake of the drugs into the c.n.s. in the diabetic state, but may also involve altered c.n.s. mechanisms.

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