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Reduced sensitivity to L-tryptophan and *p*-chloroamphetamine in streptozotocin-diabetic rats

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Rats made diabetic by injections of streptozotocin exhibit normal brain concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) despite a reduction of approximately 30% in the concentration of brain tryptophan (Curzon & Fernando, 1977; MacKenzie & Trulson, 1978). The reduction in brain tryptophan in diabetic rats is due to decreased uptake of the amino acid by brain as evidenced by a 200–300% greater accumulation of tryptophan in various brain regions in normal rats 1 h after a systemic load of L-tryptophan (50 mg kg^{-1} i.p.) (MacKenzie & Trulson, unpublished). However, the 5-HT and 5-HIAA produced from the accumulated tryptophan is only 20–50% greater in normal rats (MacKenzie & Trulson, unpublished) because the higher tryptophan concentrations attained in the normal rats probably saturate tryptophan hydroxylase (Fernstrom & Wurtman, 1971) which would attenuate the impact of greatly increased precursor concentrations on 5-HT metabolism. Therefore, although diabetes sharply restricts the accumulation of tryptophan by brain, it was not clear whether this restriction had any functional significance vis-à-vis the activation of 5-HT receptors. To test this question, normal and diabetic rats were submitted to drug treatments known to induce a behavioural syndrome that specifically reflects the activity in central 5-HT mediated synapses (Jacobs, 1976). Two drug treatments capable of inducing the syndrome are systemic loads of L-tryptophan preceded by pargyline to inhibit monoamine oxidase and injections of *p*-chloroamphetamine (PCA), a potent 5-HT releaser (Trulson & Jacobs, 1976; Trulson, Eubanks & Jacobs, 1976).

Since it had been reported that diabetes does not significantly affect the brain uptake of amphetamine (Marshall, Friedman & Heffner, 1976) but severely restricts the central accumulation of tryptophan, we predicted a shift to the right of the dose-response curve for the induction of the syndrome by tryptophan in diabetics, but no difference between normal and diabetic animals when the syndrome was induced by PCA.

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. At low drug doses diabetic were paired with normal animals, at high doses only diabetics were run and were thus paired with other diabetics. Following administration of the drugs, the rats were examined for signs of the behavioural syndrome consisting of resting tremor, rigidity or hypertonicity, hindlimb abduction, Straub tail, lateral head weaving and reciprocal forepaw treading (for a detailed description of these characteristics, see Jacobs, 1976). If at least four of these six signs were observed the syndrome was rated as present.

Female Sprague-Dawley rats (250–300 g) were made diabetic by injections of streptozotocin (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. Two weeks after injections of streptozotocin or buffer, the rats were submitted to one of two drug treatments consisting of either pargyline (50 mg kg^{-1}, i.p.) as hydrochloride followed 30 min later by one of the following doses of L-tryptophan (50, 75, 100, 125, 150, 200, 300, 450 or 600 mg kg^{-1}, i.p.) or one of the following doses of PCA (2·5, 5, 7·5, 10, 12·5, 15 or 17·5 mg kg^{-1}, i.p.). The rats were observed for signs of the syndrome for 1 h after injection of L-tryptophan or PCA. Estimates of the ED50 for each drug were obtained by probit analysis (Bliss, 1952). Differences between

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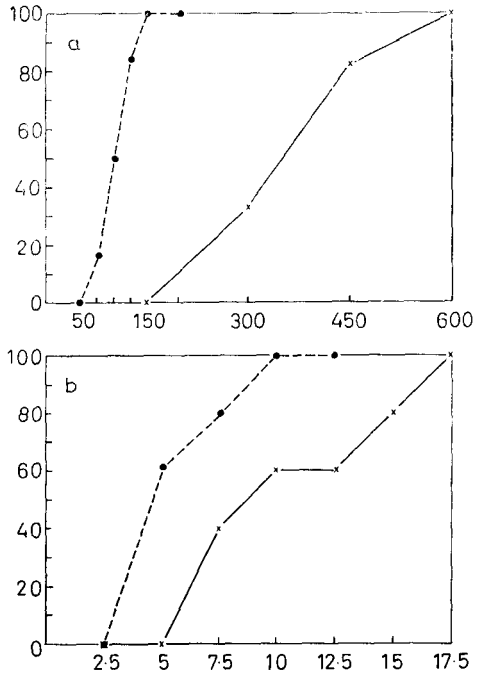

FIG. 1. Percent of animals displaying behavioural syndrome after a—pargyline (50 mg kg^{-1}) + L-tryptophan or b—*p*-chloroamphetamine. Overall curves of the two groups differ significantly: L-tryptophan $P<0{\cdot}025$; PCA $P<0{\cdot}05$ by two-tailed Student's *t*-test. Ratios of diabetic ED50/control ED50 for L-tryptophan = 3·6; for PCA = 1·76. Each point represents results obtained from 5–8 rats. ●– – ● Controls. ×–× Diabetics.

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

As predicted, the dose response curve for the diabetics receiving pargyline + tryptophan exhibited a large shift to the right from control data (ED50 of tryptophan for controls 102 mg kg^{-1}; for diabetics 367 mg kg^{-1}) (Fig. 1). This large effect could not be due to a differential brain uptake of pargyline between the two groups since the disappearance rate of central 5-HIAA following a given dose of pargyline is similar in diabetic and normal animals (MacKenzie & Trulson, unpublished). Therefore, the data indicate that the restricted central accumulation of tryptophan in diabetics leads to a reduced synthesis of functionally active 5-HT following the administration of pargyline + tryptophan.

Contrary to the predicted outcome, the dose-response curve for diabetics receiving PCA also showed a significant, although smaller, shift to the right (ED50 for controls 5·9 mg kg^{-1}; for diabetics 10·4 mg kg^{-1}) (Fig. 1). Examination of the (+)-[^{3}H]amphetamine uptake data produced by Marshall & others (1976) indicates that although the differences in the brain concentrations of amphetamine in diabetic and normal rats were not statistically significant, amphetamine concentrations in the diabetic group were lower at all times and doses tested. This tendency may have contributed to their reported subsensitivity of alloxan-diabetic rats to the anorectic and locomotor-stimulating actions of amphetamine. Curzon has found actual decreases in amphetamine uptake in the brains of streptozotocin-diabetic rats (personal communication). However, when these differences were corrected, by injecting the diabetics with larger amphetamine doses, diabetic rats continued to show an impaired amphetamine-induced hyperthermic response (Fernando & Curzon, 1977).

In conclusion, the reduced behavioural responsiveness of diabetic rats to pargyline + tryptophan appears to be due to restricted central accumulation of tryptophan, whereas the mechanism for subsensitivity to PCA in diabetic rats remains to be clarified.

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