Plasma tryptophan changes on environmental disturbance and their prevention by propranolol and nicotinic acid

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Plasma tryptophan is largely bound to albumin and the concentration of the small free (ultrafilterable) fraction is considerably influenced by unesterified fatty acids (UFA) which impair the albumin binding. Thus, food deprivation, various lipolytic drugs (Curzon & Knott, 1974) and liver damage (Curzon, Kantamaneni, Winch, Rojas-Bueno, Murray-Lyon & Williams, 1973; Knott & Curzon, 1975) all increase both plasma UFA and free tryptophan and also increase brain tryptophan and 5-hydroxytryptamine (5HT) turnover as indicated by increase of the 5HT metabolite 5-hydroxyindoleacetic acid (5HIAA).

The fall in plasma tryptophan was not explicable by increased entry into erythrocytes as erythrocyte tryptophan did not increase. In contrast with tryptophan, plasma tyrosine (which is not bound to albumin) was unaltered. The rapid plasma UFA and tryptophan changes were prevented by intraperitoneal injection of the antilipolytic drugs propranolol (1 mg/kg) or nicotinic acid (50 mg/kg) 1 h before killing. Propranolol did not significantly affect plasma corticosterone while nicotinic acid caused a large increase in fasted rats. This indicates that increases in UFA rather than corticosterone were directly responsible for the tryptophan changes. While nicotinic acid decreased plasma UFA of fasted rats to levels comparable to those of fed animals, propranolol only prevented the increase of UFA due to the environmental disturbance and in agreement with Brodie, Krishna & Hynie (1969) did not alter the underlying elevation of plasma UFA on fasting.

These findings point to propranolol and nicotinic acid as useful pharmacological tools in research on tryptophan metabolism. For example, they could be used to prevent tryptophan changes mediated by the sympathetic–UFA system and thus facilitate study of purely centrally provoked changes of brain 5-HT metabolism.

Table 1 Effect of order of removal from cage on plasma unesterified fatty acids and tryptophan concentrations in group caged fasted rats

Order of removal from cage	*UFA mEq/l*	*Total μg/ml*	*Tryptophan free μg/ml*	*% free/l*
1st (5)	0.672 ± 0.106	23.65 ± 2.95	2.75 ± 0.61	11.7 ± 2.39
4th (6) (Killed 6 min later)	0.967 ± 0.161*	17.13 ± 2.64**	3.51 ± 0.46 ns	20.81 ± 3.79**

Results are expressed as means ± one sd. Numbers of determinations are shown in parentheses. Results compared by Student's t-test; * $P < 0.02$; ** $P < 0.01$; ns not significant. Methods were as previously described (Curzon & Knott, 1974).

We now find that when rats (male, Sprague-Dawley, 180-200 g) are housed in groups of 4 and fasted for 24 h their removal from the cages caused UFA and the percent free tryptophan to rise in the plasma of the remaining rats (Table 1). Corticosterone also tended to rise. As total plasma tryptophan fell, absolute changes of free tryptophan were relatively small. The fall of plasma total tryptophan suggests that tryptophan newly freed from albumin binding rapidly leaves the plasma. In contrast to the previous longer term experiments, it did not appear to have entered the brain to any significant extent. Thus, brain tryptophan concentration of first killed fasted rats was 4.12 ± 0.52 μg/g wet wt, (n = 5) which though significantly greater ($P < 0.001$) than that of fed rats (2.19 ± 0.10, n = 3) is essentially the same as that of 4th killed fasted rats (4.05 ± 0.56, n = 6). (Results are ± one s.d.).

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Histochemical demonstration of an additional form of rat brain MAO

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Monoamine oxidase (MAO) is known to exist in a number of forms, and in the rat brain two types (A & B) have been described by Johnston (1968). Type A-MAO deaminates 5-HT and tyramine and is highly sensitive to inhibition by clorgyline, while the B form deaminates tyramine but not 5-HT and is relatively insensitive to clorgyline. These different forms may well be of considerable importance in the metabolism of monoamines in mammalian brain, and may well have differing distributions (Collins, Sandler, Williams & Youdim, 1970; Goridis & Neff, 1971).

A histochemical method has been developed which permits the demonstration of MAO in rat brain, using both 5-HT and tyramine as substrates. Using clorgyline as an inhibitor A- & B-MAO have been separately demonstrated. Both types are broadly distributed in the rat brain, with a basically similar distribution. Both are present in high amounts in areas known to be rich in monoamines.

A third type of MAO which readily utilizes 5-HT as substrate but is relatively clorgyline insensitive has also been demonstrated. It is predominantly circumventricular in distribution. It is suggested that this form, differing from A- & B-MAO not only in substrate and inhibitor characteristics, but also in distribution should be designated C-MAO. Its distribution suggests a potentially important role for this form of MAO in regulating the movement of biogenic amines between the CSF and the brain.

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Some studies on the purification of monoamine oxidase by affinity chromatography

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Partially purified preparations of monoamine oxidase (MAO) have been prepared by a number of workers (Youdim & Sourkes, 1966; Tipton, 1968; Oreland, 1971) using conventional purification procedures which utilize differences in the chemico-physical properties between MAO and other proteins. Over the past few years affinity chromatographic techniques have been developed using selective adsorbents which have biological affinity for a particular protein and this technique has been utilized to purify enzymes (Cautrecasas, Wilchek & Anfinsen, 1968; Wilchek & Gorecki, 1969).

In the present experiments a number of inhibitors of MAO were used as ligands and were attached to sepharose columns in an attempt to purify MAO by a single step experimental procedure.

An organomercurial-sepharose column was prepared by utilizing p-chloromercuribenzoate as the ligand. p-Chloromercuribenzoate was added to aminohexane sepharose suspended in 40% dimethyl formamide. 1-Ethyl-3-(3-dimethylamino propyl) carbodiimide was added, the pH maintained at 4.8 and the mixture allowed to react