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INHIBITION OF RAT LIVER TRYPTOPHAN PYRROLASE ACTIVITY AND ELEVATION OF BRAIN TRYPTOPHAN CONCENTRATION BY ADMINISTRATION OF ANTIDEPRESSANTS

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Abstract—The apoenzyme activity of rat liver tryptophan pyrrolase is decreased *in vitro* by 16–100% by concentrations of many antidepressants of 0.01–1 mM. Apo-(tryptophan pyrrolase) activity is also decreased by 37–86% at 2 hr after administration of a 10 mg/kg dose of many antidepressants. This inhibition appears to be due to the prevention of the conjugation of the apoenzyme with its cofactor haem. Brain tryptophan concentration is elevated by 19–39% at 3.5 hr after administration of the above dose of antidepressants. Isocarboxazid is the only antidepressant tested that affects neither liver pyrrolase activity nor brain tryptophan concentration. The non-antidepressants chlorpromazine, β-flupenthixol, mefenamic acid and pargyline are also ineffective in both respects. The increase in brain tryptophan concentration caused by administration of mianserin, viloxazine, desipramine or tranylcypromine is associated with an accumulation of tryptophan in the liver and an increased availability of the circulating amino acid to the brain. It is suggested that antidepressants increase brain tryptophan concentration by inhibiting liver tryptophan pyrrolase activity. The results are briefly discussed in relation to the therapeutic effects of the drugs.

There is considerable evidence [1] that the metabolism of biogenic amines is disturbed in depressive illness and, although there is no doubt that there exist mechanisms by which the various biogenic amines and other neurotransmitters are mutually regulated [2–3], there is reasonable evidence that 5-hydroxytryptamine (5-HT) may play an important role in the mood changes characteristic of this illness [4–10]. In investigating the mode of action of antidepressants, little attention has, however, been given to the possible effects of these drugs on brain tryptophan concentration, which plays an important role in 5-HT synthesis [11], or on peripheral factors influencing the availability of this amino acid to the brain. Only a few reports have appeared in this context. Brain tryptophan concentration has been reported to be elevated after acute administration of some tricyclics [12] or of tranylcypromine, but not pargyline [13] and after chronic administration of mianserin [14].

Brain tryptophan concentration could be increased when the availability of the circulating amino acid to the brain is enhanced by any of the following three peripheral mechanisms: (a) decreased liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11) activity leading to a deficient hepatic tryptophan catabolism; (b) increased release of protein-bound serum (or plasma) tryptophan; (c) a decrease in concentration of plasma neutral amino acids that compete with tryptophan for the same cerebral uptake mechanism. An inverse relationship can exist between liver tryptophan pyrrolase activity and brain 5-HT synthesis in which the former can affect the availability of circulating tryptophan for the latter. Evidence for this inverse relationship has been obtained from experiments in which pyrrolase activity has been enhanced by acute administration of cortisol [15–16] or ethanol [17–18] or during the ethanol-withdrawal-induced release of corticosterone [19] or when pyrrolase activity has been inhibited by chronic administration of dieldrin [20] or ethanol or nicotinamide [19, 21]. As regards antidepressants, it has been reported that pyrrolase activity is inhibited after acute and/or chronic administration of amitriptyline, clomipramine, imipramine or tranylcypromine [22–23]. It is also known that brain tryptophan concentration is increased when pyrrolase activity is inhibited by allopurinol, glucose, nicotinamide [24], carbidopa [25], dieldrin [20] or chronic administration of ethanol [21]. It should, however, be pointed out here that no systematic study of the effects of various antidepressants on pyrrolase activity (or brain tryptophan concentration) has been performed, and that, in the above work with a few antidepressants [22–23], only the holoenzyme activity of tryptophan pyrrolase was determined. In the initial stages of this investigation, we found that antidepressants do not inhibit the holoenzyme activity *in vitro*, but cause a decrease in that of the total enzyme (and hence the apoenzyme). Simultaneous measurements of both the holoenzyme and total pyrrolase activities are clearly of importance [26]. We have therefore performed a detailed investigation of the effects *in vitro* and after

administration of a large number of antidepressants on the various activities of rat liver tryptophan pyrrolase and have also examined the effects of administration of these drugs on brain tryptophan concentration and metabolism. The results described and discussed in the present paper suggest that many antidepressants inhibit liver tryptophan pyrrolase activity and increase brain tryptophan concentration.

MATERIALS AND METHODS

Animals. Male Wistar rats (150–170 g) were locally bred and were maintained on cube diet 41B (oxoid, Basingstoke, Hants., U.K.) and water. The animals were killed either by stunning and cervical dislocation between 13:30 and 15:15 hr (for the determination of tryptophan pyrrolase activity in fresh liver) or by decapitation at 13:30–14:00 hr (for all other determinations). When rats were killed by the latter method, the livers were isolated by a locally manufactured freeze-clamp, whereas the brains were simultaneously removed rapidly (within 20 sec of the death of the animals), immersed in liquid nitrogen for 3 min and stored (along with the livers) at −20° for 18 hr before analysis.

Drugs. Except for sodium salicylate (BDH Chemicals), the following drugs were gifts from the sources indicated in parentheses: allopurinol (The Wellcome Foundation), amitryptyline and protriptyline (Merck Sharp and Dohme), clomipramine, desipramine and imipramine (Geigy), chlorpromazine (May & Baker), α- and β-flupenthixols (Lundbeck), fluphenazine (Squibb), iprindole (Wyeth), isocarboxazid (Roche), ludiomil or maprotiline (Ciba), mefenamic acid (Park Davis & Co.), mianserin (Organon), nialamide (Pfizer), nomifensine (Hoechst), nortriptyline (Eli-Lilly), pargyline (Abbott), pemoline or kethamed (Medo-Chemicals), phenelzine (Warner), tranylcypromine (Smith Kline and French) and viloxazine (I.C.I.).

Treatments. When tested *in vitro*, drugs were dissolved in 0.2 M sodium phosphate buffer (pH 7.0), and were added in final concentrations of 0.01–1 mM each, whereas when administered (intraperitoneally), they were dissolved in 0.9% (w/v) NaCl (with neutralization to pH 7.3 if necessary) and were given in a standard dose of 10 mg/kg each. Control rats received an equal volume (2 ml/kg) of 0.9% NaCl by the same route.

Chemical, enzymic and other determinations. Tryptophan pyrrolase activity was determined in fresh liver homogenates [27] either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2 μM) haematin. The apoenzyme activity was obtained by difference. In some experiments, larger concentrations of haematin (up to 8 μM) were used.

Concentrations of glucose [28] and corticosterone [29] were determined in serum by fluorimetric procedures. Tryptophan concentrations in liver, serum and brain were also determined fluorimetrically [30] by a modification [31] as described previously [26]. Free serum tryptophan concentration was determined in ultrafiltrates obtained by using Amicon Centriflo CF-50A membrane cones, whereas that of total serum tryptophan was determined in supernatants obtained after precipitation of serum proteins with trichloroacetic acid. Brain tryptophan was analysed in the extract containing 5-HT. This and the extract containing 5-hydroxyindol-3-ylacetic acid (5-HIAA) were prepared and analysed as described [32].

Statistical analysis of results was performed by using Student's *t*-test.

RESULTS

Effects in vitro *of antidepressants and other compounds on rat liver tryptophan pyrrolase activity*

Thirteen clinically-established antidepressants were examined *in vitro* (0.01–1 mM each) for a possible direct inhibitory effect on the enzyme activity. All antidepressants tested (except isocarboxazid, which was stimulatory) decreased the total pyrrolase activity without altering that of the holoenzyme. The apoenzyme was therefore the form specifically inactivated by the drugs. The extent of this inactivation is shown in Table 1. Inhibition by concentrations of antidepressants of 0.5–1 mM varied between 77 and 100 per cent. At 0.1 mM, the inhibition was 63–100 per cent, whereas that by the 0.05 mM concentration was 31–100 per cent. A 16–99 per cent inhibition of the apoenzyme activity was achieved by a 10 μM concentration of antidepressants. The four most potent inhibitors (in decreasing order of potency) were mianserin, tranylcypromine, protriptyline and clomipramine. By contrast, the non-antidepressants pargyline and β-flupenthixol were also poor inhibitors of pyrrolase activity *in vitro*. The results in Table 1 also show that fluphenazine is an effective pyrrolase

Table 1. Effects of antidepressants and other drugs on the activity of rat liver apo-(tryptophan pyrrolase) *in vitro**

	Inhibition (%) Drug concentration (mM)				
	0.01	0.05	0.1	0.5	1.0
Tranylcypromine	60	96	92	100	100
Nialamide	33	67	72	83	100
Isocarboxazid	−400	−157	−100	−57	14
Imipramine	40	50	73	77	82
Desipramine	16	63	63	79	79
Clomipramine	73	73	80	80	100
Amitriptyline	45	59	64	82	91
Nortriptyline	61	65	77	85	86
Protriptyline	62	81	100	100	100
Mianserin	99	100	100	100	100
Nomifensine	35	59	71	100	100
Viloxazine	32	60	72	100	100
α-Flupenthixol	35	39	100	100	100
β-Flupenthixol	1	2	0	1	1
Pargyline	0	0	5	15	15
Fluphenazine	35	58	83	100	100

* The data are based on single experiments in which the various concentrations of each drug were tested in the presence of their own control. The apoenzyme activity was calculated by difference (total activity-holoenzyme activity).

inhibitor *in vitro*. This drug has been used [33] in combination with nortriptyline in the treatment of anxiety-depressive states.

Effects of administration of antidepressants and other compounds on rat liver tryptophan pyrrolase activity

These effects were examined at 2 hr because this is the time-interval at which we found four antidepressants of different chemical classes to cause the maximum inhibition of the pyrrolase activity (results not shown). The effects of 14 clinically-established antidepressants, 4 non-antidepressants and 4 potential antidepressants (10 mg/kg each) are shown in Table 2. Administration of isocarboxazid did not inhibit pyrrolase activity. By contrast, all the other 13 clinically-established antidepressants exerted significant inhibitory effects on the activities of the total enzyme and apoenzyme, but not on that of the holoenzyme. The inhibition of the former two activities by monoamine oxidase (MAO) inhibitors was 23–36 per cent and 37–69 per cent respectively, whereas the corresponding inhibition by tricyclics was 30–51 per cent and 52–86 per cent respectively. Other clinically-established antidepressants inhibited the total enzyme and apoenzyme activities by 36–44 per cent and 66–74 per cent respectively.

By contrast, the non-antidepressants chlorpromazine, β-flupenthixol, mefenamic acid and pargyline exerted no effects or caused only small decreases in pyrrolase activities. The results in Table 2 also show that pyrrolase activities were inhibited by 24–52 per cent by pemoline and salicylate, and more strongly by fluphenazine and allopurinol. Pemoline has been suggested to be a useful antidepressant [34], whereas the possible antidepressant actions of allopurinol (alone) or salicylate have not been investigated.

To find out if antidepressants inhibit pyrrolase activity by preventing the conjugation of the apoenzyme with its cofactor haem, or by another mechanism(s), liver homogenates from rats treated 2 hr previously with a 10 mg/kg dose of mianserin or tranylcypromine were incubated in the absence and presence of concentrations of added haematin of 2, 4, 6 and 8 μM. Pyrrolase activities (in μmoles of kynurenine formed/hr per g wet wt of liver) were as follows: mianserin-treated rats (1.66, 2.82, 4.30, 4.34 and 4.20 respectively); tranylcypromine-treated rats (2.01, 2.66, 5.00, 4.82 and 4.73 respectively). These results therefore show that the inhibition of the total pyrrolase activity (that assayed in the presence of a 2 μM-haematin concentration) is reversed by an excess of added cofactor.

Table 2. Effects of administration of antidepressants and other drugs on rat liver tryptophan pyrrolase activity

Treatment	μmoles Kynurenine formed/hr per g wet wt of liver Holoenzyme activity	Total enzyme activity	Apoenzyme activity
0.9% NaCl	2.9 ± 0.16	6.1 ± 0.32	3.2 ± 0.17
Tranylcypromine	2.9 ± 0.12	3.9 ± 0.09‖	1.0 ± 0.22‖
Pargyline	2.9 ± 0.10	5.9 ± 0.43	3.0 ± 0.39
Imipramine	2.6 ± 0.04	3.4 ± 0.10‖	0.8 ± 0.12‖
Viloxazine	2.8 ± 0.00	3.9 ± 0.15‖	1.1 ± 0.15‖
Mianserin	2.8 ± 0.11	3.9 ± 0.21‖	1.1 ± 0.21‖
0.9% NaCl	2.4 ± 0.09	5.9 ± 0.37	3.5 ± 0.41
Desipramine	2.4 ± 0.05	2.9 ± 0.11‖	0.5 ± 0.09‖
Phenelzine	2.5 ± 0.07	3.8 ± 0.15¶	1.3 ± 0.20¶
α-Flupenthixol	2.4 ± 0.14	3.3 ± 0.18‖	0.9 ± 0.11‖
β-Flupenthixol	2.3 ± 0.06	5.1 ± 0.37	2.8 ± 0.43
Clomipramine	2.3 ± 0.11	3.2 ± 0.23‖	0.9 ± 0.20¶
0.9% NaCl	2.4 ± 0.08	5.6 ± 0.24	3.2 ± 0.21
Nomifensine	2.4 ± 0.11	3.4 ± 0.18‖	1.0 ± 0.19‖
Nialamide	2.3 ± 0.10	4.3 ± 0.44*	2.0 ± 0.44‡
Isocarboxazid	2.8 ± 0.16	6.2 ± 0.19	3.4 ± 0.14
Amitriptyline	2.2 ± 0.06	2.9 ± 0.12‖	0.7 ± 0.13‖
Protriptyline	2.4 ± 0.13	3.3 ± 0.11‖	0.9 ± 0.07‖
0.9% NaCl	2.2 ± 0.09	4.7 ± 0.23	2.5 ± 0.14
Chlorpromazine	1.7 ± 0.02**	3.5 ± 0.14¶	1.8 ± 0.13†
Pemoline	1.8 ± 0.05*	3.0 ± 0.12‖	1.2 ± 0.12‖
0.9% NaCl	1.7 ± 0.05	3.8 ± 0.13	2.1 ± 0.10
Nortriptyline	1.7 ± 0.09	2.1 ± 0.13‖	0.4 ± 0.10‖
Mefenamic acid	1.8 ± 0.05	3.9 ± 0.24	2.1 ± 0.26
Allopurinol	1.7 ± 0.08	1.7 ± 0.08‖	0.0 ± 0.00‖
Fluphenazine	1.4 ± 0.09*	1.5 ± 0.05‖	0.1 ± 0.05‖
Sodium salicylate	1.3 ± 0.05¶	2.2 ± 0.10‖	0.9 ± 0.10‖

Assays were performed at 2 hr after intraperitoneal administration of drugs (10 mg/kg each) or an equal volume (2 ml/kg) of 0.9% NaCl. Values are means ± S.E.M. for each group of four rats. Significance of differences between each group of drugs and their respective 0.9% NaCl-treated controls is indicated as follows: *$P < 0.05$; †$P < 0.02$; ‡$P < 0.01$; ¶$P < 0.005$; ‖$P < 0.001$.

Table 3. Effects of administration of antidepressants and other drugs on rat brain tryptophan (Trp.) concentration

Expt. No.	Treatment	Brain Trp. (μg/g)	Expt. No.	Treatment	Brain Trp. (μg/g)
1	0.9% NaCl	2.48 ± 0.07	4	0.9% NaCl	2.65 ± 0.12
	Nialamide	3.08 ± 0.23†		α-Flupenthixol	3.62 ± 0.17¶
	Phenelzine	3.17 ± 0.16¶		β-Flupenthixol	2.57 ± 0.09
	Viloxazine	2.92 ± 0.18*		Ludiomil	3.41 ± 0.10¶
	Nomifensine	3.03 ± 0.12¶		Amitriptyline	3.43 ± 0.11¶
	Pemoline	3.10 ± 0.20‡		Nortriptyline	3.30 ± 0.16‡
2	0.9% NaCl	1.87 ± 0.02		Iprindole	3.28 ± 0.17‡
	Tranylcypromine	2.25 ± 0.13†	5	0.9% NaCl	2.78 ± 0.10
	Pargyline	1.94 ± 0.06		Fluphenazine	3.86 ± 0.25¶
	Mianserin	2.32 ± 0.10¶		Allopurinol	3.45 ± 0.10¶
	Isocarboxazid	1.56 ± 0.04‖		Sodium salicylate	3.21 ± 0.12¶
	Chlorpromazine	1.66 ± 0.06‖		Mefenamic acid	2.62 ± 0.09
3	0.9% NaCl	2.43 ± 0.11		Protriptyline	3.87 ± 0.17‖
	Imipramine	3.15 ± 0.07‖			
	Desipramine	3.16 ± 0.05‖			
	Clomipramine	2.93 ± 0.08¶			

Brain tryptophan concentration was determined at 3.5 hr after intraperitoneal administration of drugs (10 mg/kg each) or an equal volume (2 ml/kg) of 0.9% NaCl. To minimize the effects of animal and daily variations, each experiment was performed on a different day and the results with each group of drugs have been compared with those obtained in control (0.9% NaCl-treated) rats tested in the same experiment. Values are means ± S.E.M. for each group of five rats. Significance of differences is indicated as follows: *$P < 0.05$; †$P < 0.025$; ‡$P < 0.01$; ¶$P < 0.005$; ‖$P < 0.001$.

Effects of administration of antidepressants and other compounds on rat brain tryptophan concentration

The effects on brain tryptophan concentration of 16 clinically-established antidepressants, 4 potential antidepressants and 4 non-antidepressants were examined at 3.5 hr after administration of a 10 mg/kg dose. This time-interval was selected as a suitable time at which the maximum inhibition of pyrrolase activity (which occurs at 2 hr) is likely to lead to an increase in tryptophan availability to the brain. As shown in Table 3, isocarboxazid failed to increase brain tryptophan concentration. By contrast, increases of 19–39 per cent were observed with the other 15 antidepressants tested. The non-antidepressants chlorpromazine, β-flupenthixol, mefenamic acid and pargyline did not elevate brain tryptophan concentration. Salicylate, allopurinol and pemoline increased brain tryptophan concentration by 15–25 per cent, whereas fluphenazine caused the largest increase (39%), thus resembling protriptyline.

Effects of administration of some antidepressants on various aspects of rat tryptophan metabolism and on serum glucose and corticosterone concentrations

These effects are shown in Table 4. At 3.5 hr after

Table 4. Effects of administration of antidepressants on various aspects of rat tryptophan metabolism and on serum glucose and corticosterone concentrations

Test	0.9% NaCl	Desipramine	Tranylcypromine	Mianserin	Viloxazine
Serum glucose	134.00 ± 3.00	126.00 ± 1.00*	136.00 ± 2.00	133.00 ± 2.00	128.00 ± 1.00
Serum corticosterone	62.70 ± 3.50	62.80 ± 3.10	61.50 ± 4.50	32.20 ± 2.20**	40.20 ± 1.90**
Liver Trp.	4.73 ± 0.19	5.58 ± 0.13‖	6.25 ± 0.15**	5.68 ± 0.22¶	5.87 ± 0.18‖
Free serum Trp.	1.06 ± 0.04	1.42 ± 0.07‖	1.23 ± 0.07*	1.26 ± 0.07*	1.24 ± 0.04‖
Total serum Trp.	14.35 ± 0.59	16.62 ± 0.56‡	17.90 ± 0.94¶	16.33 ± 0.71*	17.53 ± 0.47
Free serum Trp. (%)	7.39 ± 0.19	8.54 ± 0.48	6.87 ± 0.61	7.72 ± 0.44	7.07 ± 0.31
Brain Trp.	1.42 ± 0.05	1.73 ± 0.04**	1.91 ± 0.07**	1.79 ± 0.06**	1.79 ± 0.06**
Brain 5-HT	0.68 ± 0.012	1.04 ± 0.054**	1.12 ± 0.075**	0.81 ± 0.023**	0.85 ± 0.019**
Brain 5-HIAA	0.28 ± 0.010	0.34 ± 0.019†	0.21 ± 0.010**	0.39 ± 0.007**	0.41 ± 0.030‡

Measurements were performed at 3.5 hr after intraperitoneal administration of drugs (10 mg/kg each) or an equal volume (2 ml/kg) of 0.9% NaCl. Values are means ± S.E.M. for each group of six rats. Serum glucose is in mg/dl, serum corticosterone is in μg/l and all other expressions (except the free serum Trp. %) are in μg/ml of serum or μg/g wet wt of tissue. Significance of differences is indicated as follows: *$P < 0.05$; †$P < 0.025$; ‡$P < 0.02$; ¶$P < 0.01$; ‖$P < 0.005$; **$P < 0.001$.

administration of a 10 mg/kg dose of four antidepressants representative of various classes, liver tryptophan concentration was increased by 18–32 per cent. Concentrations of free serum and total serum tryptophan were also increased by the drugs by 16–34 per cent and 16–25 per cent respectively. Binding of tryptophan to serum proteins (expressed as the percentage free serum tryptophan) was not significantly altered by any of the four drugs. Under these conditions, brain tryptophan concentration was increased by 22–34 per cent. Mianserin and viloxazine increased brain 5-HT concentration by 19–25 per cent, whereas desipramine and tranylcypromine caused larger increase (53–65%). The latter drug decreased brain 5-HIAA concentration by 25 per cent, whereas the former three drugs increased it by 21–46 per cent. Serum glucose concentration was not much altered by any of the four drugs. Serum corticosterone concentration was also not significantly altered by desipramine or tranylcypromine, but was decreased by 36 and 49 per cent by viloxazine and mianserin respectively.

DISCUSSION

Effects of antidepressants on liver tryptophan pyrrolase activity

The results in Table 2 demonstrate the ability of many antidepressants to inhibit tryptophan pyrrolase activity after administration to rats. That the drugs exert a direct inhibitory effect is suggested by their ability to act *in vitro* (Table 1). Because this inhibition appears to be caused by an interference with the conjugation of the apoenzyme with its cofactor haem, it may be suggested that: (a) the inhibition is not due to a defective apoenzyme synthesis, nor the lowering by antidepressants of serum corticosterone concentration, as has been proposed [23]; (b) antidepressants resemble allopurinol [35], but differ from ethanol, glucose or nicotinamide, whose chronic administration inhibits pyrrolase activity by increasing the hepatic concentrations of the allosteric inhibitors NAD(P)H [36, 37]; (c) the ability of administered antidepressants to inhibit pyrrolase activity may depend on the extent of the haem saturation of the apoenzyme, and, therefore, on the status of liver haem *in vivo*.

The failure of the non-antidepressants chlorpromazine, β-flupenthixol, mefenamic acid and pargyline to inhibit pyrrolase activity (Tables 1 and 2) suggests a relationship between the clinical antidepressant action and the inhibition of pyrrolase activity. Isocarboxazid is the only exception in this context, but it is possible that this drug may be effective after chronic administration. The results discussed so far suggest that, on a molar basis, MAO inhibitors are the weakest inhibitors of pyrrolase activity and this parallels their status as antidepressants in general terms. No further attempt can be made here to correlate the relative effectiveness of the drugs as pyrrolase inhibitors and as antidepressants, because of the lack of a definite consensus among clinical psychiatrists on the relative potencies of antidepressants in clinical practice.

Pyrrolase activity is also inhibited by fluphenazine, pemoline, allopurinol and salicylate (Table 2). The possible usefulness of the former two drugs as antidepressants has been mentioned earlier, and it remains to be seen whether allopurinol or salicylate possess antidepressant properties. The allopurinol inhibition of pyrrolase activity confirms a previous finding [35]. Also salicylate has previously been reported [38] to inhibit pyrrolase activity *in vitro* and early after administration (although it causes a subsequent enhancement).

We have not examined the effects of antidepressants on the cortisol induction or the tryptophan activation of tryptophan pyrrolase. However, in view of the similarity of actions of antidepressants and allopurinol (i.e. in preventing the haem conjugation of the apoenzyme), it may be suggested that antidepressants may completely block the tryptophan activation of the pyrrolase, whereas they may only prevent the cortisol-induced increase in the apoenzyme, but not in the holoenzyme, activity. These suggestions are largely borne out by previous findings [23, 39], although some aspects require clarification.

Elevation of brain tryptophan concentration by antidepressants

The findings (Table 3) that many antidepressants (except isocarboxazid) increase brain tryptophan concentration, whereas non-antidepressants (chlorpromazine, β-flupenthixol, mefenamic acid and pargyline) do not, suggest that the antidepressant-induced elevation of brain tryptophan concentration correlates qualitatively not only with their clinical action, but also with the inhibition of pyrrolase activity. The findings (Table 3) that brain tryptophan concentration is increased by allopurinol and tranylcypromine, but not pargyline, confirm previous reports [13, 24]. Although doses of salicylate of 50 mg/kg and above displace tryptophan from its binding sites on serum proteins *in vivo* [40] and thereby increase brain tryptophan concentration [41], it is not clear whether the latter effect observed here (Table 3) with the 10 mg/kg dose is caused by tryptophan displacement, pyrrolase inhibition (Table 2) or both. Further work on tryptophan binding is therefore required with this small dose of salicylate.

The correlation between the ability of antidepressants to elevate brain tryptophan concentration and to inhibit pyrrolase activity suggests that the latter effect may cause the former one. If so, then it is necessary to demonstrate that antidepressants increase tryptophan concentrations in liver and serum without altering the amino acid binding to serum proteins. This is indeed demonstrated by the results in Table 4. The failure of the drugs to alter serum glucose concentration provides further evidence that their inhibition of pyrrolase activity and elevation of brain tryptophan concentration are not mediated by glucose or insulin respectively. These findings therefore strongly suggest that antidepressants increase brain tryptophan concentration by increasing tryptophan availability to the brain secondarily to the inhibition of liver pyrrolase activity. Similar findings have been reported under conditions involving pyrrolase inhibition by chronic administration of ethanol or nicotinamide [19, 21].

Brain tryptophan concentration plays an important role in 5-HT synthesis [11]. It is also known [13]

that inhibition of MAO activity *per se* does not increase brain tryptophan concentration. On the basis of these findings and of known actions of antidepressants, it may be suggested that all four antidepressants tested (Table 4) enhance 5-HT synthesis by a tryptophan-mediated mechanism, and that the extra increases in 5-HT concentration caused by tranylcypromine and desipramine are due to the inhibition of MAO activity and amine reuptake respectively.

Relationship between the present results and the mechanism(s) of action of antidepressants

The present results are the first to establish that a large number of antidepressant drugs increase brain tryptophan concentration, by inhibiting liver tryptophan pyrrolase activity. Human liver tryptophan pyrrolase appears to resemble the rat enzyme in possessing both the holoenzyme and apoenzyme forms in roughly equal proportions [42], and it is therefore possible that antidepressants may also be effective inhibitors in man. The doses of antidepressants used in the present work were at least 4-times the human therapeutic ones, and it therefore remains to be seen whether smaller doses will also be capable of inhibiting pyrrolase activity and elevating brain tryptophan concentration after both acute and chronic administration. In view of the evidence (see the introduction) that 5-HT metabolism may be impaired in depression, it is not unreasonable to suggest that the ability of many antidepressants to increase brain tryptophan concentration (and hence 5-HT synthesis) may be relevant to their clinical mode of action. Work with patients may therefore throw light on this possibility.

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