Br. J. Pharmac. (1982), **77**, 059–067

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

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1 Administration to rats of a 0.5 mg/kg dose of any of 19 antidepressants, but not that of many other drugs, causes a significant inhibition of the total enzyme and apoenzyme activities of liver tryptophan pyrrolase (of 24–48% and 37–65% respectively) and elevates brain tryptophan concentration by 13–66%.

2 When liver tryptophan pyrrolase activity is enhanced by pretreatment with cortisol or haematin, subsequent administration of a 0.5 mg/kg dose of some, but not other, antidepressants causes inhibition, which is weak (up to 38%).

3 This weak inhibition of the enhanced pyrrolase activity together with other pharmacological and physiological factors could explain the time lag between the start of antidepressant medication and the occurrence of a therapeutic response.

4 The cortisol-induced and haematin-activated pyrrolases respond differentially to inhibition by imipramine and amitriptyline, and this may explain the differential response to these two drugs of depressed patients in relation to urinary excretion of the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol.

5 The results are discussed in relation to the mechanism of action of antidepressants and the possible involvement of disturbed hepatic tryptophan metabolism in depressive illness.

Introduction

We have shown (Badawy & Evans, 1981) that acute administration to rats of a 10 mg/kg dose of a large number of antidepressants, but not of many other drugs, inhibits the activity of liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11), which is quantitatively the most important tryptophan-degrading enzyme in man and rat (Badawy & Evans, 1976; Young, 1981), and elevates brain tryptophan concentration. Evidence was also presented by Badawy & Evans (1981) that this latter effect, which led to an enhancement of brain 5-hydroxytryptamine synthesis, was caused by an increase in the availability of circulating tryptophan to the brain secondarily to the above inhibition of liver pyrrolase activity.

Because the previously used doses of antidepressants were several fold higher than any possible single therapeutic dose, it was considered important to find out whether liver tryptophan pyrrolase activity and brain tryptophan concentration could be specifically influenced significantly by administration of a dose (0.5 mg/kg) similar to, or smaller than, the single therapeutic dose of antidepressants, but not other drugs. Also, because hepatic tryptophan metabolism (which is controlled by tryptophan pyrrolase activity) is enhanced in depressive illness (Cazzullo, Mangoni & Mascherpa, 1966; Rubin, 1967; Curzon, 1972; Mangoni, 1974; Hullin, 1976), it was equally desirable to find out if the above small dose of antidepressants can exert a significant inhibitory effect on liver pyrrolase activity that had previously been enhanced by, e.g., hormonal induction by cortisol or cofactor activation by haematin. Induction by cortisol was chosen because the circulating concentration of this hormone is known to be increased in depressed patients, whereas cofactor activation by haematin was considered a more appropriate means of enhancing the pyrrolase by an alternative mechanism in preference to administration of tryptophan, whose overall levels are unlikely to be elevated in depressed patients in view of the above enhancement of hepatic tryptophan catabolism.

The present paper describes the results of these experiments and discusses them in relation to the mechanism of action of antidepressants and the possible involvement of disturbed hepatic tryptophan metabolism in depressive illness.

0007-1188/82/090059-09 $01.00

Methods

Animals

Locally bred male Wistar rats (150–170 g) were housed four per cage (at 22 ± 1°C; under natural light-dark cycles) and were maintained on cube diet 41B (Oxoid, Basingstoke, Hants.) and water. The animals were killed between 13 h 00 min and 14 h 00 min either by stunning and cervical dislocation (for the determination of tryptophan pyrrolase activity in fresh-liver homogenates) or by decapitation (for all other determinations).

Drug treatments

Antidepressants and certain other drugs were dissolved in 0.9% w/v NaCl solution (saline) and were administered intraperitoneally. Control rats received an equal volume (2 ml/kg) of saline by the same route. In some experiments, liver tryptophan pyrrolase activity was enhanced before treatment with the above drugs by administration (4 h before death) of either cortisol acetate (20 mg/kg) or haematin hydrochloride (5 mg/kg), both of which were dissolved in dimethylformamide (1 ml/kg). Appropriate control rats for these latter treatments received an equal volume of this solvent by the same (intraperitoneal) route.

For the determination of liver pyrrolase activity, the animals were killed at 2 h, because this was found (Badawy & Evans, 1981) to be the time interval after administration of antidepressants at which maximum inhibition of the enzyme activity occurred, whereas for brain tryptophan and other determinations, the rats were killed at 3.5 h after administration of antidepressants and other drugs for reasons given with the relevant results.

Determination of liver tryptophan pyrrolase activity

Tryptophan pyrrolase activity was determined in fresh-liver homogenates (Badawy & Evans, 1975) 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. The holoenzyme and apoenzyme are respectively the haem-containing and haem-free forms of tryptophan pyrrolase in rat and human liver, and the activity of the total enzyme is the sum of those of the above two forms. In the experiments with administered haematin, the saturation of tryptophan pyrrolase with its cofactor haem was expressed as the percentage haem saturation (100 × holoenzyme activity/total enzyme activity).

Determination of serum and brain tryptophan concentrations

Free (ultrafiltrable) serum, total (acid-soluble) serum and brain tryptophan concentrations were determined by a modification (Bloxam & Warren, 1974) of the fluorimetric method of Denckla & Dewey (1967) as described previously (Badawy & Evans, 1976). Brain tryptophan was extracted by the method of Curzon & Green (1970) from frozen brains that had previously been isolated within 10 s of the death of the animals, immersed in liquid N_2 for 3 min and stored at −20°C for 20 h before analysis.

Statistical analysis of results

This was performed by use of Student's *t* test.

Drugs and chemicals

The sources of various antidepressants and other drugs have previously been described (Badawy & Evans, 1981). Mianserin hydrochloride was obtained from Bencard as well as Organon Laboratories. The following additional drugs were gifts from the sources indicated in parentheses: dothiepin or prothiaden (Boots), trazodone (Roussel), zimelidine (Astra), lorazepam (Wyeth), phenytoin or diphenylhydantoin (Parke Davis & Co.). Cortisol acetate, haematin hydrochloride and all other chemicals (of the purest commercially available grades) were purchased from BDH and Sigma (both of Poole, Dorset).

Results

Effects of administration of various doses of some antidepressants on the basal activity of rat liver tryptophan pyrrolase

The effects, at 2 h, of administration of various doses of four antidepressants (mianserin, nomifensine, tranylcypromine and desipramine) on rat liver tryptophan pyrrolase activity are shown in Figure 1. The holoenzyme activity was not much altered by any of the doses of antidepressants administered. By contrast, the total enzyme and apoenzyme activities were significantly inhibited ($P = 0.025–0.001$) by all the doses of antidepressants given, including the smallest dose (0.5 mg/kg). These results therefore establish the ability of a 0.5 mg/kg dose of the above four antidepressants to inhibit the basal activity of rat liver tryptophan pyrrolase. This inhibition has previously been shown (Badawy & Evans, 1981) to involve prevention of the conjugation of the apoenzyme with its cofactor haem.

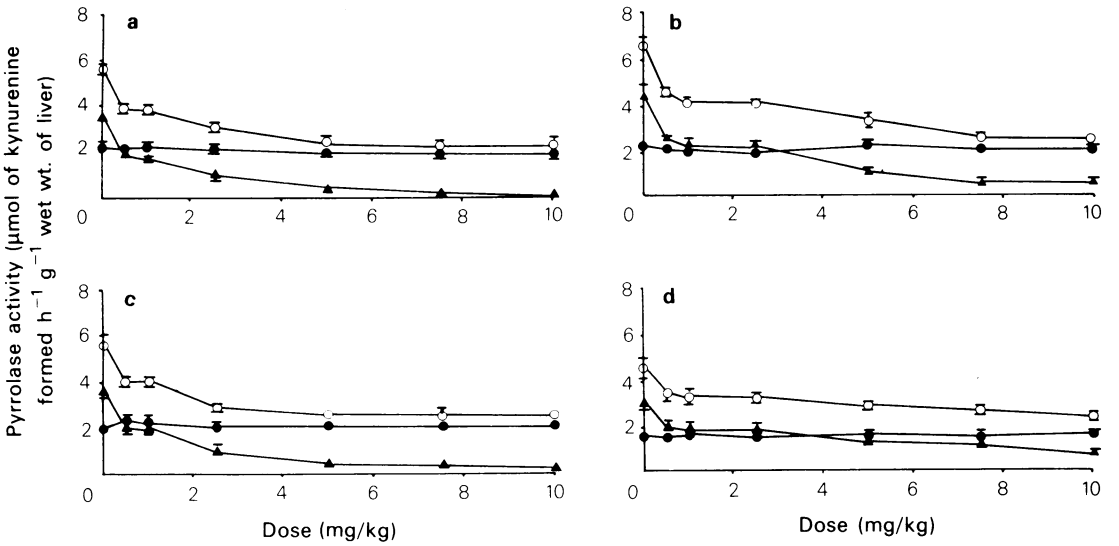

Figure 1 Effects of administration of various doses of some antidepressants on the basal activity of rat liver tryptophan pyrrolase. Rats received at 2 h before death an intraperitoneal injection of saline (2 ml/kg) or of various doses (0.5–10 mg/kg) of mianserin (a), nomifensine (b), tranylcypromine (c) or desipramine (d). Pyrrolase activity was determined as described in the Methods section either in the absence (holoenzyme activity; ●) or in the presence (total enzyme activity; ○) of added cofactor (haematin). The apoenzyme activity (▲) was obtained by difference. Values are means for each group of four rats; vertical bars show s.e.mean.

Effects of administration of a 0.5 mg/kg dose of various antidepressants and other drugs on the basal activity of rat liver tryptophan pyrrolase

The effects of 15 antidepressants, other than those used in the experiments shown in Figure 1, and those of ten other drugs are given in Table 1. Except for trazodone, a 0.5 mg/kg dose of 14 antidepressants caused a significant inhibition of liver tryptophan pyrrolase activity ($P = 0.02-0.001$). The total pyrrolase activity was inhibited by these 14 antidepressants by 27–48%, whereas that of the apoenzyme was decreased by 39–65%. Trazodone was, however, capable of inhibiting both activities when administered in a 10 mg/kg dose. The pyrrolase inhibition by this latter new antidepressant and also that by dothiepin and zimelidine are described here for the first time.

The results in Table 1 also show that no significant inhibition of pyrrolase activities was produced by a 0.5 mg/kg dose of the following non-antidepressant drugs: β-flupenthixol, fluphenazine, lorazepam, mefenamic acid, pargyline and phenytoin. The ineffectiveness of a 0.5 mg/kg dose of fluphenazine and also of that of the stimulant pemoline, which has been suggested (Kagan, 1974) as a useful antidepressant, contrasts with the ability of a 10 mg/kg dose of either drug to inhibit the enzyme activity (Badawy & Evans, 1981). The non-antidepressant chlorpromazine, however, caused only a moderate inhibition of pyrrolase activities (of up to 24%). By contrast, the total pyrrolase and apoenzyme activities were significantly ($P < 0.001$) inhibited (by 44–75%) by a 0.5 mg/kg dose of allopurinol or sodium salicylate, and the latter drug inhibited the holoenzyme activity by 35% ($P < 0.001$). The possible usefulness of these latter two drugs as antidepressants has not been explored.

Effects of administration of some antidepressants on the enhanced activity of cortisol-induced tryptophan pyrrolase in rat liver

The effects of administration of a number of antidepressants (in doses of 0.5 and 10 mg/kg) on the activity of cortisol-induced tryptophan pyrrolase are shown in Table 2. Cortisol alone increased the pyrrolase holoenzyme, total enzyme and apoenzyme activities by 378, 313 and 259% respectively ($P < 0.001$). All three activities of the cortisol-induced pyrrolase were significantly inhibited by the 0.5 mg/kg dose of mianserin or tranylcypromine, but not by that of nomifensine or desipramine. The 10 mg/kg dose of all four antidepressants was, however, effective in inhibiting the total enzyme and apoenzyme activities of cortisol-induced tryptophan pyrrolase; additionally, the holoenzyme activity was significantly inhibited by this latter dose of mianserin.

Table 1 Effects of various antidepressants and other drugs on the basal activity of rat liver tryptophan pyrrolase

Experiment No	*Treatment*	*Tryptophan pyrrolase activity* (μmol of kynurenine formed h^{-1} g^{-1} wet wt. of liver) *Holoenzyme*	*Total enzyme*	*Apoenzyme*
1	Saline	1.3 ± 0.04	5.0 ± 0.16	3.7 ± 0.16
	Imipramine	1.3 ± 0.07	3.1 ± 0.19***	1.8 ± 0.14***
	Clomipramine	1.3 ± 0.06	3.1 ± 0.29**	1.8 ± 0.25***
	α-Flupenthixol	1.4 ± 0.08	3.1 ± 0.19***	1.7 ± 0.12***
	(β-Flupenthixol)	1.3 ± 0.09	5.6 ± 0.31	4.3 ± 0.23
	Viloxazine	1.3 ± 0.12	3.3 ± 0.29**	2.0 ± 0.19***
2	Saline	1.3 ± 0.04	4.4 ± 0.13	3.1 ± 0.14
	Amitriptyline	1.2 ± 0.10	2.8 ± 0.17***	1.6 ± 0.08***
	Iprindole	1.3 ± 0.13	3.2 ± 0.17**	1.9 ± 0.06***
	Protriptyline	1.3 ± 0.07	2.4 ± 0.04***	1.1 ± 0.07***
3	Saline	1.7 ± 0.12	6.3 ± 0.42	4.6 ± 0.31
	Phenelzine	1.7 ± 0.09	3.8 ± 0.39**	2.1 ± 0.31**
	Nialamide	1.5 ± 0.05	4.3 ± 0.20*	2.8 ± 0.19**
	Ludiomil	1.4 ± 0.03	3.5 ± 0.45**	2.1 ± 0.26***
	(Pargyline)	1.8 ± 0.11	5.6 ± 0.22	3.8 ± 0.21
4	Saline	2.6 ± 0.09	7.5 ± 0.25	4.9 ± 0.19
	Isocarboxazid	1.9 ± 0.00***	4.1 ± 0.25***	2.2 ± 0.25***
	(Pemoline)	2.4 ± 0.26	7.0 ± 0.36	4.6 ± 0.23
	(Fluphenazine)	2.6 ± 0.15	7.2 ± 0.17	4.6 ± 0.23
	(Mefenamic acid)	2.4 ± 0.12	7.7 ± 0.55	5.3 ± 0.43
	(Salicylate)	1.7 ± 0.07***	4.2 ± 0.12***	2.5 ± 0.06***
5	Saline	2.4 ± 0.15	7.5 ± 0.31	5.1 ± 0.22
	Dothiepin	2.5 ± 0.25	5.1 ± 0.15***	2.6 ± 0.12***
	Dothiepin (10)*	2.6 ± 0.10	4.4 ± 0.10***	1.8 ± 0.10***
6	Saline	2.2 ± 0.15	7.5 ± 0.28	5.3 ± 0.19
	Nortriptyline	1.8 ± 0.11	3.9 ± 0.09***	2.1 ± 0.07***
	(Allopurinol)	2.2 ± 0.15	3.5 ± 0.11***	1.3 ± 0.09***
	(Chlorpromazine)	2.0 ± 0.09	6.0 ± 0.49†	4.0 ± 0.43†
	(Lorazepam)	2.1 ± 0.11	7.0 ± 0.16	4.9 ± 0.20
	(Phenytoin)	2.3 ± 0.10	7.8 ± 0.05	5.5 ± 0.14
7	Saline	1.5 ± 0.02	4.9 ± 0.38	3.4 ± 0.35
	Zimelidine	1.5 ± 0.05	3.5 ± 0.16†††	2.0 ± 0.17†††
	Zimelidine (10)*	1.7 ± 0.15	3.3 ± 0.15*	1.6 ± 0.11**
8	Saline	2.0 ± 0.07	5.2 ± 0.10	3.2 ± 0.09
	Trazodone	2.0 ± 0.19	6.1 ± 0.11***	4.1 ± 0.14**
	Trazodone (10)*	2.0 ± 0.09	3.2 ± 0.15***	1.2 ± 0.03***

Rats received at 2 h before death an intraperitoneal injection of a 0.5 mg/kg dose of antidepressants and other drugs or an equal volume (2 ml/kg) of saline. *Three antidepressants have also been administered in a 10 mg/kg dose each, because their effects are reported here for the first time. The non-antidepressant drugs used in this work are indicated in the Table in parentheses. Pyrrolase activity was determined as described in the Methods section either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added haematin. The apoenzyme activity was obtained by difference. Values are means ± s.e.mean for each group of 4 rats per each experiment. The effects of drugs are compared with the results in saline-treated controls, which were repeated in each experiment on separate weeks to minimize animal variations. The significance of the differences is indicated as follows: $\dagger P < 0.05$; $\dagger\dagger\dagger P < 0.02$; $^{*}P < 0.01$; $^{**}P < 0.005$; $^{***}P < 0.001$.

Table 2 Effects of administration of some antidepressants on the enhanced activity of cortisol-induced rat liver tryptophan pyrrolase

First injection	*Second injection*	(Dose: mg/kg)	*Tryptophan pyrrolase activity* (μmol of kynurenine formed h^{-1} g^{-1} wet wt. of liver) *Holoenzyme*	*Total enzyme*	*Apoenzyme*
DMF	Saline		1.8±0.22	4.0±0.19	2.2±0.11
Cortisol	Saline		8.6±0.31***	16.5±0.96***	7.9±0.53***
Cortisol	Tranylcypromine	(0.5)	7.6±0.11††	13.3±0.32†††	5.7±0.39†††
Cortisol	Tranylcypromine	(10)	7.5±0.48	11.9±0.71*	4.4±0.36**
Cortisol	Desipramine	(0.5)	8.4±0.19	15.7±1.04	7.3±1.05
Cortisol	Desipramine	(10)	7.6±0.37	12.0±0.81†††	4.4±0.54**
Cortisol	Mianserin	(0.5)	7.2±0.15*	12.1±0.96†††	4.9±0.89†
Cortisol	Mianserin	(10)	6.7±0.30**	11.1±0.54**	4.4±0.19***
Cortisol	Nomifensine	(0.5)	8.0±0.57	14.9±0.62	6.9±0.26
Cortisol	Nomifensine	(10)	7.9±0.56	12.7±0.10*	4.8±0.47**

Rats received an intraperitoneal injection of either cortisol acetate (20 mg/kg) or an equal volume (1 ml/kg) of the solvent dimethylformamide and were killed 4 h later. The animals also received, at 2 h after the above injection(s), a second injection of saline (2 ml/kg) or of a 0.5 mg/kg or a 10 mg/kg dose of tranylcypromine, desipramine, mianserin and nomifensine. Pyrrolase activity was determined as described in the Methods section either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added cofactor (haematin). The apoenzyme activity was obtained by difference. Values are means ± s.e.mean for each group of 4 rats. The effects of cortisol are compared with those of the solvent dimethylformamide (DMF) in the saline experiments, whereas those of the above four drugs are compared with those in the cortisol-saline experiments, and the significance of the differences is indicated as follows: †$P<0.05$; ††$P<0.025$; †††$P<0.02$; *$P<0.01$; **$P<0.005$; ***$P<0.001$.

Effects of administration of some antidepressants on the enhanced activity of haematin-activated tryptophan pyrrolase in rat liver

When the same doses of the above four antidepressants were given to rats whose pyrrolase activity has been enhanced by pretreatment with haematin, it was found (Table 3) that the holoenzyme activity was significantly inhibited by the 0.5 mg/kg dose of nomifensine and by the 10 mg/kg dose of nomifensine and of the other three antidepressants. All four antidepressants at both dose levels significantly inhibited the increase in the percentage haem saturation of tryptophan pyrrolase, but failed to alter signific-

Table 3 Effects of administration of some antidepressants on the enhanced activity of haematin-activated rat liver tryptopan pyrrolase

First injection	*Second injection*	(Dose: mg/kg)	*Tryptophan pyrrolase activity* (μmol of kynurenine formed h^{-1} g^{-1} wet wt. of liver) *Holoenzyme*	*Total enzyme*	*Saturation of pyrrolase with haem* (%)
DMF	Saline		4.1±0.17	8.8±0.17	47±1
Haematin	Saline		8.3±0.57***	8.6±0.57	96±1***
Haematin	Tranylcypromine	(0.5)	8.0±0.38	9.3±0.52	86±3†††
Haematin	Tranylcypromine	(10)	6.0±0.33†††	8.7±0.49	69±3***
Haematin	Desipramine	(0.5)	6.9±0.39	8.9±0.48	77±2***
Haematin	Desipramine	(10)	5.2±0.36**	8.8±0.50	59±3***
Haematin	Mianserin	(0.5)	7.2±0.74	8.9±0.63	81±3**
Haematin	Mianserin	(10)	5.7±0.30*	8.8±0.72	65±3***
Haematin	Nomifensine	(0.5)	5.8±0.61††	8.7±0.72	67±2***
Haematin	Nomifensine	(10)	5.2±0.32**	8.8±0.49	59±2***

Designs, details, expression of results and their comparisons are as described in Table 2, except that haematin hydrochloride (5 mg/kg) was injected instead of cortisol, and the percentage haem saturation of the pyrrolase (100 × holoenzyme activity/total enzyme activity) is given instead of the apoenzyme activity, because this percentage saturation expresses the haematin effect better. Values are means ± s.e.mean for each group of 4 rats. The significance of the differences is indicated as follows: ††$P<0.025$; †††$P<0.02$; *$P<0.01$; **$P<0.005$; ***$P<0.001$.

Table 4 Differential responses of cortisol-induced and haematin-activated tryptophan pyrrolases of rat liver to inhibition by imipramine and amitriptyline

First injection	*Second injection*	(Dose: mg/kg)	*Tryptophan pyrrolase activity* *Holoenzyme*	*Total enzyme*	*Apoenzyme or saturation with haem (%)*
Cortisol	Saline		9.2±0.13	18.9±0.64	9.7±0.64
Cortisol	Imipramine	(10)	6.0±0.42***	13.2±0.96**	7.2±0.75†
Cortisol	Amitriptyline	(10)	9.2±0.53	18.1±1.09	8.9±0.79
Haematin	Saline		6.6±0.64	8.5±0.43	78 ±2
Haematin	Imipramine	(10)	6.5±0.37	8.9±0.98	73 ±4
Haematin	Amitriptyline	(10)	4.6±0.20††	7.0±0.36†	66 ±4†

Designs, details, expressions of results and comparisons of their differences are as described in Tables 2 and 3, except that the two antidepressants were given in a 10 mg/kg dose each. Values are means ± s.e.mean for each group of 4 rats. The significance of the differences is indicated as follows: †$P<0.05$; ††$P<0.025$; **$P<0.005$; ***$P<0.001$.

antly the total enzyme activity, in haematin-pretreated rats.

Differential inhibition by imipramine and amitriptyline of the enhanced activities of cortisol-induced and haematin-activated tryptophan pyrrolases in rat liver

The results in Table 4 show that imipramine, but not amitriptyline, significantly inhibited the activities of the cortisol-induced pyrrolase holoenzyme, total enzyme and apoenzyme, whereas amitriptyline, but not imipramine, significantly inhibited the haematin-induced increases in the pyrrolase holoenzyme and total enzyme activities and the percentage haem saturation of the enzyme.

Time course of the effects of administration of a 10 mg/kg dose of some antidepressants on rat brain tryptophan concentration

Although Badawy & Evans (1981) demonstrated the ability of a 10 mg/kg dose of antidepressants to elevate rat brain tryptophan concentration at a time interval (3.5 h) arbitrarily chosen as likely to show such elevation after maximum inhibition of liver pyrrolase activity has occurred (at 2 h), it has now been found (Figure 2) that 3.5 h is the interval at which a 10 mg/kg dose of four antidepressants (mianserin, nomifensine, tranylcypromine and desipramine) causes the maximum increase in brain tryptophan concentration.

Effects of administration of a 0.5 mg/kg dose of various antidepressants and other drugs on rat brain tryptophan concentration

The effects, at 3.5 h, of administration of a 0.5 mg/kg dose of 19 antidepressants and 10 other drugs on rat brain tryptophan concentration are shown in Table 5. All 19 antidepressants administered caused significant increases in brain tryptophan concentration of 13–66% ($P=0.02-0.001$). By contrast, brain tryp-

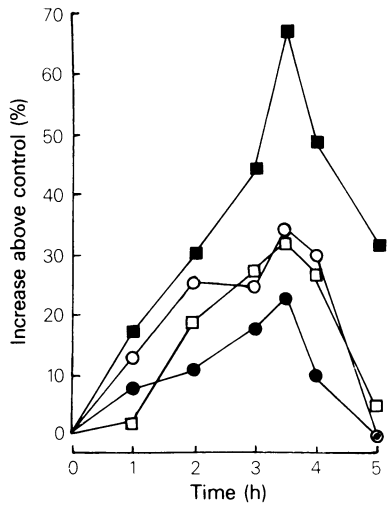

Figure 2 Time course of the effects of administration of a 10 mg/kg dose of some antidepressants on rat brain tryptophan concentration. Rats received at various times before death an intraperitoneal injection of a 10 mg/kg dose of desipramine (●), nomifensine (□), mianserin (○), tranylcypromine (■) or an equal volume (2 ml/kg) of saline. Brain tryptophan concentration was determined as described in the Methods section. The results are expressed as the percentage increases above control values and are derived from the means of 5 rats per each individual group at each time interval. The control values in saline-treated rats (in μg/g wet wt. of brain for each group of 5 rats; vertical bars show s.e.mean) at 1, 2, 3, 3.5, 4 and 5 h were 2.34±0.017, 2.17±0.048, 2.14±0.052, 2.07±0.060, 2.27±0.032 and 2.37±0.094 respectively.

Table 5 Effects of administration of a 0.5 mg/kg dose of various antidepressants and other drugs on rat brain tryptophan (Trp) concentration

Experiment No.	*Treatment*	*Brain Trp (μg/g)*	*Experiment No.*	*Treatment*	*Brain Trp (μg/g)*
1	Saline	2.40 ± 0.08	4	Saline	2.03 ± 0.04
	Amitriptyline	3.20 ± 0.07***		Iprindole	2.37 ± 0.03***
	Nortriptyline	3.52 ± 0.07***		Viloxazine	2.35 ± 0.05**
	Protriptyline	3.14 ± 0.05***		(Fluphenazine)	2.03 ± 0.05
	α-Flupenthixol	3.46 ± 0.07***		(Mefenamic acid)	2.12 ± 0.08
	(β-Flupenthixol)	2.61 ± 0.07		(Sodium salicylate)	2.34 ± 0.05**
2	Saline	2.21 ± 0.05	5	Saline	2.05 ± 0.02
	Desipramine	2.54 ± 0.06**		Clomipramine	2.45 ± 0.05***
	Mianserin	2.63 ± 0.07**		Imipramine	2.46 ± 0.04***
	Nomifensine	2.49 ± 0.08*		(Chlorpromazine)	2.09 ± 0.10
	Tranylcypromine	3.67 ± 0.18***		(Lorazepam)	2.17 ± 0.01***
	(Pargyline)	2.32 ± 0.06		(Allopurinol)	2.39 ± 0.04***
3	Saline	1.97 ± 0.07	6	Saline	2.10 ± 0.04
	Ludiomil	2.61 ± 0.07***		(Phenytoin)	2.19 ± 0.07
	Isocarboxazid	2.28 ± 0.04*		Zimelidine	2.49 ± 0.03***
	Nialamide	2.56 ± 0.06***		Zimelidine (10)*	2.68 ± 0.08***
	Phenelzine	2.72 ± 0.08***		Trazodone	2.37 ± 0.03**
	(Pemoline)	2.31 ± 0.05*		Trazodone (10)*	2.40 ± 0.04***
			7	Saline	2.03 ± 0.06
				Dothiepin	2.29 ± 0.05*
				Dothiepin (10)*	2.60 ± 0.13**

Rats received an intraperitoneal injection of drugs (0.5 mg/kg each) or an equal volume (2 ml/kg) of saline and were killed 3.5 h later. *Some antidepressants were also given in a 10 mg/kg dose each as their effects are reported here for the first time. Each experiment had its own saline-treated control group and was performed on a separate day to minimize animal variations. Values are means ± s.e.mean for each group of 5 rats, and the significance of the differences is indicated as follows: $*P<0.02$; $**P<0.005$; $***P<0.001$. Drugs that are not established antidepressants are indicated in parentheses.

tophan concentration was not significantly altered by a similar dose of the following non-antidepressant drugs: chlorpromazine, β-flupenthixol, fluphenazine, mefenamic acid, pargyline and phenytoin. The remaining four other drugs (lorazepam, salicylate, pemoline and allopurinol) increased brain tryptophan concentration by 6, 15, 17 and 17% respectively ($P = 0.02-0.001$).

Mechanisms of elevation of rat brain tryptophan concentration by administration of a 0.5 mg/kg dose of lorazepam, pemoline, salicylate and trazodone

Because lorazepam, pemoline and trazodone did not inhibit liver pyrrolase activity at a 0.5 mg/kg dose level (see Table 1), it was considered important to find out if their elevation of brain tryptophan concentration (see Table 5) at this dose level is caused by another mechanism, such as displacement of the serum-protein-bound amino acid. It was found that, at 3.5 h, the total serum tryptophan concentration (in μg/ml, means ± s.e.mean for 5 rats) of saline-treated controls of 22.34 ± 0.75 was not significantly altered by the 0.5 mg/kg dose of lorazepam, pemoline and trazodone (the corresponding values in these groups of animals were 23.32 ± 0.27, 23.90 ± 1.44 and 22.30 ± 0.58 respectively. By contrast, free serum tryptophan concentration was significantly increased from a control value (expressed as above) of 1.12 ± 0.02 to values of 1.30 ± 0.02, 1.55 ± 0.09 and 1.37 ± 0.05 after administration of lorazepam, pemoline and trazodone respectively. As a result, the percentage free serum tryptophan (means ± s.e.mean for each group of 5 rats) was increased from a control value of 5.01 ± 0.13 to values of 5.57 ± 0.08, 6.48 ± 0.17 and 6.14 ± 0.22 respectively by the above three drugs. These results therefore indicate the ability of a 0.5 mg/kg dose of lorazepam, pemoline and trazodone to displace serum-protein-bound tryptophan. Another displacer of bound tryptophan is sodium salicylate (see Badawy & Smith, 1972), but it has been shown (Badawy, 1982) that such displacement does not occur after administration of a 0.5 mg/kg dose of this drug, and that the elevation of

brain tryptophan concentration by this dose (see Table 5) is caused by the inhibition of liver tryptophan pyrrolase activity only.

Discussion

Effects of antidepressants on liver and brain tryptophan metabolism

The present results have established the ability of a dose (0.5 mg/kg) similar to, or even-smaller than, any single therapeutic dose of a large number of antidepressants, but not of many other drugs, to inhibit liver tryptophan pyrrolase activity and to elevate brain tryptophan concentration in the rat. Badawy & Evans (1981) found that this latter effect leads to an enhancement of cerebral 5-hydroxytryptamine synthesis and is caused by an increase in the availability of circulating tryptophan to the brain secondarily to the above inhibition of liver pyrrolase activity, which is produced by prevention of the conjugation of the apoenzyme with its cofactor haem. Of 19 antidepressants examined in the present work, only trazodone failed to inhibit pyrrolase activity at a 0.5 mg/kg, but not at a higher, dose level (see Table 1). This small dose of trazodone, however, increased brain tryptophan concentration (Table 5), by displacing the serum-protein-bound amino acid (see the text), as did the tranquillizer lorazepam and the stimulant pemoline (kethamed), which has been suggested (Kagan, 1974) as a useful antidepressant, and which was previously shown (Badawy & Evans, 1981) to inhibit pyrrolase activity at a 10 mg/kg dose level. The finding by these latter authors that a similar dose of fluphenazine inhibits pyrrolase activity could not be demonstrated in the present work with a 0.5 mg/kg dose (Table 1). By contrast, pyrrolase activity was inhibited and brain tryptophan concentration was increased by a 0.5 mg/kg dose of either allopurinol or salicylate, and it therefore remains to be seen whether these two drugs possess antidepressant properties of their own.

Mechanism of action of antidepressants and the role of the liver in depressive illness

It is generally thought that antidepressants act by inhibiting monoamine oxidase activity or amine reuptake or by affecting the functions of central noradrenergic and possibly also other monoaminergic systems. None of these mechanisms is, however, likely to play a major role in the mode of action of antidepressants for the following reasons: (1) the time lag between the start of antidepressant medication and the occurrence of a therapeutic response; (2) the lack of any quantitative (or qualitative) correlation between therapeutic efficacy and the extent (or occurrence) of the above effects; (3) the failure of investigators to demonstrate that a large number of antidepressants of various classes share any of these mechanisms. Our finding that 18–19 antidepressants, including the so-called second-generation antidepressants, share the single property of inhibiting liver tryptophan pyrrolase activity and, thereby, increase brain tryptophan concentration (and hence 5-hydroxytryptamine synthesis) is therefore a unique observation. Such common effect of antidepressants together with evidence for a defective cerebral 5-hydroxytryptamine synthesis in depressive illness (see van Praag, 1978; Badawy & Evans, 1981 and references cited in both) raise the not unreasonable possibility that antidepressants may exert their therapeutic action, at least in part, by inhibiting hepatic tryptophan metabolism, which appears to be disturbed (see the introduction), possibly as a result of pituitary-adrenal activation (for this latter effect in depressed patients, see Carroll, Curtis & Mendels, 1976; Schlesser, Winokur & Sherman, 1980 and references cited therein).

It is known (Schildkraut, 1973; Maas, 1978) that depressed patients with low urinary output of the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol respond to therapy with imipramine, but not amitriptyline, whereas the latter, but not the former, drug is effective in patients with enhanced excretion of the above metabolite. The differential response of tryptophan pyrrolase to inhibition by imipramine and amitriptyline in rats pretreated with cortisol or haematin (Table 4) provides an interesting animal model for the above response of depressed patients to these two drugs, but the possible relevance of these findings in the rat to the clinical situation requires extensive investigation in man.

The result shown in Table 4 and those in Tables 2 and 3 suggest, however, that enhanced liver tryptophan pyrrolase activity may or may not be inhibited by antidepressants and that any observed inhibition is relatively weaker than that of the basal enzyme (see Table 1). Such a possible weak inhibition in man together with factors such as: (1) the status of liver haem *in vivo* (because antidepressants inhibit pyrrolase activity by preventing the haem conjugation of the apoenzyme); (2) the ability of antidepressants to lower circulating corticosteroid levels (Samsonova & Lapin, 1973; Badawy & Evans, 1981); (3) the half-life of basal, induced and activated tryptophan pyrrolase (see Badawy & Evans, 1975); (4) the interval(s) between, and duration of pyrrolase inhibition by, administered therapeutic doses of the drugs, can explain the well known time lag between the start of antidepressant medication and the occurrence of a significant therapeutic response.

In conclusion, the present results and those previously reported (Badawy & Evans, 1981) strongly suggest that antidepressants may in part exert their therapeutic action by inhibiting liver tryptophan pyrrolase activity and thereby increase brain tryptophan concentration and 5-hydroxytryptamine synthesis. This suggestion is subject to experimental evaluation in man taking into consideration the wider biological implications of pituitary-adrenal activation.

We thank Mrs Madeline Warren for her personal support, Mr C.J. Morgan and Miss Nazeera F. Punjani for skilful assistance, Dr L. Rowlands (South Glamorgan Institute of Higher Education, Cardiff) for provision of technical assistance by his students Messrs H. Nabavi and B. Shomali, the various companies for generous gifts of drugs and Mr A. Dacey for animal maintenance.

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(Received December 14, 1981.
Revised April 20, 1982.)