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THE EFFECTS OF LOFEPRAMINE AND DESMETHYLIMIPRAMINE ON TRYPTOPHAN METABOLISM AND DISPOSITION IN THE RAT

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Abstract—Acute and chronic administration of lofepramine and its major metabolite desmethylimipramine (DMI) to rats elevates brain tryptophan concentration, thereby enhancing cerebral 5-hydroxytryptamine (5-HT) synthesis, by increasing the availability of circulating tryptophan to the brain, secondarily to inhibition of liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, L-tryptophan:O_2 oxidoreductase, decyclizing; EC 1.13.11.11) activity. The pyrrolase inhibition by lofepramine occurs independently of metabolism to DMI, because it can be demonstrated directly *in vitro*. Lofepramine also differs from DMI in its action profile on the above and related aspects of tryptophan metabolism and disposition. These results demonstrate that lofepramine influences tryptophan and 5-HT metabolism and disposition independently of its major metabolite DMI, and are discussed briefly in relation to the mechanism of action of antidepressants.

The tricyclic antidepressant drug lofepramine [*N*-methyl-*N*-(4-chlorophenacyl)-3-(10,11-dihydro-5*H*-dibenz(*b*,*f*)azepin-5-yl)-propylaminehydrochloride] is metabolized mainly in the liver to desmethylimipramine (DMI). Because the latter compound is also an antidepressant, the question has arisen as to the extent to which it contributes to the overall antidepressant effects of lofepramine. Although both compounds possess properties in common, including clinical efficacy, they also differ markedly in many other respects, notably cardiovascular and other toxic effects (for recent reviews, see Refs 1 and 2). We have previously shown [3–5] that 23 antidepressant drugs (including DMI) of different chemical classes and pharmacological profiles share the single property of elevating brain tryptophan concentration after acute administration to rats, thereby enhancing cerebral 5-hydroxytryptamine (5-HT) synthesis and possibly also turnover, by increasing the availability of circulating tryptophan to the brain, secondarily to inhibition of activity of the major tryptophan-degrading enzyme, hepatic tryptophan pyrrolase (tryptophan 2,3-dioxygenase, L-tryptophan:O_2 oxidoreductase, decyclizing; EC 1.13.11.11). It was therefore considered of interest to find out in the present work if lofepramine is also capable of exerting similar effects on tryptophan metabolism and disposition, and whether any of its likely effects occurs independently of its major metabolite DMI. Brief accounts of parts of this work have appeared in abstract form [6, 7].

MATERIALS AND METHODS

Animals and treatments. Locally bred Male Wistar rats (150–170 g at the start of experiments) were maintained on cube diet 41B (Oxoid) and water under a natural light:dark cycle and at 22 ± 1°. Rats were killed by decapitation between 1:00 and 3:00 p.m. Lofepramine HCl and desmethylimipramine (DMI) HCl were each dissolved in a mixture of dimethylformamide:saline (1:3, v/v) and were administered intraperitoneally at various dose levels. In the acute experiments, the dose range for both drugs was 0.5 to 10 mg/kg body weight and the animals were killed at various times as indicated with the relevant results, whereas in chronic experiments, the two drugs were given in a single daily dose of 3 or 6 mg/kg body weight (between 11:00 and 11:30 a.m.) and the animals were killed 2 hr after the last injection. Control rats received an equal volume (2 mL/kg) of the above vehicle by the same route.

Chemicals. The hydrochlorides of lofepramine and DMI were gifts from E. Merck Ltd (Darmstadt, F.R.G.), whereas all other chemicals were purchased from the Sigma Chemical Co. (Poole, U.K.) and BDH Chemicals (Poole, U.K.) and were of the purest commercially available grades.

Chemical, enzymic and other determinations. Tryptophan pyrrolase activity was determined in fresh-liver homogenates ([8]; see also the fuller description [9] and additional comments [10]) either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2 μM) cofactor (haematin). The activity of the apoenzyme (the haem-free predominant form of the pyrrolase in rat and human liver) was obtained by difference. The saturation of the enzyme with its cofactor haem was expressed as the percentage haem saturation (100 × holoenzyme activity/total enzyme activity). Concentrations of free (ultrafiltrable) serum, total (acid-soluble) serum, liver and brain tryptophan and

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Table 1. Inhibition of rat liver tryptophan pyrrolase activity *in vitro* by lofepramine and desmethylimipramine

Addition	Concentration	Liver tryptophan pyrrolase activity (μmol of kynurenine formed/hr/g wet wt) Holoenzyme	Total enzyme	Apoenzyme
Nil		1.8 ± 0.07	4.9 ± 0.32	3.1 ± 0.28
Lofepramine	10 μM	1.5 ± 0.09*	3.7 ± 0.09‡	2.2 ± 0.07†
	50 μM	1.6 ± 0.00*	2.9 ± 0.27§	1.3 ± 0.27§
	0.1 mM	1.6 ± 0.06	2.1 ± 0.12‖	0.5 ± 0.12‖
	1 mM	1.5 ± 0.06‡	1.7 ± 0.07‖	0.2 ± 0.03‖
DMI	10 μM	1.9 ± 0.12	3.7 ± 0.19‡	1.8 ± 0.30‡
	50 μM	1.9 ± 0.10	3.0 ± 0.09§	1.1 ± 0.18‖
	0.1 mM	1.7 ± 0.15	2.6 ± 0.06‖	0.9 ± 0.20‖
	1 mM	1.7 ± 0.12	2.1 ± 0.17‖	0.4 ± 0.09‖

Tryptophan pyrrolase activity was determined as described in Materials and Methods 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.

Values are means ± SEM for each group of four separate liver homogenates from normal untreated rats. The significance of the differences between control and test values is indicated as follows:

* $P < 0.05$; † $P < 0.025$; ‡ $P < 0.02$; § $P < 0.005$; ‖ $P < 0.001$.

DMI, desmethylimipramine.

those of brain 5-hydroxytryptamine (5-HT) and its major metabolite 5-hydroxyindol-3-ylacetic acid (5-HIAA) and of serum albumin, non-esterified fatty acids and corticosterone were all determined by standard procedures (for references, see Refs 11 and 12). Statistical analysis of results was by Student's *t*-test.

RESULTS

Effects of lofepramine and desmethylimipramine on rat liver tryptophan pyrrolase activity in vitro

The results in Table 1 show that both lofepramine and DMI inhibited the activity of rat liver tryptophan pyrrolase after addition *in vitro*. Thus, although the holoenzyme activity was either unaffected or only moderately decreased, that of the total enzyme was inhibited by concentrations of 10 μM–1 mM of lofepramine and DMI by 24–65% and 24–57%, respectively. The corresponding inhibition of the apoenzyme activity was therefore 29–94% and 42–87% by the two drugs, respectively.

Effects of acute lofepramine and desmethylimipramine administration on rat liver tryptophan pyrrolase activity

The time course of the effects of intraperitoneal administration of a 10 mg/kg body weight dose of lofepramine or DMI on rat liver tryptophan pyrrolase activity was examined (results not shown). We found that maximum inhibition occurred at 2 hr. With lofepramine, the inhibition (at 2 hr) of the total enzyme and apoenzyme activities was 57% and 91%, respectively, whereas that by DMI was 47% and 75%, respectively. With DMI, the inhibition was no longer significant at 5 hr, whereas the corresponding recovery point after lofepramine was 7 hr. No further changes in enzyme activity were observed during the subsequent 17–19 hr (i.e. up until the 24 hr point) after administration of either drug.

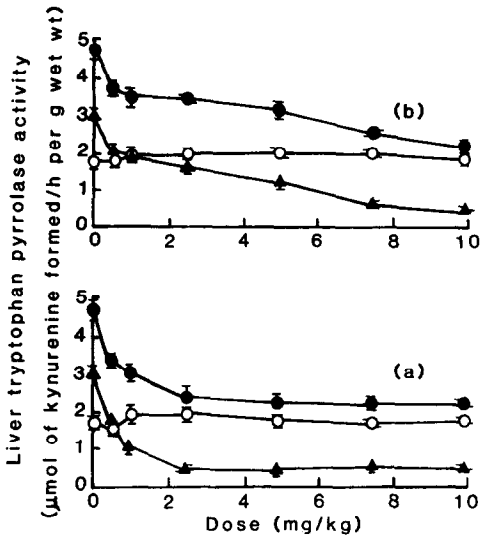

Fig. 1. Effects of acute administration of various doses of lofepramine and desmethylimipramine on the basal activity of rat liver tryptophan pyrrolase. Rats received an acute single intraperitoneal injection of various doses of lofepramine or desmethylimipramine (0.5–10 mg/kg body wt) or an equal volume (2 mL/kg) of the vehicle (dimethylformamide : saline, 1 : 3, v/v) and were killed 2 hr later. Tryptophan pyrrolase activity was determined as described in Materials and Methods, 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. Values are means ± SEM (bars) for each group of four rats. (a) Denotes lofepramine, whereas (b) denotes desmethylimipramine.

The effects of various doses of lofepramine and DMI were then examined at 2 hr (Fig. 1). The total pyrrolase and apoenzyme activities were significantly inhibited by 30% and 43% by a dose of lofepramine

Table 2. Reversal by an excess of added haematin of the inhibition of rat liver tryptophan pyrrolase activity by lofepramine or desmethylimipramine *in vitro* and after administration

Expt No.	Line No.	Treatment	Liver tryptophan pyrrolase activity (μmol of kynurenine formed/hr/g wet wt) Concentration of added haematin (μM) 0	2	4	6
1	1	Nil	2.5 ± 0.03	5.5 ± 0.06	5.5 ± 0.33	5.2 ± 0.26
	2	Lofepramine (1 mM)	2.4 ± 0.26	2.9 ± 0.22*	5.6 ± 0.12	5.5 ± 0.18
	3	DMI (1 mM)	2.3 ± 0.17	2.7 ± 0.24*	5.4 ± 0.32	4.8 ± 0.37
2	4	Vehicle	1.4 ± 0.16	3.3 ± 0.20	3.5 ± 0.32	3.5 ± 0.25
	5	Lofepramine (10 mg/kg)	1.5 ± 0.09	1.9 ± 0.10*	3.5 ± 0.24	3.7 ± 0.17
	6	DMI (10 mg/kg)	1.6 ± 0.09	2.1 ± 0.17*	3.8 ± 0.31	4.0 ± 0.31

Lofepramine and desmethylimipramine were either added *in vitro* (1 mM each) to normal rat liver homogenates, or administered intraperitoneally (10 mg/kg body wt each) 2 hr previously. The control group for the experiment *in vitro* consisted of portions of the same homogenates that did not receive any additions, whereas that for the administered drugs consisted of rats receiving an equal volume (2 mL/kg) of the vehicle (dimethylformamide:saline, 1:3, v/v). Tryptophan pyrrolase activity was determined as described in Materials and Methods either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2 μM) haematin. Additionally an excess of this source of the haem cofactor, of 4 and 6 μM concentrations, was also added.

Values are means ± SEM for each group of four rats. The values in lines 2 and 3 have been compared with those in line 1, whereas those in lines 5 and 6 were compared with those in line 4, and the significance of the differences is indicated as follows: * $P < 0.001$.

DMI, desmethylimipramine.

as little as 0.5 mg/kg, with maximum inhibition (of 51% and 87%, respectively) being caused by the 2.5 mg/kg dose. By contrast, inhibition after DMI administration was dose-dependent for the entire 0.5–10 mg/kg dose range, with inhibition of the total enzyme and apoenzyme activities caused by the 0.5 mg/kg dose being 21% and 37%, respectively.

Mechanism of inhibition of the basal activity of rat liver tryptophan pyrrolase by lofepramine and desmethylimipramine

We have previously shown [3, 5] that inhibition of rat liver tryptophan pyrrolase activity both *in vitro* and after administration of antidepressant drugs is caused by interference with the conjugation of the apoenzyme with its haem cofactor, because the inhibition was reversed by the addition *in vitro* of an excess of the cofactor source haematin. Similar results were obtained in the present work (Table 2) both *in vitro* (1 mM) and after acute administration (10 mg/kg body wt) of lofepramine and DMI.

Role of drug metabolism in the inhibition of rat liver tryptophan pyrrolase activity by acute lofepramine administration

As stated in the introduction, lofepramine is metabolized in the liver to DMI. The microsomal cytochrome P450-dependent mixed-function oxidase

Table 3. Effect of pretreatment of rats with compound SKF 525-A on the inhibition of liver tryptophan pyrrolase activity by lofepramine and desmethylimipramine administration

Pretreatment	Treatment	Line No.	Liver tryptophan pyrrolase activity (μmol of kynurenine formed/hr/g wet wt) Holoenzyme	Total enzyme	Apoenzyme
Saline	Vehicle	1	1.8 ± 0.12	4.1 ± 0.26	2.3 ± 0.19
SKF 525-A	Vehicle	2	1.8 ± 0.12	4.1 ± 0.24	2.3 ± 0.13
Saline	Lofepramine	3	1.8 ± 0.17	2.4 ± 0.15†	0.6 ± 0.13‡
SKF 525-A	Lofepramine	4	2.0 ± 0.06	2.6 ± 0.16†	0.6 ± 0.15‡
Saline	DMI	5	2.1 ± 0.11	2.5 ± 0.09†	0.4 ± 0.04‡
SKF 525-A	DMI	6	2.0 ± 0.25	2.5 ± 0.25*	0.5 ± 0.06‡

Rats received an intraperitoneal injection of lofepramine, desmethylimipramine (DMI) (10 mg/kg each) or an equal volume (2 mL/kg) of the vehicle (dimethylformamide:saline; 1:3, v/v) and were killed 2 hr later. The animals also received, 0.5 hr before the above treatments, a similar injection of either compound SKF 525-A (45 mg/kg) or an equal volume (1 mL/kg) of saline. Tryptophan pyrrolase activity was determined as described in Materials and Methods 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.

Values are means ± SEM for each group of four rats. The results in lines 2, 3 and 5 have been compared with those in line 1, whereas those in lines 4 and 6 were compared with those in line 2. The significance of the differences is indicated as follows: * $P < 0.01$; † $P < 0.005$; ‡ $P < 0.001$.

Table 4. Effects of administration of lofepramine and desmethylimipramine on the activity of rat liver tryptophan pyrrolase previously enhanced by hormonal induction by cortisol or cofactor activation by haematin

First injection	Second injection	Dose (mg/kg)	Line No.	Liver tryptophan pyrrolase activity (μmol of kynurenine formed/hr/g wet wt) Holoenzyme	Total enzyme	Apoenzyme
DMF	Vehicle	—	1	1.8 ± 0.09	5.2 ± 0.04	3.4 ± 0.07
DMF	LOF	0.5	2	1.7 ± 0.12	3.2 ± 0.06†	1.5 ± 0.17†
DMF	LOF	10	3	1.8 ± 0.10	2.4 ± 0.10†	0.6 ± 0.04†
DMF	DMI	0.5	4	1.8 ± 0.09	3.4 ± 0.08†	1.6 ± 0.17†
DMF	DMI	10	5	1.9 ± 0.11	2.6 ± 0.14†	0.7 ± 0.05†
Cortisol	Vehicle	—	6	5.8 ± 0.16†	11.9 ± 0.37†	6.1 ± 0.24†
Cortisol	LOF	0.5	7	3.4 ± 0.16†	5.8 ± 0.06†	2.4 ± 0.20†
Cortisol	LOF	10	8	2.1 ± 0.18†	5.8 ± 0.37†	3.7 ± 0.36*
Cortisol	DMI	0.5	9	4.7 ± 0.10*	5.8 ± 0.13†	1.1 ± 0.18†
Cortisol	DMI	10	10	2.5 ± 0.15†	5.3 ± 0.25†	2.8 ± 0.17†
Haematin	Vehicle	—	11	4.7 ± 0.18†	4.9 ± 0.11	—
Haematin	LOF	0.5	12	2.7 ± 0.03†	3.1 ± 0.12†	—
Haematin	LOF	10	13	2.3 ± 0.10†	2.8 ± 0.26†	—
Haematin	DMI	0.5	14	3.0 ± 0.19†	4.0 ± 0.12*	—
Haematin	DMI	10	15	2.6 ± 0.21†	3.1 ± 0.17†	—

Rats received an intraperitoneal injection of cortisol acetate (20 mg/kg), haematin hydrochloride (5 mg/kg) or an equal volume (1 mL/kg) of the solvent dimethylformamide (DMF) and were killed 4 hr later. The animals also received, at 2 hr after the above injections, a similar injection of lofepramine (LOF), desmethylimipramine (DMI) (0.5 or 10 mg/kg) or an equal volume (2 mL/kg) of the vehicle (DMF:saline, 1:3, v/v). Tryptophan pyrrolase activity was determined as described in Materials and Methods 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 the experiments with haematin, which acts by saturating the apoenzyme with haem, it is not appropriate to express the apoenzyme activity, hence the symbol (—).

Values are means ± SEM for each group of four rats. The values in lines 2, 3, 4, 5, 6 and 11 have been compared with those in line 1, those in lines 7, 8, 9 and 10 were compared with those in line 6 and those in lines 12, 13, 14 and 15 were compared with those in line 11. The significance of the differences is indicated as follows: * $P < 0.005$; † $P < 0.001$.

Table 5. Effects of pretreatment of rats with lofepramine and desmethylimipramine on the cortisol induction of liver tryptophan pyrrolase activity

Pretreatment	Treatment	Line No.	Liver tryptophan pyrrolase activity (μmol of kynurenine formed/hr/g wet wt) Haematin added (μM) 0	2	4	6
Vehicle	DMF	1	1.6 ± 0.07	4.2 ± 0.32	—	—
LOF	DMF	2	1.6 ± 0.09	3.2 ± 0.23*	—	—
DMI	DMF	3	1.7 ± 0.13	3.0 ± 0.10†	—	—
Vehicle	Cortisol	4	3.9 ± 0.28§	11.9 ± 0.98§	—	—
LOF	Cortisol	5	2.8 ± 0.37*	6.3 ± 0.73‡	11.8 ± 0.98	12.0 ± 1.03
DMI	Cortisol	6	3.5 ± 0.28	6.2 ± 0.66‡	11.8 ± 2.05	11.9 ± 1.94

Rats received an intraperitoneal injection of either cortisol acetate (20 mg/kg) or an equal volume (1 mL/kg) of the solvent dimethylformamide (DMF) and were killed 4 hr later. The animals also received, at 0.5 hr before these treatments, a similar injection of lofepramine (LOF), desmethylimipramine (DMI) (10 mg/kg each) or an equal volume (2 mL/kg) of the vehicle (DMF:saline, 1:3, v/v). Tryptophan pyrrolase activity was determined as described in Materials and Methods either in the absence (holoenzyme activity) or in the presence of concentrations of added haematin of 2 (total enzyme activity), 4 and 6 μM (excess).

Values are means ± SEM for each group of four rats. The values in lines 2, 3 and 4 have been compared with those in line 1, whereas those in lines 5 and 6 were compared with those in line 4. The significance of the differences is indicated as follows: * $P < 0.05$; † $P < 0.02$; ‡ $P < 0.005$; § $P < 0.001$.

The apoenzyme activity can be obtained from the difference between total activity (in presence of 2 μM haematin) and that of holoenzyme.

system appears to be responsible for such metabolism [13]. The results in Table 3 show that pretreatment of rats with compound SKF 525-A, an inhibitor of the above mixed-function oxidase xystem [14], which inhibits the conversion of lofepramine to DMI [13], did not influence the extent of inhibition of activity of liver tryptophan pyrrolase by subsequent lofepramine administration. The results in Table 3 also show that SKF 525-A alone did not alter tryptophan pyrrolase activity and was also unable to influence the inhibition caused by DMI administration.

Effects of acute lofepramine and desmethylimipramine administration on rat liver tryptophan pyrrolase activity previously enhanced by hormonal induction or cofactor activation

The results described so far have demonstrated that both lofepramine and DMI inhibit the basal activity of rat liver tryptophan pyrrolase. Pyrrolase activity can be enhanced by a variety of mechanisms [8], one of which, hormonal induction by glucocorticoids, may be of particular importance in depression, in view of the well known disturbance of the hypothalamic–pituitary–adrenal axis and the consequent elevation of circulating cortisol concentration in some 50% of depressed patients. It was therefore considered important to find out if acute lofepramine and DMI administration to rats will also cause a significant degree of inhibition of pyrrolase activity that has been or was being enhanced by one or more of the above mechanisms. In addition to enhancing pyrrolase activity by hormonal induction by cortisol, cofactor activation by haematin administration was also used as the alternative mechanism. Substrate activation by tryptophan was considered inappropriate here, because there is no evidence that circulating tryptophan concentration is elevated in depression. The results are shown in Table 4. It is clear that both lofepramine and DMI at the 0.5 and 10 mg/kg dose levels caused a significant inhibition of activity of the pyrrolase that has been induced by cortisol, or activated by haematin, pretreatment.

Absence of effect of acute lofepramine or desmethylimipramine administration on the cortisol induction of rat liver tryptophan pyrrolase

The results in Table 5 show that pretreatment of rats 0.5 hr earlier with a 10 mg/kg dose of lofepramine caused significant decreases (of 28% and 47%; P = 0.05–0.005) in the holoenzyme and total enzyme activities, respectively, of the pyrrolase that had been induced 4 hr later by subsequent cortisol administration. Pretreatment of rats with a 10 mg/kg dose of DMI also caused a 48% decrease in the total activity of the cortisol-induced pyrrolase, but did not alter significantly that of the holenzyme. Such decreases could imply an inhibition of either synthesis (induction) or activity. That synthesis is not impaired by lofepramine or DMI is suggested by the ability of an added excess of haematin to reverse the above inhibition and thus to restore fully

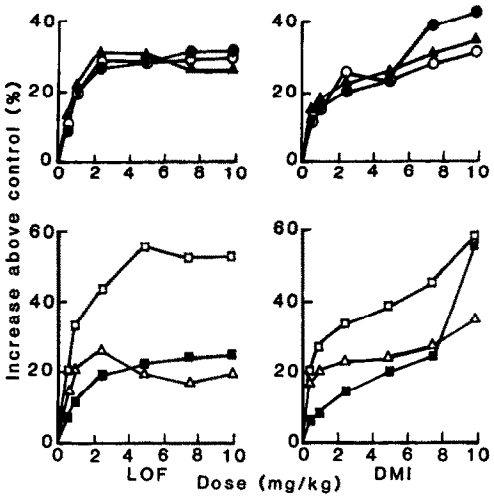

Fig. 2. Summary of the effects of acute administration of various doses of lofepramine and desmethylimipramine on rat tryptophan metabolism and disposition. Rats received an acute single intraperitoneal injection of various doses of lofepramine (LOF) or desmethylimipramine (DMI) (0.5–10 mg/kg) or an equal volume (2 mL/mg) of the vehicle (dimethylformamide:saline, 1:3, v/v) and were killed 3.5 hr later. Free serum (●), total serum (○), liver (▲) and brain (△) tryptophan and brain 5-HT (■) and 5-HIAA (□) concentrations were all determined as described in Materials and Methods. The results are expressed as percentage increases above the control values which have been obtained from six rats per group.

the expression of the enzyme induced by cortisol. The above inhibition is therefore confined to inactivation of the enzyme by lofepramine or DMI at a time interval after administration (4.5 hr) at which both agents are known to inhibit the enzyme, as is also shown by the results in Table 3 with the un-induced enzyme.

Effects of acute lofepramine and desmethylimipramine administration on rat tryptophan metabolism and disposition and related metabolic processes

We have previously shown [4] that the maximum increase in rat brain tryptophan concentration following acute intraperitoneal administration of a 10 mg/kg dose of antidepressant drugs occurred at 3.5 hr, i.e. 1.5 hr after maximum inhibition of liver tryptophan pyrrolase activity. In the present work, a 10 mg/kg intraperitoneal dose of lofepramine or DMI also caused maximum increases in concentrations of brain tryptophan, 5-HT and 5-HIAA and in those of liver, free serum and total serum tryptophan at 3.5 hr (results not shown). All subsequent experiments were therefore performed at this latter time interval. Dose–response experiments with lofepramine or DMI (0.5–10 mg/kg each) were performed at 3.5 hr and the results for tryptophan metabolic parameters are summarized in Fig. 2. As shown, both drugs caused increases in concentrations of free serum, total serum, liver and brain tryptophan

Table 6. Effects of acute lofepramine and desmethylimipramine administration on rat tryptophan metabolism and disposition

Parameter	Control	Lofepramine	DMI
Free serum Trp (μg/mL)	1.16 ± 0.02	1.27 ± 0.03†	1.30 ± 0.02§
Total serum Trp (μg/mL)	22.08 ± 0.30	24.60 ± 0.10§	24.63 ± 0.33§
Free serum Trp (%)	5.21 ± 0.09	5.16 ± 0.14	5.28 ± 0.11
Liver Trp (μg/g)	5.92 ± 0.25	6.70 ± 0.08†	6.82 ± 0.16†
Brain Trp (μg/g)	2.11 ± 0.11	2.40 ± 0.06*	2.26 ± 0.09
Brain 5-HT (μg/g)	0.84 ± 0.007	0.90 ± 0.016‡	0.89 ± 0.013‡
Brain 5-HIAA (μg/g)	0.30 ± 0.020	0.36 ± 0.020*	0.36 ± 0.019*

Rats received an intraperitoneal injection of lofepramine, desmethylimipramine (0.5 mg/kg each) or an equal volume (2 mL/kg) of the vehicle (dimethylformamide:saline, 1:3, v/v) and were killed 3.5 hr later. The parameters listed were determined as described in Materials and Methods.

Values are means ± SEM for each group of six rats. The significance of the differences between control and test results is indicated as follows: * $P < 0.05$; † $P < 0.02$; ‡ $P < 0.01$; § $P < 0.001$.

DMI, desmethylimipramine, Trp (tryptophan), 5-HT (5-hydroxytryptamine), 5-HIAA (5-hydroxyindol-3-ylacetic acid).

and in those of brain 5-HT and 5-HIAA, all of which were significant (P = 0.05–0.001) However, whereas the maximum increases induced by lofepramine were caused generally by a 2.5 mg/kg dose, the DMI-induced increases were dose-dependent for the entire dose range. It is noteworthy that these dose–response differences between lofepramine and DMI parallel those in the inhibition of liver tryptophan pyrrolase activity illustrated in Fig. 1.

The results in Table 6 give details of the 3.5 hr effects of intraperitoneal administration of a small (0.5 mg/kg) dose of lofepramine and DMI. Both drugs increased the concentrations of brain 5-HT and 5-HIAA (by 6–7% and 20%, respectively). Additionally, lofepramine administration resulted in an increase in brain tryptophan concentration, of 14%. These increases were associated with an increase in tryptophan availability to the brain, as suggested by the observed rises in both free serum and total serum tryptophan concentrations, of 9% and 11%, respectively, after lofepramine and 12% and 11%, respectively, after DMI. Because of these proportionate increases in both free serum and total serum tryptophan concentrations, tryptophan binding to serum proteins was not significantly altered by either drug treatment, as is suggested by the absence of any significant changes in the percentage of free serum tryptophan. This amino acid circulates mainly bound to albumin and, in experiments not shown here, we found that circulating albumin concentration was not significantly altered by acute administration of any of the doses of lofepramine or DMI used in the present work. Similarly, neither drug exerted any significant effect on the concentration of the physiological displacers of albumin-bound tryptophan, non-esterified fatty acids (results not shown). The results in Table 6 also show that liver tryptophan concentration was significantly elevated by both lofepramine and DMI (0.5 mg/kg each), by 13% and 15%, respectively.

Effects of chronic lofepramine and desmethylimipramine administration of rat liver tryptophan pyrrolase activity and on tryptophan metabolism and disposition and related metabolic processes

These effects were examined at weekly intervals for up to 4 weeks following intraperitoneal administration of single daily doses of either drug of 3 or 6 mg/kg body weight. Similar results were obtained for all the time intervals examined. The results for the 1-week experiment are shown in Table 7. As shown, liver tryptophan pyrrolase activity was significantly inhibited after 1 week of chronic administration of lofepramine or DMI at both dose levels. Thus the inhibition by the 3 and 6 mg/kg doses of lofepramine of the total enzyme activity was 35% and 55%, respectively, whereas that of apoenzyme activity was 64% and 91%, respectively. The corresponding inhibition by the 3 and 6 mg/kg doses of DMI was exactly the same as with lofepramine. Liver tryptophan concentration was elevated by 31% and 51% at 1 week after chronic administration of the 3 and 6 mg/kg doses of lofepramine, respectively, and by 20% and 64%, respectively, after the corresponding doses of DMI. Free (ultrafiltrable) serum tryptophan concentration was increased by the 3 and 6 mg/kg doses of lofepramine by 19% and 39%, respectively, whereas the corresponding increases in that of total (acid-soluble) tryptophan were 28% and 47%, respectively. These proportionate increases in free and total serum tryptophan concentrations were therefore not associated with altered binding of tryptophan to circulating albumin, as is suggested by the absence of any significant changes in the percentage free serum tryptophan. The chronic effects of the 3 mg/kg dose of DMI on circulating tryptophan concentrations and binding were similar to those of the corresponding dose of lofepramine. The 6 mg/kg dose of DMI, however, was associated with a much greater increase in free serum (92%), than in

Table 7. Effects of chronic lofepramine and desmethylimipramine administration on rat liver tryptophan pyrrolase activity and tryptophan metabolism and disposition and related metabolic processes

Parameter	Control (—)	Lofepramine (3 mg/kg)	Lofepramine (6 mg/kg)	Desmethylimipramine (3 mg/kg)	Desmethylimipramine (6 mg/kg)
Liver TP					
Holoenzyme	1.8 ± 0.08	1.8 ± 0.17	1.6 ± 0.02	1.8 ± 0.20	1.6 ± 0.10
Total enzyme	4.0 ± 0.26	2.6 ± 0.25§	1.8 ± 0.05¶	2.6 ± 0.21§	1.8 ± 0.09¶
Apoenzyme	2.2 ± 0.18	0.8 ± 0.21‖	0.2 ± 0.06¶	0.8 ± 0.09‖	0.2 ± 0.03¶
Liver Trp	9.00 ± 0.11	11.83 ± 0.57¶	13.57 ± 0.47¶	10.77 ± 0.65†	14.78 ± 0.46¶
Free serum Trp	1.78 ± 0.08	2.11 ± 0.06§	2.47 ± 0.14‖	2.10 ± 0.15*	3.41 ± 0.14¶
Total serum Trp	29.12 ± 1.43	37.23 ± 1.75‖	42.68 ± 0.92¶	37.23 ± 1.65‖	41.55 ± 0.79¶
Free serum Trp (%)	6.11 ± 0.52	5.67 ± 0.80	5.79 ± 0.22	5.64 ± 0.54	8.21 ± 0.48‡
Brain Trp	1.74 ± 0.04	2.29 ± 0.12‖	2.47 ± 0.17‖	2.20 ± 0.12‖	3.86 ± 0.22¶
Brain 5-HT	0.52 ± 0.021	0.67 ± 0.027‖	0.81 ± 0.028¶	0.77 ± 0.032¶	0.99 ± 0.060¶
Brain 5-HIAA	0.28 ± 0.016	0.33 ± 0.014*	0.41 ± 0.030‖	0.39 ± 0.022‖	0.45 ± 0.012¶
Serum NEFA	0.41 ± 0.05	0.44 ± 0.01	0.44 ± 0.05	0.43 ± 0.02	0.55 ± 0.02*
Serum albumin	41.4 ± 0.8	38.9 ± 1.4	41.3 ± 0.5	39.7 ± 0.8	40.3 ± 2.7
Serum corticosterone	52.5 ± 3.2	25.4 ± 3.4¶	15.3 ± 1.3¶	36.6 ± 1.5‖	37.8 ± 0.9‖

Lofepramine and desmethylimipramine were administered chronically by single daily intraperitoneal injections of either 3 or 6 mg/kg each and control rats received an equal volume (2 mL/kg) of the vehicle (dimethylformamide : saline, 1:3, v/v) by the same route. Animals were killed after 7 full days and at 2 hr after the last injection. The parameters listed were determined as described in Materials and Methods. Liver tryptophan pyrrolase activity is in μmol of kynurenine formed/hr/g wet wt, whereas serum non-esterified fatty acid (NEFA), albumin and corticosterone concentrations are in mM, g/L and nM, respectively. All other expressions (except the % free serum Trp) are in μg/mL of serum or per g wet weight of tissue.

Values are means ± SEM for each group of six rats. The significance of the differences between control and test results is indicated as follows: * $P < 0.05$; † $P < 0.025$; ‡ $P < 0.02$; § $P < 0.01$; ‖ $P < 0.005$; ¶ $P < 0.001$.

TP, tryptophan pyrrolase; Trp, tryptophan; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindol-3-ylacetic acid; NEFA, non-esterified fatty acids.

total serum (43%), tryptophan concentration, thus leading to a significant decrease in tryptophan binding to serum albumin, as suggested by the 34% increase in the percentage free serum tryptophan. As the results in Table 7 show, this latter increase is not caused by a decrease in circulating albumin concentration, but is most likely the result of the 34% elevation of that of serum non-esterified fatty acids induced by this dose (6 mg/kg) of DMI.

The results in Table 7 also show that the increase in circulating tryptophan availability described above was reflected in increases in brain tryptophan concentration, of 32% and 42% by the 3 and 6 mg/kg doses of lofepramine, respectively, and by 26% and 122% by the corresponding doses of DMI, respectively. This latter increase reflects the greater rise in free serum tryptophan concentration induced by the 6 mg/kg dose of DMI. The above two doses of lofepramine increased brain 5-HT and 5-HIAA concentrations by 29% and 56% and by 18% and 46%, respectively. The increases induced by the corresponding doses of DMI were 48% and 72%, and 39% and 61%, respectively.

The results in Table 7 additionally show that treatment of rats for 1 week with lofepramine or DMI lowered serum corticosterone concentration. Thus the 3 and 6 mg/kg doses of lofepramine decreased circulating corticosterone concentration by 52% and 71%, respectively, whereas the decreases caused by the corresponding doses of DMI were 30% and 28%, respectively.

DISCUSSION

The present results have demonstrated the effectiveness of the tricyclic antidepressant drug lofepramine as an inhibitor of tryptophan pyrrolase activity in rat liver. In this respect, lofepramine resembles 23 other antidepressants (including its major metabolite DMI) of various chemical structures and pharmacological classes [3–5]. Pyrrolase inhibition is therefore a property shared by a large number of different antidepressants and it remains to be seen if other existing and potential antidepressants could be added to this list. Pyrrolase inhibition by antidepressants should increase circulating tryptophan availability to the brain, which may be important in the treatment of depression, in view of recent evidence for decreased plasma tryptophan levels in major depression [15] and for the reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan [16].

That the pyrrolase inhibition by lofepramine administration is a direct effect, rather than being caused via metabolism to DMI or any other indirect mechanism, is suggested by the ability of the drug to inhibit the basal enzyme activity *in vitro* (Table 1). The ability of an excess of added haematin to reverse the inhibition (Table 2) suggests that lofepramine acts by preventing the conjugation of the apoenzyme with its cofactor haem, as is the case with other antidepressants [3, 5]. The inability of lofepramine to inhibit the cortisol induction (as distinct from inhibition of activity of the cortisol-induced enzyme, which is readily reversible by addition of an excess of haematin, see Table 5) suggests that the drug does not block enzyme (protein) synthesis and is thus free from this undesirable property.

Liver tryptophan pyrrolase activity could be enhanced by a number of mechanisms [8], of which hormonal induction by glucocorticoids is of particular importance in depression, in view of disturbed hypothalamic–pituitary–adrenal function in this illness. The ability of lofepramine to inhibit liver tryptophan pyrrolase activity that had previously been enhanced by hormonal induction by cortisol (or cofactor activation by haematin) (Table 4) suggests that this drug should be an effective pyrrolase inhibitor under conditions expected in depressed subjects.

Liver tryptophan pyrrolase is a major peripheral determinant of tryptophan availability (see, e.g., Ref. 11) and the results summarized in Fig. 2 show clearly that tryptophan accumulation in liver, serum and brain after acute administration was maximum with a dose of lofepramine (2.5 mg/kg body wt) known to cause the maximal inhibition of liver tryptophan pyrrolase activity (Fig. 1). A similar correlation can also be deduced from the corresponding data with DMI, which showed dose-dependent effects throughout the entire 0.5–10 mg/kg dose range. The changes in tryptophan disposition summarized in Fig. 2 and those exemplified in Table 6, and the associated absence of altered binding of the amino acid to circulating proteins are therefore typical consequences of liver tryptophan pyrrolase inhibition and may best be explained by this inhibition by lofepramine or DMI decreasing the rate of hepatic tryptophan degradation, thus leading to accumulation of the amino acid in the liver and a consequent increase in its availability in the circulation and, hence, to the brain.

Cerebral 5-HT synthesis is controlled mainly by brain tryptophan concentration, because the rate-limiting enzyme of the 5-HT biosynthetic pathway, tryptophan hydroxylase, is unsaturated with its tryptophan substrate [17–19]. The increases in brain 5-HT and 5-HIAA concentrations after administration of lofepramine and DMI (Fig. 2 and Table 6) may therefore be simply explained by the drugs enhancing 5-HT synthesis and possibly also turnover, secondarily to the associated increase in brain tryptophan concentration.

The above discussion has so far been concerned with the acute effects of lofepramine and DMI on tryptophan metabolism and disposition. Similar conclusions concerning all the aspects discussed could be drawn from the results of the chronic experiments shown in Table 7, except for the finding that the larger dose of DMI (6 mg/kg body wt) increases tryptophan availability to the brain additionally by causing displacement of albumin-bound tryptophan, most probably via non-esterified fatty acids. Furthermore, as the results in Table 7 show, lofepramine (and to a lesser extent DMI) can lower circulating corticosterone concentration. A possible decrease in circulating cortisol concentration in patients with depression from an elevated to a normal value would be an additional advantage of therapy with lofepramine or other antidepressants, as this should limit excessive hepatic tryptophan degradation (and also that of the tyrosine precursor of brain catecholamines).

As stated in the introduction, lofepramine is metabolized mainly to DMI and the question has therefore arisen as to what extent DMI contributes to the antidepressant action of its parent compound. The present results show clearly that lofepramine exerts a number of effects on tryptophan metabolism and disposition, which may be of importance in relation to its antidepressant action, differently from, and independently of, DMI. Thus lofepramine exerts a direct inhibitory effect *in vitro* on liver tryptophan pyrrolase activity (Table 1) and the inhibition observed after its acute administration is not influenced by prevention of its metabolism to DMI by compound SKF 525-A (Table 3). Furthermore, the dose–response profile of the lofepramine effects on liver tryptophan pyrrolase activity, which is reflected in the associated modulation of tryptophan metabolism and disposition, is different from that of DMI (Figs 1 and 2). In view of the importance of tryptophan and 5-HT in depressive illness and the above differences between lofepramine and DMI and also those concerning the cardiovascular and other toxic effects mentioned in the introduction and the fact that the DMI therapeutic blood concentration range (108–158 μg/L) is much higher than the levels (up to 50 μg/L) achieved after lofepramine intake (see Ref. 12 and references cited therein), it is not unreasonable to suggest that lofepramine exerts its therapeutic antidepressant action independently from its metabolite DMI.

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