Biochemical Pharmacology, Vol. 22, pp. 1499–1507. Pergamon Press, 1973. Printed in Great Britain.

ANTIDEPRESSANTS AND LIVER TRYPTOPHAN PYRROLASE ACTIVITY

MAYA L. SAMSONOVA and IZYASLAV P. LAPIN

Laboratory of Psychopharmacology, Bekhterev Psychoneurological Research Institute, Leningrad, Ul. Bekhtereva 3, Leningrad C-19, U.S.S.R.

(*Received* 12 *August* 1972; *accepted* 23 *November* 1972)

Abstract—Tricyclic antidepressants were found to diminish liver tryptophan pyrrolase activity in rats. Neuroleptics and anticholinergics, used for comparison, did not change the enzyme activity. No effects of imipramine were observed *in vitro* nor in adrenalectomized animals. Imipramine did not influence tryptophan or hydrocortisone induction of tryptophan pyrrolase. Antidepressants in doses which lower the enzyme activity also lowered blood plasma concentration of 11-hydroxycorticosteroids. This effect may be responsible for inhibition of tryptophan pyrrolase activity by antidepressants.

Inhibitors of monoamine oxidase did not decrease liver tryptophan pyrrolase activity in rats and mice but suppressed tryptophan induction of this enzyme.

The possible relations between reduction by antidepressants of liver tryptophan pyrrolase activity and enhanced serotonin synthesis in the central nervous system and the thymoanaleptic effect are discussed.

ANTIDEPRESSANTS strongly affect metabolism of serotonin (5HT).[1] The most marked effect of tricyclic antidepressants (TAD) is the inhibition of reuptake of 5HT into neurons which results in accumulation of extraneuronal amine. Inhibitors of monoamine oxidase (IMAO) diminish deamination and thereby increase the level of intraneuronal 5HT. Activation of the central serotoninergic processes induced by antidepressants could underlie selective mood-elevating or thymoanaleptic action of these drugs in depressive patients.[1]

The rate of synthesis of 5HT in brain is dependent on the level of tryptophan (Try) in the tissue and plasma. It is well-known that the kynurenine pathway is the major route of metabolism of Try. Since this pathway is regulated by the activity of liver tryptophan pyrrolase (TP), we have examined the influence of antidepressants on this enzyme.

The effects of two antidepressant drugs (imipramine and tranylcypromine) on the TP activity in the liver of rats have been studied[2–4] and it has been found an increase in activity of the enzyme after administration of a single dose of imipramine (6 mg/kg), a decrease after administration of both drugs in a dose of 40 mg/kg in stressed animals and no change after the animals were given both drugs during 3 weeks. Because of the multiple possibilities for control of TP activity we felt it was essential to examine the effects of various dosages of most typical antidepressants on the level of TP activity.

Abbreviations used: 5HT, serotonin; MAO, monoamine oxidase; IMAO, inhibitors of MAO; TAD, tricyclic antidepressants; TP, tryptophan pyrrolase; Try, tryptophan.

METHODS

Albino male and female mice weighing 17–25 g and albino male and female rats weighing 150–250 g from the Rappolovo farm were used. Drugs were injected i.p. Controls received distilled water. Because of the difficulty of maintaining solutions of the aminoacid, immediately before injection, DL-Try was dissolved in distilled water by addition of 6 N NaOH, and the pH adjusted to neutral (pH 7).

IMAO: pargyline (Abbot), phenelzine (made in the USSR) and niamide (Pfizer) were dissolved in water immediately before injection, 2 hr prior to Try administration. Solutions of TAD: imipramine ("Melipramine"), chlorimipramine ("Anafranil") and amitriptyline ("Damilen"), of neuroleptics chlorpromazine ("Aminazine") and haloperidol (Gedeon Richter) and of anticholinergics atropine and benactyzine ("Amyzil") were injected 4 hr prior to decapitation.

Bilateral adrenalectomy was performed under aether anesthesia and sham-operated rats were used as controls. Animals were used in experiments 5 days after operation.

Liver TP activity was measured according to method of Knox.[5] L-Try (Hoffmann-La-Roche Ltd.) was used as a substrate. Enzyme activity was calculated according to the calibration curve made with kynurenine base (Sigma Ltd.).

Blood plasma 11-hydroxycorticosteroids were measured by fluorimetric method.[6]

Figures in the tables show the means ± standard errors.

RESULTS

Tricyclic antidepressants

Effect of TAD on tryptophan pyrrolase activity. Effect of TAD was compared with the action of neuroleptics and anticholinergics chemically and/or pharmacologically similar to TAD (Table 1).

TABLE 1. EFFECT OF TRICYCLIC ANTIDEPRESSANTS, NEUROLEPTICS AND ANTICHOLINERGICS ON RAT LIVER TRYPTOPHAN PYRROLASE ACTIVITY

Drug	Dose (mg/kg)	Tryptophan pyrrolase activity	
		μM of kynurenine/g/hr	% Control
Distilled water		2·15 ± 0·31	100
Imipramine	15	1·26 ± 0·17*	66
Distilled water		2·59 ± 0·24	100
Chlorimipramine	2	1·64 ± 0·34*	63
Amitriptyline	2	1·34 ± 0·17†	51
Chlorpromazine	1	2·01 ± 0·52	79
Distilled water		1·05 ± 0·13	100
Haloperidol	1	1·54 ± 0·18	146
Atropine	1	1·06 ± 0·17	100
Benactyzine	1	1·46 ± 0·14	139
Distilled water		2·30 ± 0·27	100
Chlorpromazine	5	4·04 ± 0·20‡	179

Each group consisted of 10 female rats. Animals were decapitated 4 hr after injection of drugs and TP determined as described in Methods.

* $P < 0·05$; † $P < 0·01$; ‡ $P < 0·002$.

Imipramine, chlorimipramine and amitriptyline inhibited TP activity by 35–50 per cent. Anticholinergics, benactyzine and atropine, as well as the neuroleptics, chlorpromazine and haloperidol (all in a dose of 1 mg/kg which is adequate for their typical pharmacological activities), did not affect enzyme activity. Chlorpromazine, however, in a dose of 5 mg/kg significantly increased TP activity.

The relationship between dose of imipramine, taken as typical of the TAD, and inhibition of TP activity was examined in detail (Table 2).

TABLE 2. EFFECT OF VARIOUS DOSES OF IMIPRAMINE ON RAT LIVER TRYPTOPHAN PYRROLASE ACTIVITY

Drug	Dose (mg/kg)	Tryptophan pyrrolase activity	
		μM of kynurenine/g/hr	% Control
Distilled water		1·80 ± 0·10	100
Imipramine	1	1·99 ± 0·12	110
Imipramine	3	1·67 ± 0·09	92
Distilled water		2·40 ± 0·28	100
Imipramine	7·5	1·55 ± 0·08*	65
Imipramine	15	1·57 ± 0·12*	65
Imipramine	30	1·76 ± 0·22	73
Distilled water		1·15 ± 0·16	100
Imipramine	50	2·41 ± 0·19†	209

Each group consisted of six female rats. Decapitation was made 4 hr after injection of imipramine.
* $P < 0·05$; † $P < 0·002$.

While 7·5 or 15 mg/kg of imipramine decreased levels of liver TP activity, dose greater than 30 mg/kg resulted in an increase of TP activity. Thus, no close dose–response relationship could be demonstrated.

After treating rats with high doses of chlorimipramine or amitriptyline (25 mg/kg) both drugs also increased enzyme activity and again no clear dose–response relationship was apparent.

Inhibition of TP activity by imipramine was observed only *in vivo*. The enzyme activity was not changed by incubation of liver homogenates 1, 2, 3 and 4 hr with solutions of imipramine in concentrations (10^{-3} — 10^{-6} M) which correspond or exceed those *in vivo*.

Since corticosteroids are known to alter TP activity, the possibility that drug-induced alterations in TP activity are mediated through the adrenal cortex hormones was explored.

In adrenalectomized rats TP activity was 60 per cent lower than that found in sham-operated or normal rats (Table 3).

Imipramine treatment resulted in a decrease in enzyme activities in livers of sham-operated animals, but did not alter significantly those in livers of adrenalectomized rats.

Effect of TAD on hormonal or substrate induction of tryptophan pyrrolase activity. TAD, in doses which diminish liver enzyme activity, did not influence elevation of the enzyme activity produced by injection of hydrocortisone or by administration of Try (Table 4).

No TAD altered neither hormonal nor substrate induction of TP activity.

TABLE 3. LACK OF INHIBITORY EFFECT OF IMIPRAMINE ON LIVER TRYPTOPHAN PYRROLASE ACTIVITY IN ADRENALECTOMIZED RATS

Drug	Dose (mg/kg)	Tryptophan pyrrolase μM of kynurenine/g/hr			
		Sham-operated rats		Adrenalectomized rats	
		Mean ± S.E.	% Control	Mean ± S.E.	% Control
Distilled water		1·85 ± 0·15	100	0·78 ± 0·21	100
Imipramine	15	1·31 ± 0·14	76*	0·94 ± 0·28	120

Each group consisted of ten male animals. Decapitation was made 4 hr after injection.
* $P < 0·05$.

TABLE 4. EFFECT OF TRICYCLIC ANTIDEPRESSANTS ON INDUCTION OF TRYPTOPHAN PYRROLASE ACTIVITY PRODUCED BY HYDROCORTISONE OR TRYPTOPHAN

Treatment	Dose (mg/kg)	Tryptophan pyrrolase activity	
		μM of kynurenine/g/hr	% Control
Hormonal induction			
Distilled water + distilled water		2·00 ± 0·21	100
Hydrocortisone + distilled water	2·5	4·53 ± 0·38*	226
Hydrocortisone + imipramine	2·5 15·0	4·41 ± 0·40	220
Distilled water + distilled water		3·61 ± 0·30	100
Hydrocortisone + distilled water	2·5	6·15 ± 0·76*	170
Hydrocortisone + amitriptyline	2·5 2·0	8·61 ± 1·70	237
Hydrocortisone + Chlorimipramine	2·5 2·0	7·98 ± 1·29	221
Substrate induction			
Distilled water + distilled water		4·35 ± 0·39	100
Tryptophan + distilled water	450	10·32 ± 0·84*	237
Tryptophan + imipramine	450 15	9·07 ± 0·87	208
Tryptophan + chlorimipramine	450 2	9·05 ± 0·45	207
Tryptophan + amitriptyline	450 2	8·58 ± 0·79	220

Each group consisted of ten female rats. Drugs were injected 4 hr prior to decapitation and Try was injected 2 hr after the drugs.
* $P < 0·05$.

Effect of TAD on the level of 11-hydrocorticosteroids in rat blood plasma. Is the inhibitory effect of antidepressants related to their influence on the level of corticosteroids in the blood? The concentration of 11-hydroxycorticosteroids in rat blood plasma was significantly lowered (by about 35–40 per cent) 1 hr after injection of antidepressants in doses which resulted in a decrease in TP activity. This effect was no longer apparent 4 hr after injection of the drugs.

TABLE 5. EFFECT OF TRICYCLIC ANTIDEPRESSANTS ON THE LEVEL OF 11-HYDROXYCORTICOSTEROIDS IN RAT BLOOD PLASMA

Drug	Dose (mg/kg)	Concn 11-hydroxycorticosteroids (Controls γ/100 ml blood)	(Stress γ/100 ml blood)
		1 hr after injection	
Distilled water		35·41 ± 3·75	68·38 ± 5·49
Imipramine	15	22·40 ± 1·48†	34·40 ± 3·03†
Chlorimipramine	2	24·30 ± 1·69†	59·60 ± 6·60
Amitriptyline	2	22·50 ± 2·51*	67·20 ± 4·57
		4 hr after injection	
Distilled water		38·20 ± 4·45	65·10 ± 4·55
Imipramine	15	47·50 ± 3·70	65·30 ± 6·50
Chlorimipramine	2	43·50 ± 4·60	61·90 ± 6·70
Amitriptyline	2	46·20 ± 4·99	67·20 ± 5·73

Each group consisted of five male rats. Stress was produced by 30 sec swimming in a water of 20°.
* $P < 0.05$; † $P < 0.01$.

Monoamine oxidase inhibitor antidepressants

Effect of IMAO on liver tryptophan pyrrolase activity. Neither phenelzine nor iproniazid altered the levels of TP activity (Table 6).

TABLE 6. EFFECT OF INHIBITORS OF MONOAMINE OXIDASE ON LIVER TRYPTOPHAN PYRROLASE ACTIVITY IN RATS AND MICE

Drug	Dose (mg/kg)	Tryptophan pyrrolase activity (μM kyn./g/hr) Mice Mean ± S.E.	Mice % Control	Rats Mean ± S.E.	Rats % Control
Distilled water		3·91 ± 0·83	100	3·03 ± 0·49	100
Phenelzine	50	4·38 ± 0·80	112	3·36 ± 0·76	111
Distilled water		2·10 ± 0·30	100	2·39 ± 0·43	100
Niamid	100	5·24 ± 0·71†	250	5·93 ± 0·72*	248
Distilled water		5·65 ± 0·51	100	2·80 ± 0·49	100
Pargyline	100	9·40 ± 0·86*	166	7·23 ± 0·78†	257
Iproniazid	100	5·88 ± 0·65	104	Not tested	

Each group consisted of seven male animals. Decapitation was made 2 hr after injection.
* $P < 0.05$; † $P < 0.02$.

TABLE 7. EFFECT OF MONOAMINE OXIDASE INHIBITORS ON SUBSTRATE INDUCTION OF TRYPTOPHAN PYRROLASE ACTIVITY IN RATS AND MICE

Treatment	Dose (mg/kg)	Tryptophan pyrrolase activity	
		μM of kynurenine/g/hr	% Control
		Mice	
Distilled water		3·75 ± 0·33	100
Tryptophan	1000	17·76 ± 1·15	473†
Iproniazid + tryptophan	100 1000	16·36 ± 1·35	436
Niamid + tryptophan	100 1000	10·93 ± 1·60	269‖
Pargyline + tryptophan	100 1000	13·98 ± 0·75	372‡
Distilled water		2·61 ± 0·26	100
Tryptophan	1000	12·23 ± 2·02	468†
Phenelzine + tryptophan	50 1000	4·78 ± 0·54	183§
		Rats	
Distilled water		3·05 ± 0·44	100
Tryptophan	600	16·35 ± 1·34	536*
Phenelzine + tryptophan	50 600	9·00 ± 2·70	295‡

Each group consisted of seven males. Decapitation was made 2 hr after injection of Try.
* $P < 0·05$, † $P < 0·001$ (as compared with control, distilled water), ‡ $P < 0·02$, § $P < 0·01$, ‖ $P < 0·002$ (comparison was made with group treated with Try).

After administration of niamid and pargyline a significant increase in activity of the liver TP was found.

Effect of IMAO on substrate induction of tryptophan pyrrolase. Phenelzine, niamid, and pargyline diminished Try-induced increase of TP activity but iproniazid in the dose used (which normally inhibits MAO) failed to prevent elevation of the enzyme activity induced by Try (Table 7).

TABLE 8. EFFECT OF MONOAMINE OXIDASE INHIBITORS ON HYDROCORTISONE INDUCTION OF TRYPTOPHAN PYRROLASE ACTIVITY IN RATS

Treatment	Dose (mg/kg)	Tryptophan pyrrolase activity	
		μM of kynurenine/g/hr	% Control
Distilled water		2·40 ± 0·45	100
Hydrocortisone	25	5·21 ± 0·96*	218
Phenelzine + hydrocortisone	50 25	3·94 ± 0·70	164
Niamid + hydrocortisone	100 25	10·54 ± 1·61†	439

Each group consisted of six male rats. Hydrocortisone was injected, 2 hr later the IMAO was administered and 4 hr after injection of hydrocortisone the rats were killed.
* $P < 0·05$ (comparison with control group); † $P < 0·05$ (as compared with group treated with hydrocortisone).

Effect of IMAO on hydrocortisone induction of tryptophan pyrrolase. Hormonal induction of TP in rats was not changed by phenelzine and enhanced by niamid (Table 8).

Effect of IMAO on 11-hydroxycorticosteroids in rat blood plasma. IMAO did not influence the level of 11-hydroxycorticosteroids in blood plasma of control rats, but significantly lowered plasma levels of these hormones in rats stressed by 30 sec of swimming.

TABLE 9. EFFECT OF MONOAMINE OXIDASE INHIBITORS ON 11-HYDROXYCORTICOSTEROIDS IN RAT BLOOD PLASMA

Treatment	Dose (mg/kg)	Concn of 11-hydroxycorticosteroids (γ/100 ml blood)	
		Controls	Stress
Distilled water		49·20 ± 4·96	97·60 ± 6·40
Phenelzine	50	48·80 ± 5·49	73·60 ± 6·22*
Iproniazid	100	57·60 ± 6·94	65·20 ± 3·22†
Distilled water		36·50 ± 6·39	74·00 ± 5·51
Niamid	100	41·60 ± 5·59	56·80 ± 4·86*
Pargyline	100	54·80 ± 2·14	56·40 ± 2·14†

Each group consisted of five or ten (controls) male rats. Decapitation was made 2 hr after injection. Stress was produced by 30 sec swimming in a water of 20°.

* $P < 0.05$; † $P < 0.02$.

DISCUSSION

Antidepressants effects on levels of TP activity in liver appear to be correlated with their effect on plasma corticosteroids. In unstressed animals TAD diminish both plasma corticosteroids and TP activity, while IMAO produce little or no decrease in the plasma steroids or the enzyme activity. TAD lowered plasma levels of 11-hydroxy-corticosteroids* in control animals. Although a specific stress was not administered, these animals in our colony are subject to a variety of stressors (noise in the laboratory, environmental temperature, handling etc.). The diminution of corticoid levels in plasma by TAD may indicate an effect on the responses to the mild stresses. It is clear, however, that chlorimipramine or amitryptyline did not prevent the elevation of plasma corticoids produced by the stress of swimming.

IMAO are reported to either elevate or diminish the levels of TP activity.[7]

High doses of TAD, like neuroleptics, elevate levels of TP activity in unstressed animals. It is known[8] that high doses of TAD have pharmacological spectrum of activity similar to that of neuroleptics.

When TP was induced by administration of hydrocortisone or Try, TAD did not alter the effect of the inducing agent, but some IMAO decreased the induction of the TP by Try. The latter observation is in agreement with previous finding from our laboratory.[9] This effect is probably not related to inhibition of MAO, since iproniazid failed to alter the induction of TP by Try.

* Experiments on 11-hydroxycorticosteroids in plasma were carried out in collaboration with Dr. V. E. Ryzenkov from Department of Pharmacology of the Institute of Experimental Medicine.

In rats subjected to emotional stress, probably similar to that normally encountered by our animals, Pekkarinen[10] found that TAD used in doses close to the lower those in our experiments, diminish the increase in concentration of corticosteroids induced by the stress.

Because the diminishing effects of TAD on TP activity were prevented in adrenalectomized animals, it is likely that such diminutions in enzyme activity are mediated by the adrenal cortical hormones.

The role of influences on TP activity of TAD and IMAO in their therapeutic action is not known. Direct evidence for an effect of antidepressants on liver TP activity in man are lacking. However, some data suggest that this phenomenon of diminution of enzyme levels, which is observed in animals, probably appears in depressed patients treated with TAD. During treatment with TAD there is a decreased excretion of 17-hydroxycorticosteroids,[11] i.e. an effect similar to that observed in the experiments on rats reported here. There is a good correlation between clinical improvement and restoration of normal excretion of corticosteroids in depressive syndromes. This is particularly evident in depressions with anxiety, where steroid excretion is markedly increased before treatment.

The increased levels of circulating corticoids found in depression presumably are associated with an increase in TP activity. If reduction of liver TP activity during the treatment with antidepressants takes place in depressive patients, this effect may contribute to the mood-elevating action of these drugs by diminishing the "tryptophan drain". A key question, however, is a duration of effects of antidepressants. Antidepressants if only acting in short-lasting way would not be very specific and effective for serotonin levels. Unless the antidepressant would lower corticoids and TP over a long period of time under repeated administrations, it seems unlikely that serotonin levels in brain could be affected. Studies with repeated dosages would, therefore, be a necessary complement to the data shown in this paper.

Decreased formation of kynurenine probably results in an increase plasma levels of Try. Since 5HT synthesis may vary with the levels of Try in plasma and brain,[12] decrease in activity of TP may result in enhanced hydroxylation of Try. Lowered formation of 5HT has been reported after activity of TP was increased.[13,14] Furthermore, diminution of TP activity leads to decrease of production of kynurenine and its metabolites, which antagonize some central effects of 5HT and tryptamine.[15]

IMAO may elevate 5HT levels by inhibition of the deamination of the amine and/or by enhancing the rate of synthesis of 5HT. The primary action of IMAO is believed to be on the degradative process, but an effect on TP may also be of importance. It would be of interest, therefore, to examine the effect on depression of some inhibitors of TP alone and in combination with either Try or IMAO.

CONCLUSION

Imipramine (7·5 and 15 mg/kg) diminished levels of rat liver tryptophan pyrrolase activity by 30–35 per cent. This effect was observed 4, 6 and 20 hr after injection of the drug. A similar effect was found for two other antidepressants tested, amitriptyline and chlorimipramine (2 and 10 mg/kg). Neuroleptics (chlorpromazine and haloperidol) and anticholinergics (atropine and benactyzine) did not change the enzyme activity. High doses of antidepressants and neuroleptics increased tryptophan pyrrolase activity.

Imipramine did not alter the enzyme activity *in vitro*, or in adrenalectomized animals, or the substrate or hydrocortisone induction of tryptophan pyrrolase.

Imipramine, amitriptyline and chlorimipramine lowered blood level of 11-hydroxycorticosteroids. This effect may be responsible for inhibition of tryptophan pyrrolase activity produced by antidepressants.

Among the monoamine oxidase inhibitors tested, phenelzine (50 mg/kg) and iproniazid (100 mg/kg) did not change liver tryptophan pyrrolase activity in rats and mice but niamid and pargyline (100 mg/kg) increased it.

The monoamine oxidase inhibitors tested (except iproniazid) suppressed tryptophan-induced activation of tryptophan pyrrolase and did not influence hydrocortisone-induced activation.

Monoamine oxidase inhibitors lowered the elevation of blood 11-hydroxycorticosteroids produced by swimming stress but did not influence the initial (control) level of these hormones.

The possible role of inhibition of liver tryptophan pyrrolase activity by antidepressants in their therapeutic mood-elevating action is discussed.

REFERENCES

1. I. P. Lapin and G. F. Oxenkrug, *Lancet* **1,** 132 (1969).
2. A. Mangoni, G. Paracchi, F. Brambilla, M. G. Bernetti and D. Lanzara, *Boll. Soc. Ital. biol. Sper.* **42,** 1838 (1966).
3. F. Cabibbe, G. Paracchi and D. Lanzara, *Boll. Soc. Ital. biol. Sper.* **43,** 1183 (1967).
4. F. Cabibbe and G. Paracchi, *Boll. Soc. Ital. biol. Sper.* **44,** 1254 (1968).
5. W. E. Knox, *Meth. Enzymol.* **2,** 242 (1955).
6. Yu. A. Pankov and I. N. Usatova, *Methods of Study of Some Hormones and Mediators*, p. 23. Moscow (1965).
7. T. Satoh and T. Iwamoto, *Jap. J. Pharmac.* **18/1,** 89 (1968).
8. I. P. Lapin, *Therapie*, **19,** 1107 (1964).
9. M. L. Samsonova and G. F. Oxenkrug, *Vopr. med. khimii*, **18,** 198 (1972).
10. A. Pekkarinen, *Eripainos Lääketehdas Leiraksen Julkaisuja*, **20,** 111 (1970).
11. P. V. Birjukovitch, E. A. Rushkevitch and S. P. Zelinski, in *Pharmacological Backgrounds of Antidepressant Effect* (Ed. I. P. Lapin), p. 61. Leningrad (1970).
12. J. D. Fernström and R. J. Wurtman, *Science* **173,** 149 (1971).
13. G. Curzon, *Pharmakopsychiatrie/Neuropsychopharmakologie*, **2,** 234 (1969).
14. A. R. Green and G. Curzon, *Biochem. Pharmac.* **19,** 2061 (1970).
15. I. P. Lapin, *Psychopharmacologia* **26,** 237 (1972).