EFFECT OF IMIPRAMINE, AMITRIPTYLINE AND THEIR MONOMETHYL DERIVATIVES ON RESERPINE ACTIVITY

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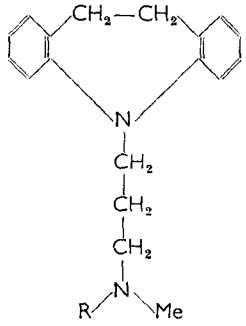
Impramine, amitriptyline and their respective *N*-monomethyl derivatives (desmethylimipramine and desmethylamitriptyline) prevent reserpine activity to varying degrees. Desmethylimipramine and desmethylamitriptyline are more effective than are imipramine and amitriptyline in reducing the hypothermia induced by reserpine in rats. Imipramine and desmethylimipramine are more effective than are amitriptyline and desmethylamitriptyline in decreasing the severity of gastric ulcers induced by reserpine in restrained rats. Desmethylimipramine does not prevent the lowering of brain amines by reserpine. The antagonistic effect of imipramine toward leptazol convulsions in mice is not shared by desmethylimipramine. Imipramine and desmethylimpramine do not potentiate the central effects of 5-hydroxytryptophan and tryptamine and do not prevent the hypothermia induced by 5-hydroxytryptamine, α-methyl-dopa or chlorpromazine. The activity of desmethylimipramine may be differentiated therefore on a pharmacological basis from that of the monoamine oxidase inhibitors and amphetamine.

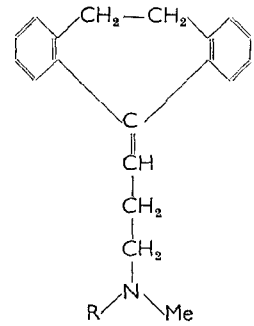
IN several animal species, imipramine causes a weak phenothiazine-like tranquillisation (Domenjoz and Theobald, 1959) in contrast to its action on the symptoms of endogenous depression reported first by Kuhn (1957) and confirmed by others. In our previous work we reported the anti-reserpine action of imipramine suggesting that this could be a clue to its antidepressant action (Garattini, 1959; Costa, Garattini and Valzelli, 1960). The anti-reserpine action of imipramine was more effective when the antagonist was given chronically; unlike chlorpromazine, imipramine greatly potentiated the anti-reserpine action of a monoamine oxidase inhibitor (Costa, 1960). We observed also that imipramine increases the brain 5-hydroxytryptamine (5-HT) concentration but we could not interpret the significance of this finding because the effect was transient, it was present in rats but not in rabbits; and imipramine was not amonoamine oxidase inhibitor (Costa, Garattini and Valzelli, 1960; Costa, 1960); Domenjoz and Theobald (1959), Sulser and Watts (1960) and Sigg, Gyermek and Soffer (1961) confirmed our pharmacological findings. More recently Brodie and his group (Sulser, Watts and Brodie, 1961; Gillette, Dingell, Sulser, Kuntzman and Brodie, 1961) have elucidated the problem of the anti-reserpine action of imipramine by showing that the antagonism was due to the accumulation in brain of the desmethylimipramine previously described and isolated among many other urinary metabolites by Hermann, Schindler and Pulver (1959). The great merit of Brodie's group (Brodie, Bickel and Sulser, 1961) was to show that this imipramine metabolite not only antagonises but reverses the reserpine syndrome, eliciting an endogenous excitation when given before reserpine; the drug is not active *per se* in normal animals. Moreover, chronic administration of imipramine is more effective against reserpine than

single injections because of the accumulation in brain of the imipramine metabolite. Finally, the fact that in clinical use desmethylimipramine acts more rapidly against endogenous depression than imipramine (Brodie, Bickel, Sulser, 1961) stresses the importance of the anti-reserpine effect as a test for antidepressant action.

This discovery by Brodie's group prompted us to investigate the effect of desmethylimipramine on a number of pharmacological and biochemical effects of reserpine in order to prove the specificity of the antagonism existing between reserpine and desmethylimipramine. For comparison amitriptyline (Vernier, 1961) and its *N*-monomethyl analogue (desmethylamitriptyline) (Hucker and Porter, 1961) were also evaluated as reserpine antagonists.



R = Me = Imipramine
R = H = Desmethylimipramine



R = Me = Amitriptyline
R = H = Desmethylamitriptyline

EXPERIMENTAL

Animals. Female Sprague-Dawley rats weighing 170 g., female Swiss mice, weighing 20 g. and the Zebra fish, *Brachydanius rerius* were used in these experiments.

Methods

Body temperature was measured by inserting an electric thermometer into the rectum. Heart rate was measured from an electrocardiogram record (speed 50 cm./min.). Experimental ulcers were produced by giving reserpine, 5 mg./kg., i.p., to rats kept under restraint according to the technique of Rossi, Bonfils, Lieffogh, and Lambling (1956). Leptazol (0·5 per cent acqueous solution) was infused into the tail vein at a constant speed of 0·14 ml./min. according to Orloff, Williams and Pfeiffer (1949) and Fingl and McQuarrie (1960).

Adrenal hypertrophy was studied 6 days after unilateral adrenalectomy under light ether anaesthesia.

Brain 5-HT and noradrenaline levels were measured by a spectrofluorimetric method according to Shore (1959).

The expansion of melanophores was observed in *Brachydanius rerius* kept in beakers containing 50 ml. of distilled water at 18–20°.

Drugs. Reserpine was kindly supplied by Ciba; imipramine and desmethylimipramine by Geigy; amitriptyline, desmethylamitriptyline,

and α-methyl-dopa by Merck, Sharp and Dohme; tetrabenazine by Hoffman La Roche; chlorpromazine by Farmitalia and 5-HT creatinine sulphate by Vister.

The doses and the route of administration used are given in the Tables.

Results

Hypothermia by reserpine. All four compounds tested, when given i.p. 1 hr. before the administration of reserpine, 2·5 mg./kg. i.v., prevent the hypothermia induced by reserpine. The results obtained are in Table I from which it is evident that imipramine and amitriptyline are less

TABLE I

EFFECT OF IMIPRAMINE AND ITS DERIVATIVES ON HYPOTHERMIA INDUCED BY RESERPINE

No. of rats	Treatment in mg./kg. i.p. (1 hr. before reserpine 2·5 mg./kg. i.v.)	Body temperature (° ± S.E.) hours after reserpine			
		0	4	6	24
13	Saline	36·8 ± 0·16	34·4 ± 0·33	33·0 ± 0·35	26·8 ± 1·49
5	Imipramine 7·5	36·0 ± 0·20	36·1 ± 0·18	36·3 ± 0·46	31·2 ± 0·94
11	Imipramine 15	35·7 ± 0·12	37·7 ± 0·30	37·0 ± 0·24	37·5 ± 0·5
6	Imipramine 30	35·0 ± 0·43	37·4 ± 0·27	37·1 ± 0·31	36·1 ± 0·32
11	*DMI 7·5	36·5 ± 0·11	37·8 ± 0·13	37·3 ± 0·16	36·3 ± 0·2
11	DMI 15	36·1 ± 0·23	38·2 ± 0·07	37·4 ± 0·14	37·3 ± 0·3
5	Amitriptyline 7·5	35·8 ± 0·36	35·1 ± 0·32	34·4 ± 0·65	30·2 ± 2·6
11	Amitriptyline 15	35·9 ± 0·30	36·3 ± 0·27	34·7 ± 0·20	33·5 ± 0·5
6	Amitriptyline 30	35·2 ± 0·67	36·3 ± 0·35	35·3 ± 0·32	31·7 ± 1·35
11	†DMA 7·5	36·3 ± 0·07	36·2 ± 0·24	35·1 ± 0·21	34·8 ± 0·2
10	DMA 15	36·7 ± 0·20	37·7 ± 0·16	35·5 ± 0·18	32·9 ± 1·0

* Desmethylimipramine.
† Desmethylamitriptyline.

effective than the respective desmethyl derivatives in preventing the hypothermia induced by reserpine.

Ulcer by restraint. Reserpine, 5 mg./kg. i.p., increases the incidence of formation of gastric ulcers in restrained rats. Pretreatment with any of the four compounds prevent this effect as it is shown in Table II.

TABLE II

EFFECT OF IMIPRAMINE AND ITS DERIVATIVES ON GASTRIC ULCER INDUCED BY RESERPINE IN RESTRAINED RATS

No. of rats	Treatment in mg./kg. i.p. (30 min. before 5 mg./kg. i.p. reserpine)	Severity of ulcer
25	Controls	100 ± 5
10	Imipramine 15	25 ± 7
10	Imipramine 7·5	50 ± 14
10	DMI 15	39 ± 15
10	DMI 7·5	64 ± 14
5	Amitriptyline 30	57 ± 18
5	Amitriptyline 15	72 ± 9
5	DMA 30	45 ± 12
5	DMA 15	93 ± 12

Other pharmacological effects. Imipramine and its desmethyl derivative, 15 mg./kg., but not amitriptyline, prevent the bradycardia induced by reserpine, 2·5 mg./kg. i.p. The compounds were injected 30 min. before, and the heart rate was measured 8 hr. after, giving reserpine.

Reserpine decreases the threshold in mice for tonic convulsions during infusion with leptazol. Imipramine and amitriptyline, 50–75 mg./kg. i.p., 3 hr. before reserpine, 5 mg./kg. i.p., and 1 hr. before leptazol, but not desmethylimipramine, counteract the effect of reserpine on leptazol-induced convulsions. However imipramine and amitriptyline at the doses tested exert a direct anticonvulsant activity, while desmethyl-imipramine is inactive.

Desmethylimipramine does not inhibit the stimulation of adrenal hypertrophy found when reserpine was given after monoadrenalectomy at a dose of 200 μg./kg. daily for 6 days.

Imipramine and desmethylimipramine differ from monoamine oxidase inhibitors, in that given in doses of 15–30 mg./kg. i.p., they do not increase the hyperthermia and tremors produced by 5-hydroxytryptophan, 50 mg./kg. i.p., in mice or the convulsions produced by tryptamine, 40 mg./kg. i.v., in rats.

Effect on melanophore expansion. Reserpine produces expansion of the melanophores of *Brachydanius rerius* (zebra fish) at doses of 0·1–0·5 μg./ml. Both desmethyl compounds, but not imipramine and amitriptyline at a dose of 2 μg./ml., prevent the effect induced by reserpine. Amitriptyline alone acts on melanophores in a manner similar to that of reserpine.

Effect on brain amines. As is shown in Table III, desmethylimipramine does not prevent the lowering of brain 5-HT and noradrenaline induced by reserpine.

TABLE III

EFFECT OF DESMETHYLIMIPRAMINE ON DEPLETION OF BRAIN 5-HT AND CATECHOLAMINE INDUCED BY RESERPINE IN RATS

No. of rats	Treatment	Brain		Body temperature	Blepharospasm
		5-HT	NOR.		
8	Controls	100 ± 3	100 ± 5·2	37·3 ± 0·22	–
8	DMI, 15 mg./kg. i.p.	104 ± 1·8	103 ± 3·4	36·8 ± 0·18	–
8	Reserpine, 2·5 mg./kg. i.v.	35 ± 2·4	22 ± 1·5	34·3 ± 0·4	+
8	DMI, 15 mg./kg. i.p. + reserpine 2·5 mg./kg.. i.v.	35 ± 2·6	25 ± 1·6	37·6 ± 0·3	–

DMI was given 1 hr. before reserpine.

Effect on other types of hypothermia. To establish the specificity of the anti-reserpine effect exerted by imipramine and desmethylimipramine, other drugs capable of inducing hypothermia were tested. The results obtained are in Table IV.

The only hypothermia prevented by imipramine and desmethylimipramine is that induced by tetrabenazine, a short acting reserpine analogue (Pletscher, Besendorf and Gey, 1959). The lowering of body temperature produced by chlorpromazine, α-methyl-dopa and 5-HT are not antagonised.

DISCUSSION

Imipramine, amitriptyline and the desmethyl derivatives prevent, with differing potency, some pharmacological effects induced by reserpine. The hypothermia induced by reserpine is, on the whole, inhibited more by the desmethyl derivatives than by imipramine and amitriptyline. This is

of interest in relation to the findings described above, that the desmethyl derivatives are the metabolites occurring in the tissues after the administration of imipramine and amitriptyline. It may therefore be suggested that the anti-reserpine effects observed after the administration of imipramine and amitriptyline are mediated through the formation of the respective desmethyl derivatives.

TABLE IV

EFFECT OF IMIPRAMINE AND DESMETHYLIMIPRAMINE ON VARIOUS AGENTS INDUCING HYPOTHERMIA

No. of rats	Treatment mg./kg. i.p.	Body temperature after hr.			
		0	2	4	6
6	Tetrabenazine 40	37·2 ± 0·18	35·8 ± 0·56	35·0 ± 0·51	36·6 ± 0·26
6	Imipramine 15 + tetrabenazine 40	35·7 ± 0·18	36·9 ± 0·13	37·5 ± 0·15	37·0 ± 0·05
6	DMI 15 + tetrabenazine 40	35·5 ± 0·47	36·6 ± 0·11	36·9 ± 0·20	36·6 ± 0·26
		0	1	4	6
15	α-Methyl-dopa 500	36·9 ± 0·18	34·7 ± 0·14	33·3 ± 0·24	34·4 ± 0·37
7	Imipramine 15 + α-methyl-dopa 500	34·9 ± 0·34	33·7 ± 0·23	33·7 ± 0·53	34·6 ± 0·75
12	DMI 30 + α-methyl-dopa 500	34·7 ± 0·33	34·3 ± 0·22	34·2 ± 0·33	34·4 ± 0·26
		0	1	2	3
6	5-HT 20	36·8 ± 0·05	32·5 ± 0·23	34·5 ± 0·60	36·0 ± 0·50
6	Imipramine 15 + 5-HT 20	35·1 ± 0·18	31·0 ± 0·40	32·2 ± 0·57	33·5 ± 0·36
6	DMI 15 + 5-HT 20	35·0 ± 0·22	30·2 ± 0·98	31·6 ± 0·73	32·7 ± 0·55
		0	1	2	5
5	Chlorpromazine 10	37·7 ± 0·19	34·1 ± 0·62	33·9 ± 0·94	35·1 ± 0·83
5	DMI 15 + chlorpromazine 10	36·2 ± 0·25	33·2 ± 0·31	32·2 ± 0·30	34·9 ± 0·48

The two derivatives counteract the expansion of melanophores induced by reserpine in zebra fish while imipramine and amitriptyline are almost inactive at the same concentration.

Imipramine and desmethylimipramine are more effective than are amitriptyline and desmethylamitriptyline in preventing the gastric ulcers induced by reserpine in restrained rats.

However not all the pharmacological effects exerted by imipramine and amitriptyline may necessarily be mediated by their *N*-monomethyl derivatives. For example, the anticonvulsant activity shown by imipramine is not shared by its derivative.

Again not all the effects of reserpine are counteracted by imipramine or its congeners. The stimulation exerted on adrenal hypertrophy is not inhibited by desmethylimipramine. This is consistent with the observation that this derivative does not prevent the release of ACTH by reserpine (B. B. Brodie, personal communication).

The antireserpine activity exerted by the derivative is different from that of the monoamine oxidase inhibitors.

Desmethylimipramine does not prevent the lowering of brain amines induced by reserpine. Furthermore, as opposed to monoamine oxidase inhibitors, it does not increase the tremors and convulsions induced respectively by 5-hydroxytryptophan (Horita and Gogerty, 1958) in mice, and tryptamine (Tedeschi, Tedeschi and Fellows, 1959) in rats.

The effects of imipramine and its derivatives on the hypothermia induced by reserpine seem to be specific since the hypothermia induced by chlorpromazine, 5-HT, and α-methyl-dopa is not prevented by imipramine or desmethylimipramine. Tetrabenazine, being a short acting reserpine-like drug, is antagonised by imipramine and its metabolite.

These results differentiate desmethylimipramine from the stimulants. Amphetamine counteracts not only reserpine and tetrabenazine, but also chlorpromazine, 5-HT and α-methyl-dopa (experiments to be reported).

In conclusion, imipramine and its congeners exhibit a pharmacological pattern which is different both from monamine oxidase inhibitors and amphetamine. This may be of interest in assessing the mechanism of action of the antidepressant effect shown by these drugs.

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