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Pharmacological studies on modaline sulphate (W 3207)

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Modaline sulphate (W 3207) has pharmacological effects which probably belong both to the group of monoamine oxidase inhibitors and the imipramine-like drugs. It differs from the monoamine oxidase inhibitors by producing an increase in body temperature in fully reserpinized animals. It differs from imipramine-like drugs in not potentiating the hyperthermic effect of isoprenaline. The effects of modaline are probably related to the formation of a metabolite because they are prevented by the administration of SKF 525 A.

MODALINE sulphate, 2-methyl-3-piperidinopyrazine monosulphate (W 3207), is a new compound with an original chemical structure, unrelated to hydrazine, iminodibenzyl derivatives or other known antidepressant agents (Gylys & Osborne, 1962), showing therapeutic antidepressive action (Feldman, 1963; Dunlop, De Felice, Bergen & Resnick, 1964). This compound has pharmacological effects which are typical both of monoamine oxidase inhibitors and imipramine-like substances (Dubnick, Morgan & Phillips, 1963; Gylys, Muccia & Taylor, 1963). The purpose of this paper is to report investigations aimed at resolving these effects and to establish whether the reported pharmacological effects are induced by the compound itself or by its metabolic products.

Experimental

MATERIALS AND METHODS

Female Sprague-Dawley rats, average weight 100 g, and female Swiss mice, average weight 22 g, were used. Modaline was given intraperitoneally to all animals. Isoprenaline was infused into the rat tail vein for 15 min at a dose of 400 or 40 μg/rat/min. In these experiments rats were kept restrained in individual cylindrical cages (diameter 4·7 cm, length 15·5 cm) of galvanised wire; the thermometer electrode cable was retained in the rectum throughout the experiment. In other experiments, body temperature was recorded by inserting the thermometer at the moment of determination. Monoamine oxidase inhibition was estimated *in vitro* according to Weissbach, Smith, Daly, Witkop & Udenfriend (1960) by measuring the oxidation of kinuramine in brain homogenates of rats pretreated *in vivo* with modaline.

Drugs used for comparison were pheniprazine (JB 516) (Horita, 1958; 1959; Spector, Prockop, Shore & Brodie, 1958), *N*-benzoyl-*N'*-phenylethylhydrazine (T-3) (Bettinetti, 1961; Jori, Bonaccorsi, Valzelli & Garattini, 1963) and desipramine (Dubnick, Leeson & Phillips, 1962; Garattini, Giachetti, Jori, Pieri & Valzelli, 1962). Other details of the experimental conditions appear in the various tables. All doses are expressed as salts. Drugs used were kindly supplied by the following

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sources: modaline (W 3207 B) (Warner-Lambert), desipramine (Geigy), nortriptyline (Pharmacia), pheniprazine (JB 516) (Farber), *N*-benzoyl-*N'*-phenylethylhydrazine (T-3) (supplied by Dr. S. Pietra), L(−)-dihydroxyphenylalanine (dopa) (Hoffmann-LaRoche), reserpine (Serpasil) (Ciba), phentolamine (Regitin) (Ciba), propranolol (Inderal) (Imperial Chemical Industries), dibenamine (Smith Kline & French), isoprenaline (Isoproterenol) (Biosintex).

Results

INHIBITION OF MONOAMINE OXIDASE ACTIVITY

Brain homogenates obtained from rats treated with various drugs were analysed for their monoamine oxidase activity. Table 1 demonstrates

TABLE 1. MONOAMINE OXIDASE INHIBITION AFTER TREATMENT WITH VARIOUS DRUGS *IN VIVO* AND POTENTIATION OF TRYPTAMINE EFFECTS BY VARIOUS DRUGS

Compound*	Dose (mg/kg i.p.)	% inhibition of brain monoamine oxidase	Compound †	ED50; mg/kg i.p. fiducial limits P <0·05)
Modaline	4	56	Modaline	4·7 (10·30–2·10)
,,	8	89	Modaline**	6·60 (12·20–3·50)
,,	16	93	Pheniprazine	0·55 (0·96–0·31)
Pheniprazine	5	82	T-3	1·85 (0·90–3·70)
Desipramine	15	−15	Desipramine	>15·0

* Given 4 hr before assessing monoamine oxidase activity.
** Given 30 min before tryptamine.
† Compounds were given 4 hr before tryptamine (5 mg/kg i.v.) ED50 = dose enhancing the effects induced by tryptamine to 50% of the maximum possible increase.

that modaline shows an effect similar to pheniprazine in blocking the activity of this enzyme, whereas desipramine, in agreement with the observations of Pulver, Exer & Hermann (1960) and Usdin & Usdin (1961), is inactive.

POTENTIATION OF THE PHARMACOLOGICAL EFFECTS OF TRYPTAMINE

Monoamine oxidase inhibitors enhanced the convulsive or tonic actions induced by intravenous injection of tryptamine (Tedeschi, Tedeschi & Fellows, 1959) which included hunching of the back, backward locomotion, Straub tail, salivation and clonic convulsions of the anterior paws. These symptoms were scored according to an arbitrary scale (1 + to 3 + for clonic convulsions and 1 + for each of the other symptoms; maximum 7+).

Table 1 also reports the ED50 of modaline, pheniprazine, and T-3 in the potentiation of tryptamine. Desipramine had no effect at doses active in other tests. Modaline was often very active when injected 30 min before tryptamine. The potentiation it induces was inhibited by pretreatment with SKF 525 A (see Table 2), a compound known to inhibit several microsomal enzymes responsible for the metabolism of various drugs (Brodie, Gillette & La Du, 1958; Gillette, 1963).

POTENTIATION OF HYPERTHERMIA INDUCED BY DOPA

In mice treated with monoamine oxidase inhibitors, dopa induced an increase in the motor activity and in the body temperature (Everett,

TABLE 2. EFFECT OF SKF 525 A ON THE POTENTIATION OF TRYPTAMINE INDUCED BY MODALINE

Treatment	Dose of modaline mg/kg	%increase of tryptamine effects after	
		30 min*	4 hr*
SKF 525 A + Modaline	16	76 ± 3	—
— ,, ...	16	100 ± 0·01	—
SKF 525 A + ,, ...	8	21 ± 3	76 ± 4
— ,, ...	8	91 ± 2	92 ± 5
SKF 525 A + saline	—	—	37 ± 8

All the animals received tryptamine (5 mg/kg i.v.).
SKF was given orally at a dose of 50 mg/kg 30 min before modaline.
* Time between modaline and tryptamine.

Davin & Toman, 1959; Van der Wende & Spoerlein, 1962). The data reported in Table 3 show that all the tested antidepressant drugs increased the hyperthermic effect of dopa.

TABLE 3. POTENTIATION OF DOPA HYPERTHERMIA

Compound	Dose mg/kg	Body temperature (°C) ± s.e. after dopa	
		30 min	60 min
Saline	—	38·2 ± 0·1	35·5 ± 0·3
Modaline	4	39·5 ± 0·5*	38·4 ± 0·5*
,, ...	8	39·2 ± 0·2*	39·6 ± 0·1*
,, ...	16	40·2 ± 0·2*	39·9 ± 0·2*
T-3	10	38·7 ± 0·4	39·9 ± 0·3*
Pheniprazine	2·5	39·3 ± 0·4*	38·5 ± 0·2*
Desipramine	7·5	39·6 ± 0·2*	35·4 ± 0·5

The compounds were given 12 hr after pheniprazine (10 mg/kg i.p.) and 30 min before dopa (50 mg/kg i.p.).
* Difference statistically significant ($P < 0·01$) in respect to the group treated with saline.

RESERPINE INHIBITION

Pretreatment with modaline inhibited the hypothermia induced by intravenous administration of reserpine. Table 4 summarizes the results

TABLE 4. EFFECT OF VARIOUS DRUGS ON THE HYPOTHERMIA INDUCED BY RESERPINE

Treatment (mg/kg)	Changes of body temperature (°C) ± s.e. after reserpine			
	40 min	4 hr	6 hr	24 hr
Saline —	+1·2 ± 0·08	−1·7 ± 0·2	−2·9 ± 0·2	−1·3 ± 3
Modaline* 16	+1·9 ± 0·1	+1·0 ± 0·7	+0·5 ± 0·1	—
,, 8	+1·2 ± 0·3	−0·3 ± 0·2	−0·7 ± 0·3	−0·5 ± 0·4
,, 4	+1·3 ± 0·4	−0·9 ± 0·3	−1·2 ± 0·2	−1·3 ± 0·5
,, 2	+0·8 ± 0·3	−2·5 ± 0·3	−2·7 ± 0·5	−0·3 ± 0·6
Desipramine 15	+1·7 ± 0·1	+0·7 ± 0·2	+0·8 ± 0·1	+0·3 ± 0·4
Saline —	+1·5 ± 0·1	−1·3 ± 0·2	−2·3 ± 0·2	−0·8 ± 0·2
Modaline** 16	+2·4 ± 0·1	−0·1 ± 0·1	−0·3 ± 0·1	−0·6 ± 0·2
,, 8	+2·0 ± 0·4	−0·5 ± 0·2	−0·9 ± 0·5	−1·0 ± 0·3
,, 4	+0·8 ± 0·9	−3·1 ± 1·4	−2·7 ± 0·7	−0·2 ± 0·6
,, 2	+1·5 ± 0·3	−2·6 ± 0·5	−3·3 ± 0·6	−1·4 ± 0·8
Desipramine 15	+1·5 ± 0·3	+0·4 ± 0·4	−1·1 ± 0·5	−0·5 ± 0·2
Pheniprazine 10	+2·8 ± 0·6	−0·2 ± 0·3	+0·5 ± 0·5	−1·1 ± 0·3

Reserpine was given intravenously (2·5 mg/kg). Drugs were given intraperitoneally 30 min* and 18 hr** before reserpine.

obtained with various doses of modaline when it was injected 30 min and 18 hr before reserpine. Modaline antagonised hypothermia in fully

reserpinised rats when it was injected 18 hr after reserpine. The data in Fig. 1 show that it paralleled the effect induced by desipramine at the

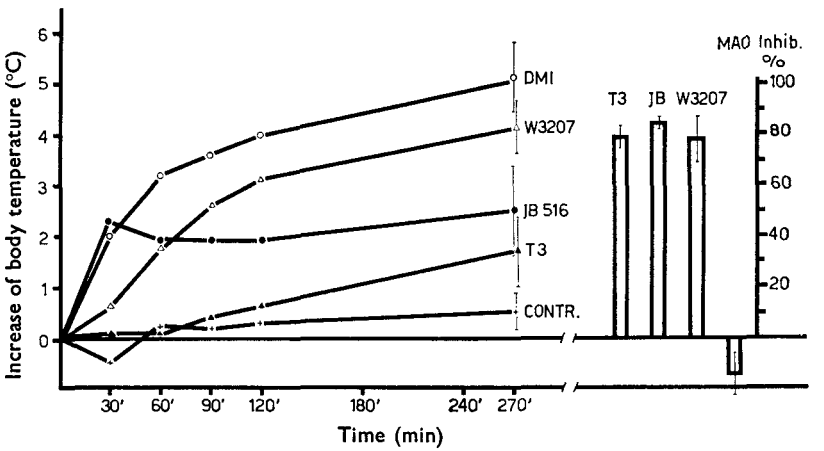

FIG. 1. Rats were treated 18 hr before the test with reserpine (5 mg/kg i.v.) at a room temperature of 20°. Drugs were injected i.p. at the beginning of the experiment: desipramine (DMI) 15 mg/kg; modaline sulphate (W3207) 16 mg/kg; pheniprazine (JB516) 2·5 mg/kg and T-3 10 mg/kg. The columns represent the % inhibition of brain monoamine oxidase. The vertical bars show the s.e. of the mean. Each point represents at least 5 determinations.

same dose level of 16 mg/kg. T-3 had no significant effect on reserpine-induced hypothermia at a dose that exhibited strong monoamine oxidase inhibition.

The reversal of reserpine hypothermia induced by modaline was almost completely inhibited by pretreatment with SKF 525 A (Fig. 2). The latter compound has no effect when injected alone in reserpinised rats.

POTENTIATION OF THE HYPERTHERMIA INDUCED BY ISOPRENALINE

Infusion with isoprenaline induced a rise in the body temperature (see Fig. 3). When infusion was at a rate of 40 μg/rat/min the peak was attained after about 90 min. No differences were noticed between the effect of a dose of 400 or 40 μg/rat/min. Desipramine and nortriptyline enhance this hyperthermia while the monoamine oxidase inhibitor pheniprazine reduced this effect (see Fig. 3).

Adrenalectomy as well as α- and β-adrenergic blocking agents, reduce the hyperthermia evoked by high concentration of isoprenaline (Table 5). Modaline slightly inhibited this hyperthermia (see Fig. 3 and Table 5).

Discussion

Our results confirm that modaline is a strong inhibitor of monoamine oxidase (Dubnick & others, 1963; Gylys & others, 1963) as shown by direct measurement of the enzymatic activity and by indirect evaluation of the pharmacological consequences of this effect (potentiation of tryptamine and dopa, inhibition of reserpine hypothermia).

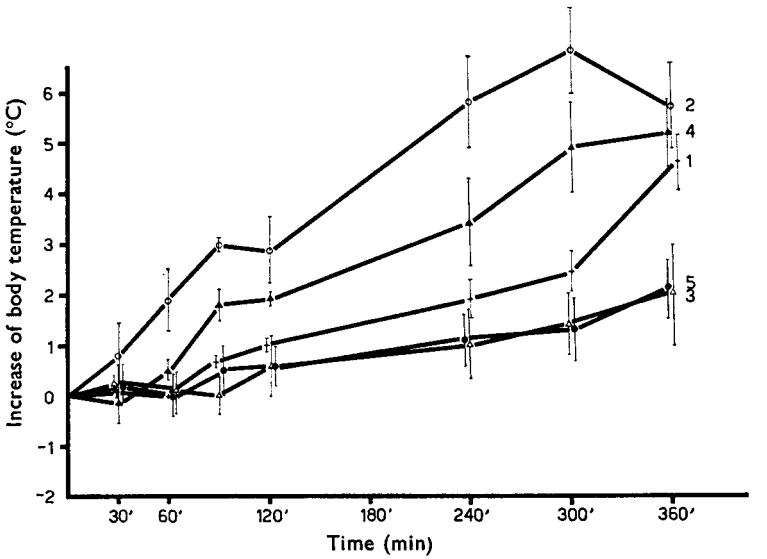

FIG. 2. Rats were treated 18 hr before the test with reserpine (5 mg/kg i.v.) at a room temperature of 20°. At the time 0 the following treatments were given: 1. SKF 525 A 50 mg/kg oral + modaline sulphate 16 mg/kg i.p. 2. Modaline sulphate 16 mg/kg. i.p. 3. SKF 525 A 50 mg/kg oral + modaline sulphate 8 mg./kg i.p. 4. modaline sulphate 8 mg/kg i.p. 5. Saline. The vertical bars show the s.e. of the mean. Each point consists of at least 5 determinations.

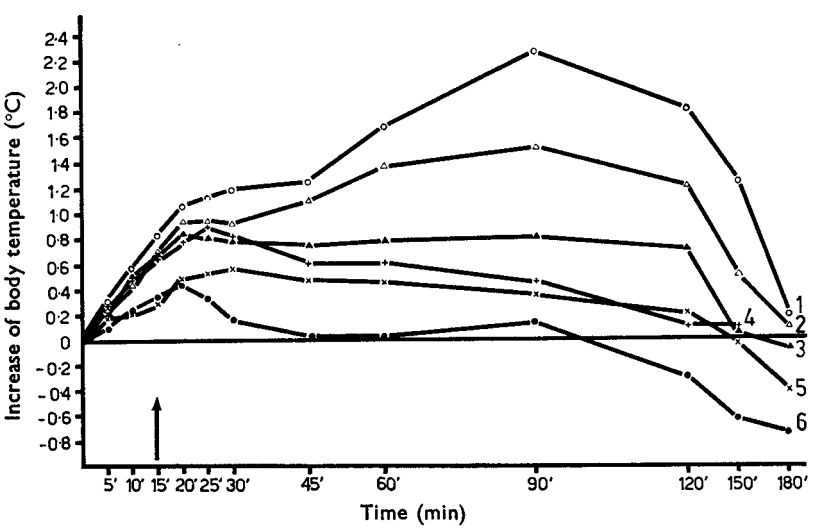

FIG. 3. Rats were infused with isoprenaline 40 μg/rat/min for 15 min. Drugs were given 1 hr before test at the following doses in mg/kg: 1. Desipramine 15. 2. Nortriptyline 10. 3. Saline. 4. Modaline sulphate 16. 5. Modaline sulphate 4. 6. Pheniprazine 10.

TABLE 5. EFFECT OF VARIOUS DRUGS ON THE HYPERTHERMIA INDUCED BY AN INFUSION OF ISOPRENALINE

Treatment	Dose (mg/kg i.p.)	Thermic index (°C) ± s.e.
Saline	—	7·4 ± 1
Desipramine	15	12·7 ± 1·4
Modaline	16	5·6 ± 1·8
Dibenamine	15	5·1 ± 1·7
Phentolamine.. ..	10	2·4 ± 0·8
Propranolol	5	2·8 ± 1·3
Adrenalectomy ..	—	2·9 ± 2·2

Thermic index is the sum of temperature difference (° C) before treatment and after: 5, 10, 15, 20, 25 30, 45, 60, 90, 120 min.
Desipramine and modaline were given 1 hr before isoprenaline; phentolamine, propranolol and dibenamine were given 15 min before the infusion. Adrenalectomy was performed three days before the test.
Isoprenaline (400 μg/ml) was infused at the rate of 0·1 ml/rat/min for 15 min.

The inhibition of monoamine oxidase by modaline is characterised by a rapid onset (see Table 1) and by a long duration of action (see Table 4).

The attempts to reveal an imipramine-like component in the pharmacological effects of modaline have been successful under one experimental condition only. Like desipramine, modaline raised the body temperature in fully reserpinised animals. This effect was not related to an action on monoamine oxidase because similar blockade of the enzyme induced by compound T-3 or by pheniprazine did not affect the body temperature of reserpinised rats.

Because of the observations in reserpinised animals, we cannot exclude the possibility that the prevention of the reserpine hypothermia obtained with modaline is the result of a separate imipramine-like effect. With the enhancement of the hyperthermic response induced by dopa in monoamine oxidase blocked animals, it was not possible to distinguish an imipramine-like effect from the monoamine oxidase inhibition. In fact a second dose of a monoamine oxidase inhibitor potentiated the dopa hyperthermia like imipramine. These results may be related to the observation that a second administration of a monoamine oxidase inhibitor further increases the level of brain amines despite the already existing block of the enzyme (Dubnick & others, 1962). Modaline did not potentiate the hyperthermia after isoprenaline infusion, a test considered to be specific for imipramine-like drugs (Jori & Garattini, 1965). It is possible that the schedule of treatment for modaline was not the most suitable because it might produce either an increase or a reduction in the intensity of a sympathetic response depending on the dose as demonstrated for imipramine (Thoenen, Huerlimann & Haefely, 1964).

Attempts to distinguish between imipramine-like effects and monoamine oxidase inhibition by inhibiting the metabolism of modaline were not successful. The use of an inhibitor (SKF 525 A) of microsomal enzymes responsible for drug metabolism resulted in the blockade of the potentiation of tryptamine (see Table 2) and of the reversal of reserpine hypothermia (see Fig. 2).

These results therefore suggest that monoamine oxidase inhibition and imipramine-like effects are both present in the metabolic products of

modaline. The limitations and importance of the imipramine-like component in explaining the antidepressive action of modaline remain to be established.

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