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THE EFFECT OF DESMETHYLIMIPRAMINE AND OTHER "ANTI-TREMORINE" DRUGS ON THE METABOLISM OF TREMORINE AND OXOTREMORINE IN RATS AND MICE

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Abstract—In rats drugs may delay or block the syndrome induced by tremorine (TMN) either by decelerating its bioactivation to oxotremorine (OTMN), e.g. SKF 525 A, Sch 5706, imipramine and desmethylimipramine (DMI), or by antagonizing the active metabolite at receptor-sites. Drugs may potentiate and prolong the action of OTMN by inhibiting its bioinactivation (DMI, SKF 525 A). Drugs may also have all these properties and the pharmacological consequences will depend upon the dose used (Sch 5706). Pronounced species differences were found in the action of anti-tremorine drugs. In the mouse, DMI did not block the metabolism of OTMN and SKF 525 A was less potent than in the rat.

TREMORINE, 1,4-dipyrrolidino-2-butyne (TMN), induces both central and peripheral cholinergic effects in animals. These effects, however, are mediated through the formation of oxotremorine (OTMN) and not elicited by the parent compound itself.[1–3] Thus, the effects of TMN should be antagonized not only by anticholinergic drugs, but also by substances which decelerate the formation of OTMN. Accordingly, several investigators independently found that a number of substances which antagonized the pharmacological effects of TMN did not counteract the symptoms induced by OTMN in animals.[4–6] Indeed, Sjöqvist and Gillette[6] found that desmethylimipramine, an "anti-tremorine"* drug, potentiated and prolonged the effects of OTMN in rats and therefore suggested that DMI was a potent inhibitor of the metabolism of both TMN and OTMN in this animal species. Strong biochemical support for this view was recently presented by Hammer and Sjöqvist.[7] The experiments reported in this paper confirm and extend the previous observations and also demonstrate a marked species difference between rats and mice in the effect of various drugs on the metabolism of TMN and OTMN. The pharmacological implications of these drug interactions are discussed.

MATERIAL AND METHODS

I. In vivo *experiments*

Animals. Male Sprague–Dawley rats weighing 180–220 g were used. Most of the

* The term "anti-tremorine" drug is used for compounds which antagonize TMN mainly by delaying its onset of action.

reported experiments were performed in Swedish rats, while the initial observations were obtained in American rats bred at the National Institutes of Health, Bethesda. The results obtained in the two kinds of rat were qualitatively the same but the American rats were somewhat more sensitive to the tremorogenic drugs.

The mouse experiments were performed in male Swiss albino mice (N.M.R.I., Bethesda). Their weights ranged from 17 to 23 g, but did not differ by more than 2 g in any single experiment.

Drugs and their administration. ^{3}H-TMN (S.A. = 240 mC/m-mole) was obtained by synthesis from ^{3}H-pyrrolidine, paraformaldehyde and *N*-propargylpyrrolidine.[8] For ^{3}H-OTMN (S.A. = 730 mC/m-mole) pyrrole was hydrogenated with tritium containing hydrogen and the ^{3}H-pyrrolidine obtained was reacted with paraformaldehyde and *N*-propargylpyrrolidone-(2). The radiochemical purity was established by paper chromatography (*n*-butanol: acetic acid: water 4:1:5). The radioactive TMN or OTMN was diluted with unlabelled compound to yield a sp. act. which was convenient for the analysis of homogenates of the carcass and the brain as described below. All drugs were injected i.p. unless otherwise stated.

The various "anti-tremorine" drugs were injected 60 min before TMN or OTMN respectively. The following dosage schedule was employed: tremorine dihydrochloride, corresponding to 10 mg/kg of the base; oxotremorine oxalate, corresponding to 0·5 mg/kg of the base, desmethylimipramine hydrochloride, 10–20 mg/kg, imipramine hydrochloride, amitriptyline hydrochloride and thiazinaminum 0·03 m-mole/kg. SKF 525 A (β-diethylaminoethyl diphenylpropylacetate), Sch 5705 (the diethylaminoethyl ester of monoethyl-ethyl-phenylmalonate) and Sch 5706 (the diethylaminoethylamide of monomethylphenyl malonate) were injected in doses of 0·1 m-mole/kg.

Recording of tremor and body temperature. Tremor was either recorded objectively as described by Holmstedt and Lundgren[9] or rated according to a simple scoring. technique,[6] in which the investigator was kept unaware of the pretreatment use. The rectal temperature was recorded with an electro-thermometer at intervals of 15 or 30 min throughout the experiments. Except when otherwise stated the experiments were run at a room temperature of 18–20°.

Determination of ^{3}H-TMN and ^{3}H-OTMN. Animals were killed by cervical dislocation. After the brain had been removed for separate analysis, the rest of the animal (carcass) was homogenized in a Warring–Blendor with 4 parts of ice-cold water. A 2 ml aliquot of the homogenate, filtered through gauze, was made alkaline with 1 ml of 0·5 N NaOH and extracted with either 6 ml of heptane containing 0·25% isoamyl alcohol (^{3}H-TMN) or toluene containing 1·5% isoamyl alcohol (^{3}H-OTMN). Under these conditions only TMN is extracted into heptane, whereas both TMN and OTMN are extracted into toluene. The samples were centrifuged and 2 ml of the organic phase was counted in a liquid scintillation spectrometer. The brain was homogenized with 7 parts of water and an aliquot of 1 ml was made alkaline with 0·2 ml of 0·5 N NaOH and extracted with 3 ml heptane (0·25% isoamyl alcohol) or toluene (1·5% isoamyl alchohol) respectively. One ml of the organic phase was counted as described above. Internal standards were prepared as follows: *Carcass*; animals were killed and homogenized immediately after the administration of ^{3}H-TMN (10 mg/kg) or ^{3}H-OTMN (0·5 mg/kg) respectively and these homogenates were considered to contain 2 μg of TMN or 0·1 μg of OTMN respectively per ml. Brain; known amounts of ^{3}H-TMN and ^{3}H-OTMN in a volume of 10 μl were added to

aliquots of 1 ml of brain homogenates from untreated animals. The standards and the samples of unknown concentrations were analyzed together.

Specificity of extraction procedures. The specificity of the extraction methods was assessed by the technique of comparative distribution ratios.[7, 10] The partition ratios of the material extracted into heptane or toluene from homogenates of animals treated with the tremorogenic drugs 20 and 120 min previously were compared with those of authentic material in a series of two phase-systems, consisting of heptane or toluene and aqueous buffers of various pH values. The solubility characteristics of apparent TMN and OTMN were similar to those of the authentic compounds.

The specificity of the methods was further ascertained by thin layer chromatography of the organic extracts on Silicagel G as described below. Subsequent scanning showed the radioactivity to be located in only one spot with an R_f-value identical to that of authentic TMN or OTMN.

Determination of ^{3}H-OTMN formed in vivo *after administration of ^{3}H-TMN.* Male rats were pretreated i.p. with either DMI (20 mg/kg, dissolved in physiologic saline) or saline. One hour later they were given 10 mg/kg of ^{3}H-TMN (S.A. = 13·5 mC/m-mole). Six rats in each group were decapitated 15 and 60 min respectively after the administration of ^{3}H-TMN. The brain was homogenized in water to yield a 20% homogenate. Seven ml of the homogenate was transferred to a 40 ml glass-stoppered centrifuge tube to which were added 0·25 ml of 5 N NaOH, 0·1 ml of an aqueous solution containing 0·1% of both non-radioactive TMN and OTMN and 25 ml of toluene (1·5% isoamylalcohol). For evaluation of the overall recovery of both ^{3}H-TMN and ^{3}H-OTMN, 10 μl of an 0·1% solution of ^{3}H-TMN (S.A. 240 mC/m-mole) and 10 μl of an 0·1% solution of ^{3}H-OTMN (S.A. 730 mC/m-mole) were added to brain homogenates from untreated rats and mixed with alkali and unlabelled drugs as described above. All samples were extracted by vigorous shaking for 30 min. Emulsions, which occasionally formed, were broken by freezing in a mixture of dry ice and acetone. After centrifugation, 20 ml of the organic phase was transferred to another glass-stoppered centrifuge tube containing 1·5 ml of 0·1 N HCl and the amines were extracted into the aqueous phase by shaking for 15 min. After centrifugation the organic phase was carefully aspirated and 1·3 ml of the aqueous phase was transferred to a 15 ml glass-stoppered centrifuge tube containing 0·25 ml of 1 N NaOH and 5 ml of toluene (1·5% isoamyl alcohol). The samples were shaken for 15 min and centrifuged. One half ml of the toluene phase was used to determine total radioactivity in a liquid scintillation counter. Four ml of the toluene phase was transferred to a 15 ml conical glass-stoppered tube. The solvent was evaporated under vacuum in a Rotavapor with the water bath kept at 30–32°. The solid matter was redissolved in 0·3 ml of toluene. For separation of ^{3}H-TMN and ^{3}H-OTMN approximately 0·05 ml of the solution was put on a thin layer plate of Silicagel G and chromatographed with a solvent system consisting of heptane, chloroform, ethanol and diethylamine (12:2:1:1). After drying in air for 30 min the plates were sprayed with an amount of Dragendorff's reagent just sufficient to visualize the position of TMN (R_f approx. 0·6) and OTMN (R_f approx. 0·45). The areas of Silicagel containing the amines were scraped off quantitatively into separate vials for liquid scintillation counting. To avoid colour quenching, the Dragendorff complex was decolourized by the addition of 0·2 ml of a 5% solution of the disodium salt of ethylenediaminetetracetic acid to each vial. Occasionally, metallic bismuth was formed but was

easily dissolved by warming the sample for a moment to 50°. After addition of the scintillation fluid the radioactivity in each amine was determined. From the total radioactivity of ^{3}H-OTMN and ^{3}H-TMN in the above 0·5 ml aliquot of the toluene phase, the ratio between the radioactivity of ^{3}H-TMN and ^{3}H-OTMN (as obtained from the thin layer separation). and the overall recovery of both radioactive amines as given by the standards, one can calculate the amount of ^{3}H-OTMN, which was formed *in vivo* from ^{3}H-TMN. Because the administered ^{3}H-TMN was randomly labelled in one pyrrolidine ring,[8] the sp. act. of the metabolite ^{3}H-OTMN was 12·5 per cent lower than that of the precursor ^{3}H-TMN.

II. In vitro *experiments*

Rats were fasted over night and injected i.p. with either saline or DMI (40 mg/kg) 1 hr before sacrifice. The livers were removed and homogenized in 3 parts of ice-cold 0·25 M sucrose in a glass tube provided with a Teflon pestle. The homogenate was centrifuged at 9000–10,000 *g* and 4° for 15 min. To 1 ml of the supernatant the following reagents were added to give a 2 ml sample with the indicated final concentrations: 0·2 mM TPN, 20 mM glucose-6-phosphate, 5 mM $MgCl_2$, 30 mM nicotinamide and 60 mM phosphate buffer pH 7·5. In some experiments various amounts of DMI were added. The concentrations of TMN and OTMN used are indicated in the respective tables.

The mixture was incubated either aerobically or under nitrogen for 30 min at 37° in a metabolic shaker. The enzymatic reaction was stopped by addition of 0·5 ml of 1 N NaOH. A 2 ml aliquot was assayed for ^{3}H-TMN or ^{3}H-OTMN as described above.

RESULTS

I. *Effect of DMI on the metabolism of TMN and OTMN in rats*

(1) *Effect of DMI on the metabolism of TMN* in vivo. Rats were pretreated with saline and then given TMN (10 mg/kg, i.p.) and killed at various times thereafter. Analysis of the carcass revealed that TMN was eliminated biphasically; about 50 percent of the drug was eliminated within the first 30 min. After the first hour, however, the biological half-life of TMN was about 150 min (Fig. 1). In rats pretreated 1 hr previously with DMI (10 mg/kg, i.p.), TMN was eliminated monophasically with a half-life of about 290 min (Fig. 1). In both control and DMI treated animals, the brain levels of TMN reached maximal values within the first 2 hr and then remained relatively constant during the next 2 hr. Maximal brain levels, however, were more than three times higher in the DMI treated animals than in controls (Fig. 2).

In another series of experiments the *in vivo* formation of OTMN from TMN was measured. Fifteen minutes after TMN administration, the brain levels of OTMN were the same in control rats and in DMI treated rats. In the latter group, however, TMN levels were considerably higher than in controls. Sixty minutes after TMN administration the brain levels of both TMN and OTMN were considerably higher in DMI pretreated animals than in controls (Table 1). These findings suggested that DMI in rats inhibits both the conversion of TMN to OTMN and the further metabolism of OTMN. Accordingly, DMI delayed the onset of tremor and hypothermia in

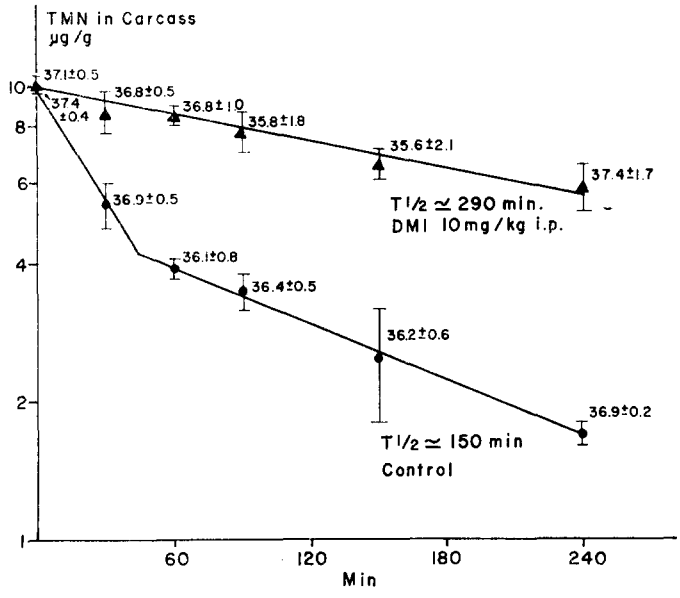

FIG. 1. Effect of DMI pretreatment on the elimination of TMN (10 mg/kg) administered i.p. to rats. The numbers in the graph refer to the rectal temperature. All data are means ± S.D. (n = 5).

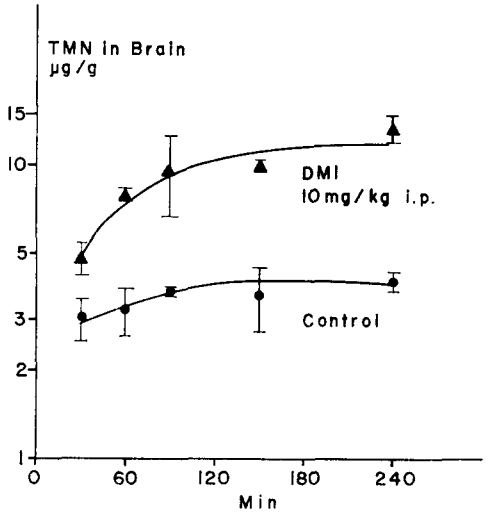

FIG. 2. Effect of DMI on brain levels of TMN (10 mg/kg) after i.p. administration of both compounds. (The animals are identical with those in Fig. 1).

rats after the injection of TMN but prolonged the duration of these effects after they had appeared (Figs. 3 and 4).

(2) *Effect of DMI on the metabolism of OTMN* in vivo. In control rats, OTMN (0·5 mg/kg, i.p.) disappeared from the carcass polyphasically (Fig. 5). About 60 per cent of the dose was eliminated within the first 8 min, but the fractional rate of elimination decreased thereafter. In animals pretreated 1 hr previously with DMI (15 mg/kg, i.p.), however, OTMN was eliminated monophasically with a half-life of about

TABLE 1. *In vivo* FORMATION OF OTMN FROM TMN IN RAT

	Brain—TMN (μg/g)		Brain—OTMN (μg/g)		Ratio TMN:OTMN	
Pretreatment	15 min	60 min	15 min	60 min	15 min	60 min
Saline	3·6 ± 0·4	6·0 ± 0·7	0·100 ± 0·012	0·052 ± 0·013	36	115
DMI 20 mg/kg	6·5 ± 0·4	15·7 ± 3·5	0·107 ± 0·006	0·173 ± 0·021	60	90

All data are means ±S.D. (n = 5).

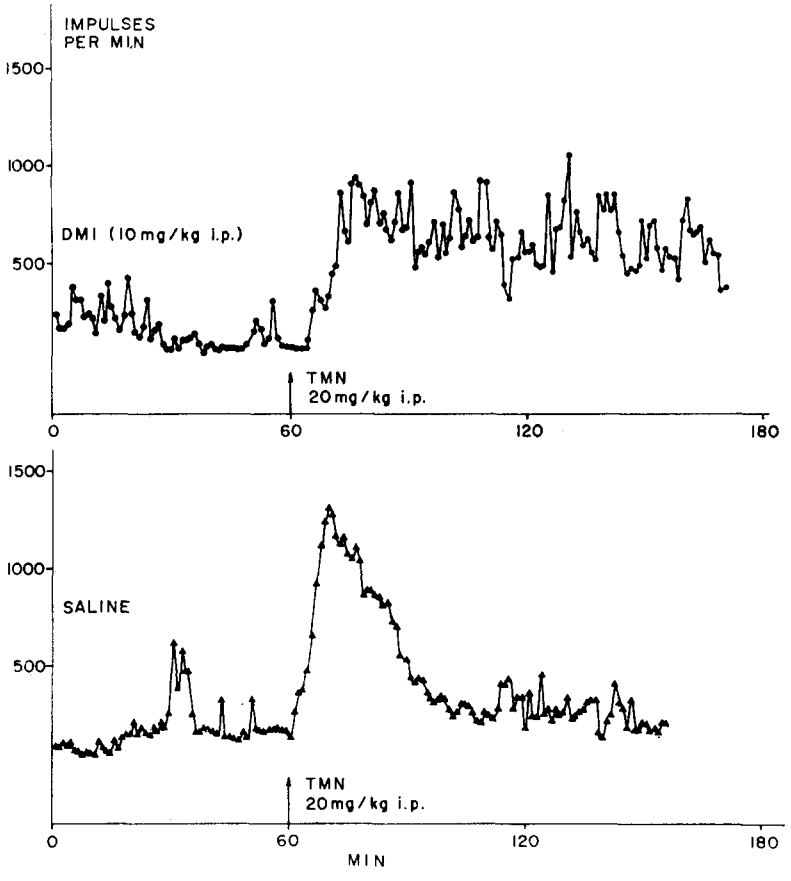

FIG. 3. Effect of DMI on onset and duration of tremor after i.p. administration of TMN. DMI delays the onset of tremor but prolongs its duration after it has appeared. The distance between two dots or triangles corresponds to one minute. The results are typical of five experiments.

80 min (Fig. 5). Accordingly, the brain levels of OTMN reached greater maximal values and were maintained longer in DMI treated than in control rats (Fig. 6). As a result, a more marked hypothermia developed in DMI pretreated animals (Figs. 5 and 6). The findings are in agreement with the observation that DMI potentiates and prolongs the action of OTMN in rats[6] (Fig. 7).

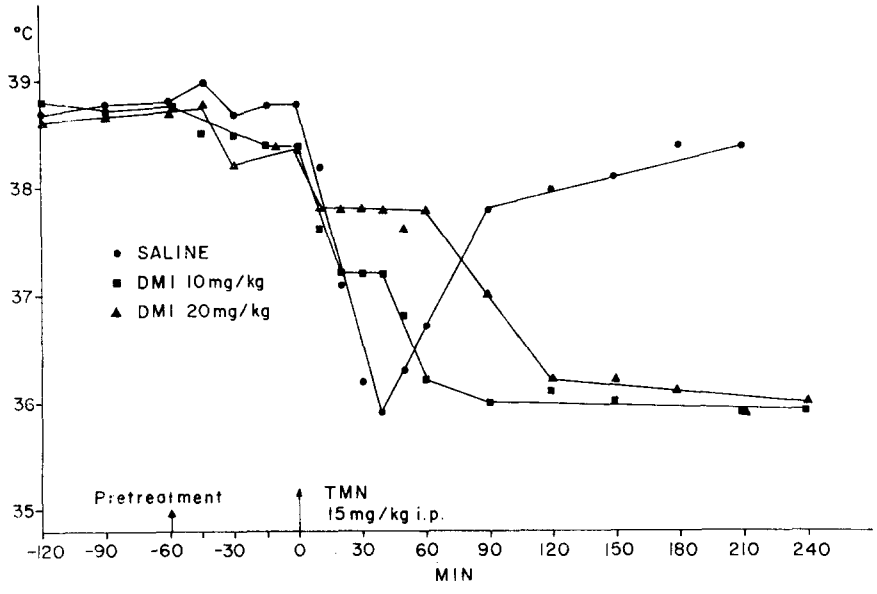

FIG. 4. Effect of DMI on the rectal temperatures in rats receiving TMN. The points represent the mean values in at least 5 animals.

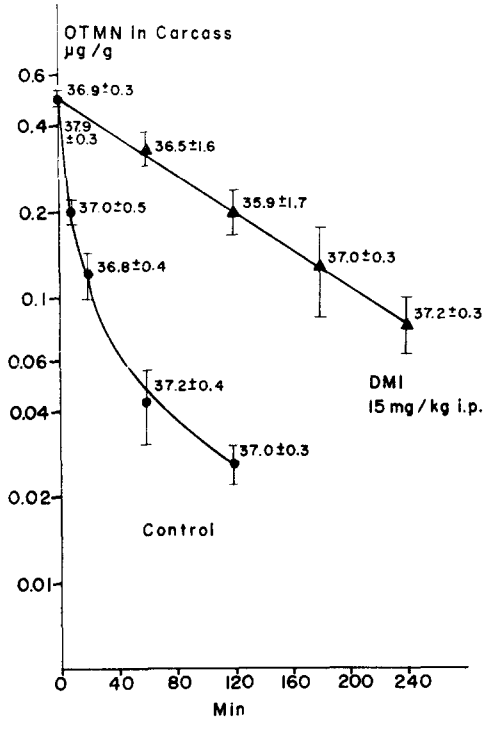

FIG. 5. Effect of DMI on the elimination of OTMN (0·5 mg/kg, i.p.) from rats. The numbers in the graph refer to the rectal temperature. All data are means ± S.D. (n = 5).

(3) *Elimination of TMN and OTMN after i.v. administration.* The elimination of TMN and OTMN was considerably slower after i.v. than after i.p. injection (Figs. 8 and 9). For example, within the first 20 min about 65 per cent of the OTMN was eliminated after i.v. administration as compared to 85 per cent after i.p. administration.

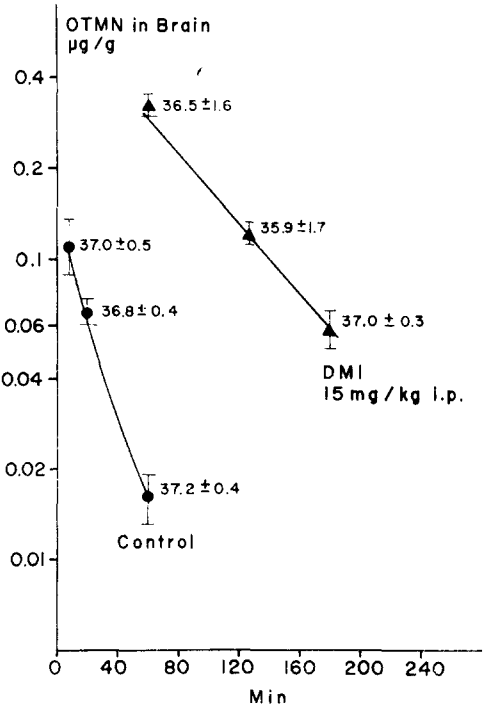

FIG. 6. The effect of DMI on brain levels of OTMN (0·5 mg/kg) in rats. (The animals are identical with those in Fig. 5.)

(4) *Effects of DMI on the* in vitro *metabolism of TMN and OTMN.* The metabolism of TMN and OTMN was found to require the presence of both the microsomal and the soluble fractions of liver. Only a small amount of OTMN was metabolized under anaerobic conditions (Table 2). Furthermore, both OTMN and TMN were metabolized more rapidly by liver preparations from rats pretreated with phenobarbital (Table 3).

As shown in Table 4, the *in vitro* metabolism of both TMN and OTMN was markedly inhibited by DMI at concentrations of 10^{-6}–10^{-4} M. Liver preparations from rats pretreated with DMI (40 mg/kg, i.p.) 1 hr before sacrifice metabolized TMN and OTMN less rapidly than did those from control animals (Table 3).

II. *Effect of other drugs on the rate of metabolism and biological action of TMN and OTMN in rats*

The finding that DMI blocked the metabolism of both TMN and OTMN in rats suggested the possibility that other "anti-tremorine" drugs might act similarly. Rats

were therefore pretreated with various "anti-tremorine" drugs 1 hr before the administration of the tremorogenic compounds and were killed 2·5 hr after the administration of TMN or 1 hr after the administration of OTMN. Analysis of the carcasses revealed that the metabolism of TMN and OTMN was markedly inhibited

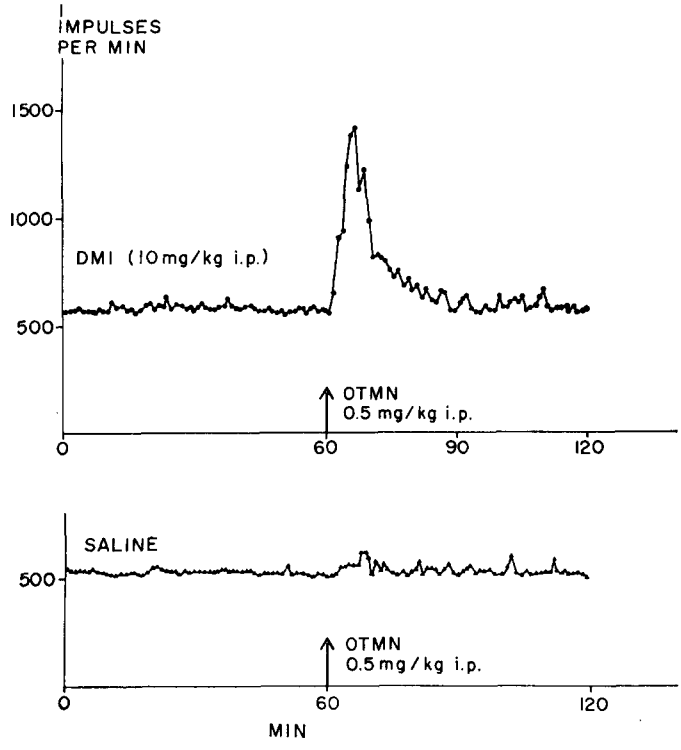

FIG. 7. Typical effect of DMI on the intensity and duration of OTMN induced tremor.

TABLE 2. INTRACELLULAR LOCALIZATION OF OXOTREMORINE METABOLIZING ENZYME SYSTEM IN RAT LIVER

Fraction	Per cent metabolism*
Whole homogenate	41
600 *g* supernatant	41
Nuclear fraction	0
9000 *g* supernatant	47
9000 *g* supernatant under N_2	8
Mitochondrial fraction	0
Soluble fraction	0
Microsomes	5

* Mean of 2 experiments. The respective fractions were incubated with 50 μg of OTMN for 30 min as described under Methods.

TABLE 3. *In vivo* METABOLISM OF TMN AND OTMN AFTER INCUBATION WITH THE 9000 *g* SUPERNATANT OF LIVER FROM RATS PRETREATED WITH SALINE, PHENOBARBITAL OR DMI

Pretreatment	Relative rates of metabolism	
	TMN*	OTMN*
Saline	100	100
Phenobarbital 100 mg/kg i.p. daily for 5 days	193 (n = 2)	247 (n = 2)
DMI 40 mg/kg i.p.	30 ± 5 (n = 5)	30 ± 9 (n = 5)

* Substrate conc. 15 μg/ml incubate.
About 7 μg/ml of TMN and 8 μg/ml of OTMN were metabolized in 30 min by the supernatant fraction of liver from control rats.

TABLE 4. INHIBITION OF TMN AND OTMN METABOLISM *in vitro* BY DESMETHYLIMIPRAMINE

Final molar conc. of DMI	Per cent inhibition	
	TMN* metabolism	OTMN* metabolism
10^{-4}	90	64
10^{-5}	37	18
10^{-6}	13	9

* 15 μg/ml was present in incubation mixture. Mean of three experiments.

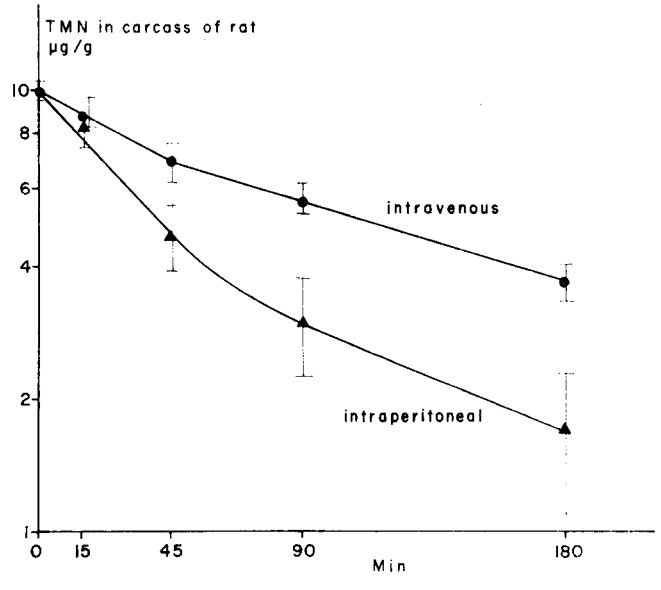

FIG. 8. Elimination of TMN (10 mg/kg) from rat carcass after i.p. and i.v. injection. All data are means ± S.D. (n = 5).

by SKF 525 A, Sch 5706, imipramine, amitriptyline and the quaternary substance thiazinaminum (Table 5).

As shown in Fig. 10, a blockade of the metabolism of OTMN by DMI and SKF 525 A enhanced and prolonged OTMN hypothermia. Other drugs that blocked

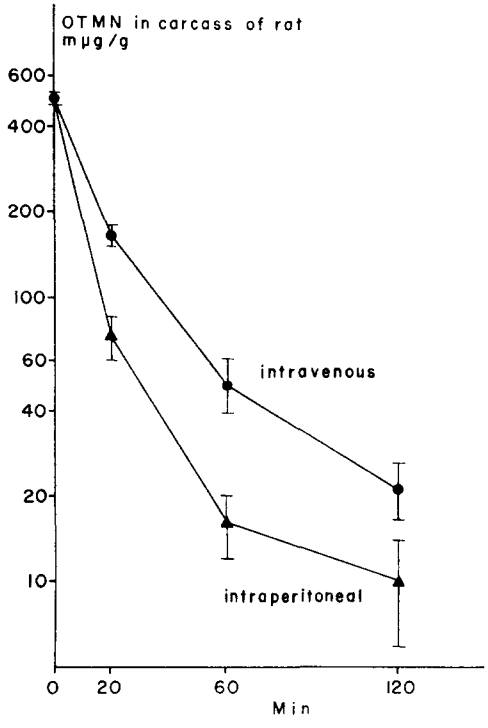

FIG. 9. Elimination of OTMN (0·5 mg/kg) from rat carcass after i.p. and i.v. injection. All data are means ± S.D. (n = 5).

OTMN metabolism also excacerbated the decrease in rectal temperature (Table 5). Apparently, there was a realationship between the amount of OTMN in the brain and the hypothermic effect. This was especially evident when comparing control rats with animals pretreated with SKF 525 A, Sch 5706 and thiazinaminum, drugs which presumably elicit no important central effect in the doses used.

III. *Species difference in the effect of various drugs on the pharmacological action and metabolism of TMN and OTMN*

It has been shown that OTMN and particularly TMN is metabolized much more slowly in mice than in rats.[11] This results in prolonged tremor and excessive hypothermia in the mouse. A species difference between rats and mice was found in the effects of DMI, SKF 525 A, Sch 5705 and Sch 5706 on the action and metabolism of TMN and OTMN (Table 6). Since the rate of metabolism of TMN is extremely slow in the mouse no attempt was made to study the effect of the various inhibitors listed in Table 6, on TMN metabolism in this species.

DMI, which delayed the onset of action of TMN and prolonged the action of OTMN in rats, did not exert these effects in mice; instead DMI shortened the effect of OTMN in the latter species (cf. Spencer[12]). Accordingly, DMI did not cause an increase in tissue levels of OTMN in mice (Table 7). In contrast SKF 525 A, a well-known inhibitor of the drug metabolizing enzyme systems in liver microsomes, delayed the

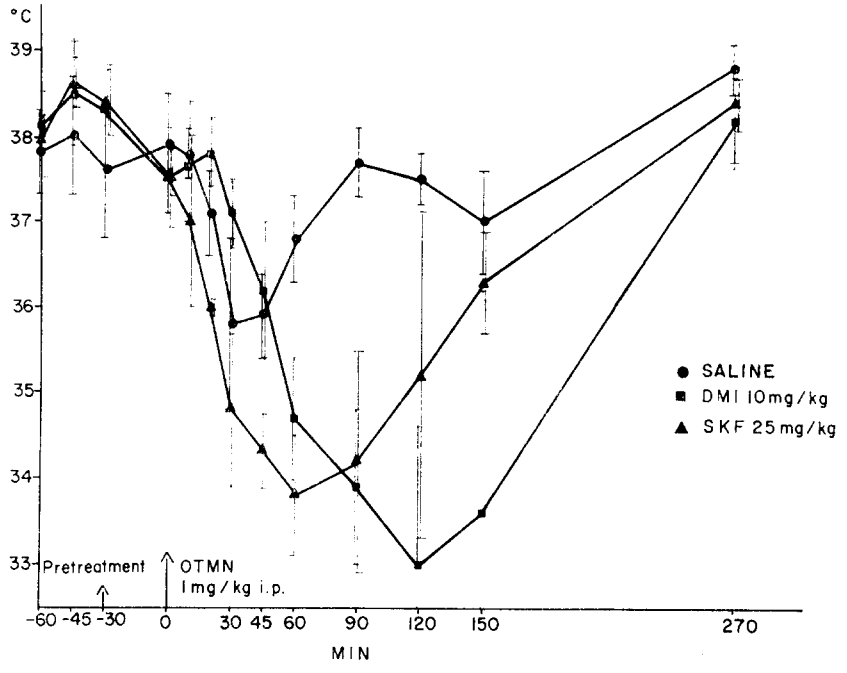

FIG. 10. Potentiation and prolongation of OTMN hypothermia in rats by DMI and SKF 525 A. All data are means ± S.D. (n = 5).

onset of action of TMN and prolonged the action of OTMN both in rats and mice. The *in vivo* metabolism of OTMN was markedly inhibited in rats (Table 5) and to a lesser degree in mice (Table 7). The inhibition in mice was observed at room temperature but not at an environmental temperature of 32° (Table 7). The marked OTMN hypothermia which develops at room temperature decelerates the rate of metabolism of OTMN[13] and probably also that of SKF 525 A, thereby permitting a sustained action on the liver microsomal enzymes.

Sch 5705 which has two ester functions in its structure had no effect on the pharmacological action and metabolism of TMN and OTMN in rats. In mice, however, this compound at doses of 100 mg/kg delayed the development of hypothermia and tremor after the administration of TMN (Fig. 11) and prolonged the effects of OTMN (Fig. 12). But at doses of 50 mg/kg it had little effect on the action of either TMN or OTMN. Since Sch 5705 at the lower dose increased the tissue concentrations of OTMN in mice (Table 8) it apparently has both an inhibitory effect on liver microsomal enzymes and a true central anticholinergic effect. Sch 5706, which is both an ester and an amide, inhibited the metabolism of TMN in rats and OTMN in rats and mice. Consequently, in rats the onset of action of TMN was delayed and the duration

TABLE 5. EFFECT OF DRUGS ON THE ELIMINATION OF TMN AND OTMN IN RATS

Pretreatment	Dose i.p. m-mole/kg	TMN-treated animals		OTMN-treated animals			Rectal temperature after administration of OTMN °C ± S.D.
				Carcass		Brain	
		TMN in carcass (μg/g ± S.D.)	Per cent of injected dose eliminated ±S.D.	OTMN mμg/g ±S.D.	Per cent of injected dose eliminated	OTMN mμg/g ±S.D.	
Controls	Saline	2·80 ± 0·2	72 ± 2	22 ± 2	96	10 ± 2	37·2 ± 0·2
SKF 525 A	0·1	7·70 ± 0·7	23 ± 7‡	157 ± 23‡	69	191 ± 36‡	33·8 ± 0·5‡
Sch 5706	0·1	6·56 ± 1·3	34 ± 13‡	106 ± 33†	82	115 ± 49†	35·3 ± 0·9†
Sch 5705	0·1	2·22 ± 0·6	78 ± 6	27 ± 5	95	9 ± 3	37·6 ± 0·03
Imipramine	0·03	7·12 ± 0·7	29 ± 7‡	119 ± 6†	76	117 ± 18‡	34·4 ± 0·4‡
Amitriptyline	0·03	4·34 ± 0·05	56 ± 5‡	39 ± 9†	92	9 ± 3	37·5 ± 0·3
Thiazinaminum	0·03	6·20 ± 0·7	38 ± 7‡	72 ± 39*	86	53 ± 39*	36·4 ± 1·0

Rats were pretreated with the various drugs 1 hr before the administration of the tremorogenic compound. Animals receiving TMN were killed 2·5 hr later whereas those given OTMN were killed 1 hr later. The rectal temperatures were determined just before sacrifice. The temperature before injection of OTMN was 38·1 ± 0·2 (S.D.). The data are expressed as means ±S.D. from 4–5 animals.

* $0{\cdot}05 > P > 0{\cdot}01$.

† $0{\cdot}01 > P > 0{\cdot}001$.

‡ $P < 0{\cdot}001$.

TABLE 6. COMPARISON OF THE EFFECT OF VARIOUS METABOLIC INHIBITORS ON THE ACTION OF TMN AND OTMN IN MICE AND RATS

	Mice					Rats					
	TMN		OTMN			TMN			OTMN		
Inhibitor	Hypotherm.	Tremor	Hypotherm.	Tremor	Metabolism	Hypotherm.	Tremor	Metabolism	Hypotherm.	Tremor	Metabolism
DMI	Partial antagonism	Unchanged	Partial antagonism	Shortened	Unchanged †*	Delayed then prolonged	Delayed	Inhibited	Potent. and prolonged	Potent. and prolonged	Inhibited
SKF 525A	Delayed and partly blocked	Delayed	Prolonged	Prolonged	Unchanged* Inhibited †	Delayed then prolonged	Delayed then prolonged	Inhibited	Potent. and prolonged	Potent. and prolonged	Inhibited
Sch 5705	Delayed (100 mg/kg)	Delayed	Prolonged (100 mg/kg)	Unchanged	Inhibited*	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Sch 5706	Blocked (100 mg/kg)	Partly blocked	Prolonged (100 mg/kg)	Unchanged	Inhibited*	Delayed then prolonged	Delayed then prolonged	Inhibited	Potent. and prolonged	Potent. and prolonged	Inhibited

Environmental temperature * 32–35°. † 20°.

Animals were pretreated 30–60 min with the respective inhibitor and then TMN (10 mg/kg) or OTMN (0·5 mg/kg) were administered. All drugs were given i.p. Potent. = Potentiated.

of action of OTMN was prolonged. In mice, the TMN syndrome was blocked (Fig. 11), and OTMN hypothermia was prolonged (Fig. 12) though OTMN tremor was unchanged as compared to controls. The data indicate that Sch 5706 in mice both inhibits the metabolism of TMN and exerts a slight anticholinergic effect, especially at high doses (Figs. 11 and 12).

TABLE 7. EFFECT OF DMI AND SKF 525 A ON THE METABOLISM OF OTMN IN THE MOUSE AT DIFFERENT ENVIRONMENTAL TEMPERATURES

Pretreatment (30 min)	Dose in mg/kg i.p.	Environmental temp. °C	OTMN in carcass $\mu g/g^*$ (n = 6)	P
Saline		35	0·12 ± 0·05	
DMI	50	35	0·15 ± 0·06	N.S.
Saline		22	0·17 ± 0·03	
DMI	20	22	0·17 ± 0·08	N.S.
DMI	40	22	0·18 ± 0·06	N.S.
Saline		20	0·17 ± 0·05	
SKF 525 A	25	20	0·29 ± 0·05	P < 0·05
Saline		32	0·24 ± 0·05†	
SKF 525 A	25	32	0·27 ± 0·06†	N.S.
SKF 525 A	50	32	0·30 ± 0·04†	N.S.

* 90 Min after the i.p. injection of 0·5 mg/kg of OTMN.
† 60 Min after the injection of OTMN.

TABLE 8. EFFECT OF SCH 5705 AND 5706 ON THE METABOLISM OF OTMN IN THE MOUSE

Pretreatment (30 min)	Dose i.p. mg/kg	OTMN in carcass $\mu g/g^*$ (n = 6)	P
Saline		0·12 ± 0·05	
Sch 5706	50	0·23 ± 0·05	<0·05
Sch 5705	50	0·23 ± 0·07	<0·05

These experiments were run at 35° to avoid any influence of the hypothermic effect of OTMN on its own metabolism.
* 90 min after i.p. injection of 0·5 mg/kg.

The *in vivo* experiments in rats thus indicated a difference in efficacy between Sch 5706 and Sch 5705 in the inhibition of the metabolism of TMN and OTMN. Accordingly, Sch 5706 was considerably more potent than Sch 5705 as an inhibitor of OTMN metabolism by rat liver preparations *in vitro*. At concentrations of 10^{-4} M Sch 5706 inhibited OTMN metabolism by about 85 per cent whereas Sch 5705 inhibited only by about 40 per cent. The metabolism of TMN by rat liver preparations was inhibited to about 50 per cent by pretreating the animals 1 hr before sacrifice with Sch 5706 (50 mg/kg, i.p.) but was unaffected by prior administration of Sch 5705 in an equivalent dose.

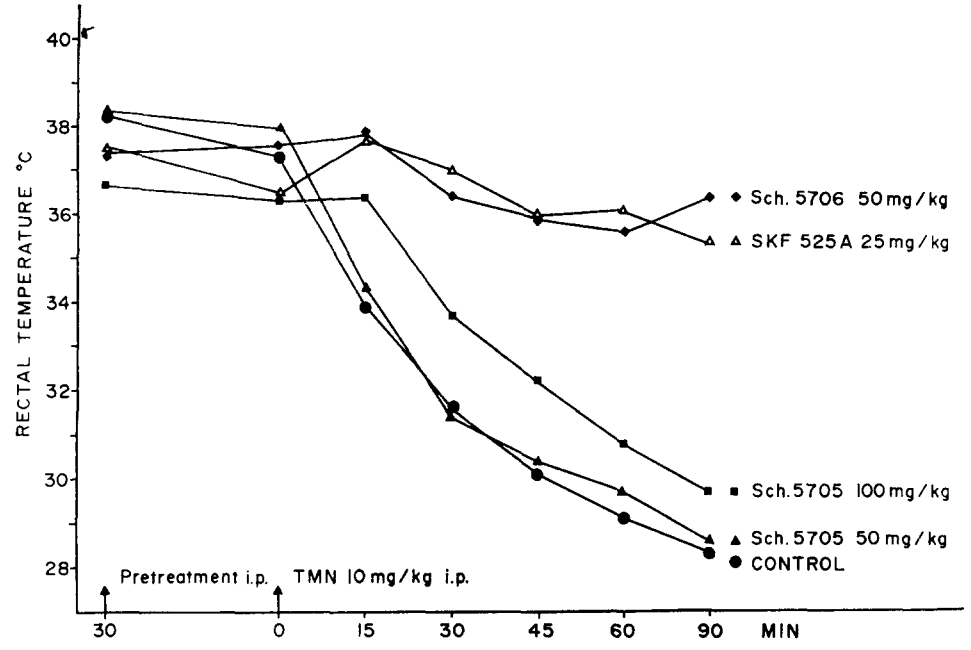

FIG. 11. Effect of SKF 525 A, Sch 5705 and Sch 5706 on TMN-hypothermia in mice. All data are means of 6 animals. S.E. was in no case greater than 1·5 per cent.

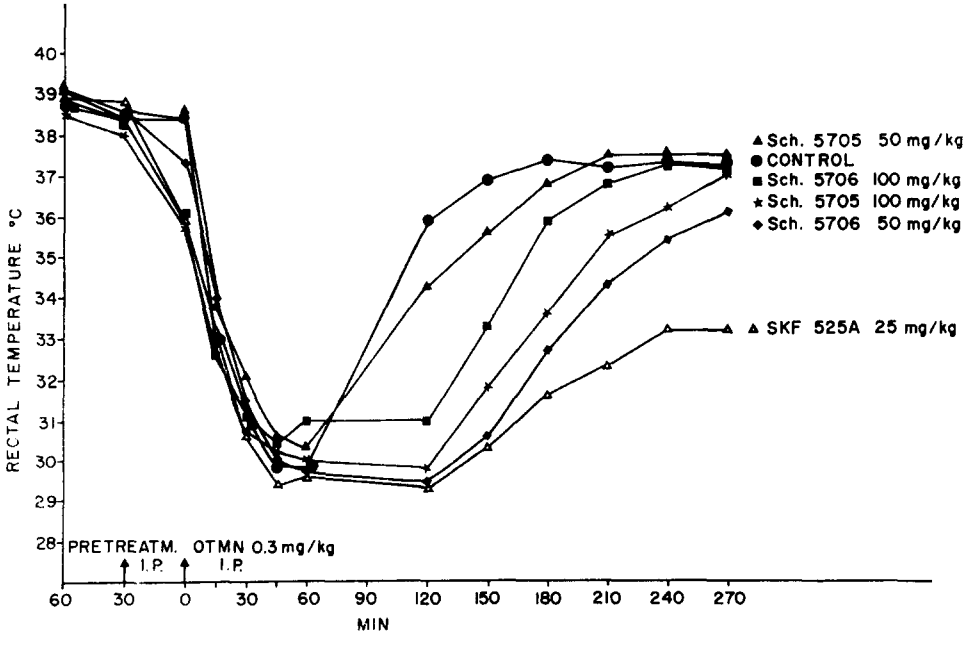

FIG. 12. Effect of SKF 525 A, Sch 5705 and Sch 5706 on OTMN-hypothermia in mice. All data are means of 6 animals. S.E. was in no case greater than 3 per cent.

DISCUSSION

Since the central effects of TMN resemble some of the symptoms seen in Parkinson's disease, e.g. ataxia, temor, and rigidity, and since these effects can be blocked by many drugs which are clinically useful in this disorder, Everett *et al.*[14] suggested TMN to be a useful tool in screening compounds for anti-Parkinson activity. The validity of this test, however, rests on the assumption that drugs interfere with the pharmacologic action of TMN solely at cholinergic receptor sites. As reported in a previous paper,[6] in rats desmethylimipramine delays the onset of action of TMN and prolongs the action of OTMN, the active metabolite of TMN. These findings suggested that, in rats, DMI altered the pharmacologic effects of these tremorogenic drugs mainly by inhibiting their metabolism. Evidence given in the present paper confirms this view.

In evaluating the present results, one should realize that only a minor fraction of OTMN is eliminated unchanged in urine,[15] and that urinary excretion of TMN should be even less important owing to its higher lipid solubility. In accordance with this view, the total radioactivity in animals receiving either ^{3}H-TMN or ^{3}H-OTMN remains practically constant for at least 2 hr.[15]

After i.p. injection, both TMN and OTMN disappeared polyphasically in the rat. About 50 per cent of a dose of TMN was metabolized during the first 30 min, but the biological half-life after 1 hr approached 150 min. Similarly, about 60 per cent of a dose of OTMN was metabolized during the first 8 min, but the fractional rate decreased thereafter. These findings suggested that considerable amounts of TMN and OTMN, absorbed from the peritoneal cavity into portal blood, were metabolized by the liver before the drugs reached the systemic circulation and hence the various tissues of the body. Accordingly, both compounds were metabolized much more slowly after i.v. than after i.p. administration. In rats pretreated with DMI, both TMN and OTMN disappeared monophasically. Thus, the inhibitory effect of DMI on the metabolism of TMN and OTMN were especially evident during the absorption phase of the tremorogenic compounds. These findings explain why DMI intensifies the syndrome induced by i.p. but not by i.v. threshold doses of OTMN.[6]

The present studies demonstrate that the metabolism of TMN and OTMN by liver microsomal preparations was inhibited by low concentrations of DMI added *in vitro*. Similarly, liver microsomes from rats pretreated with DMI metabolized both TMN and OTMN more slowly than did those from control rats. This confirms the view that in rats TMN and OTMN metabolizing enzymes are blocked by DMI in concentrations present in the experiments discussed above. Compared to other tricyclic monomethylated antidepressants, DMI is a very potent blocker of drug metabolism, indicating that it may even block the metabolism of drugs at the low doses used in man.[7]

In addition to its action on liver microsomes, DMI seems to influence the action of OTMN at neuronal sites in the brain for the following reasons:

In rats, OTMN produces an increase in acetylcholine levels in the brain.[9] In fact, quantitative recordings revealed that the magnitude and duration of tremor are correlated with the increase in brain levels of acetylcholine (ACh.)[16] Although classical anticholinergic drugs lower the amount of brain ACh, DMI *per se* has no such effect in rats.[16] In contrast, the combined administration of DMI and OTMN causes an even greater increase of the brain level of ACh. This increase persists as long as OTMN is maintained at high levels in brain, but the OTMN syndrome apparently disappears before the ACh levels in brain return to normal.[16] Experimental evidence

has recently been adduced that DMI counteracts the cholinergic effects of OTMN indirectly through an action on central monoaminergic neurons.[17] These data support the view that DMI has no important direct action on central cholinergic neurons.[6]

The conversion of TMN to OTMN and the further metabolism of OTMN in rats was also markedly blocked by SKF 525 A, imipramine and Sch 5706. Accordingly, these substances delayed the onset of action of TMN and prolonged the effects of OTMN, including hypothermia. Indeed, the decrease in body temperature may further decelerate the metabolism of the tremorogen *in vivo*.

Since thiazinaminum, which contains a quaternary nitrogen counteracts TMN-induced tremor in rats, it has been suggested to pass the blood–brain barrier despite its structure.[18] However, thiazinaminum did not counteract the hypothermia produced by OTMN, nor did it counteract temor induced by this active metabolite. The action of thiazinaminum is clarified by the finding that it inhibits the metabolism of TMN.

Pronounced species differences were found in the action of antitremorine drugs. Under the experimental conditions used DMI did not block the metabolism of OTMN in the mouse, whereas SKF 525 A seemed to be active (Table 7). Spencer[12] studying the modulation of OTMN hypothermia by drugs concluded that the metabolism of OTMN in mice was not carried out by imipramine—or SKF 525 A—sensitive enzymes. However, it is difficult to evaluate the effects of drugs on the metabolism of OTMN in mice without measuring tissue levels of the compound because hypothermia decelerates the metabolism of OTMN.[13] Thus any procedure interfering with the hypothermic effect of OTMN will secondarily change its metabolism. For example, atropine and amphetamine speed up the metabolism of OTMN in mice by blocking hypothermia.[13] In rats, however, the hypothermic effects of TMN and OTMN are much less marked and valid conclusions about the effect of DMI and SKF 525 A on the metabolism of the tremorogenes could be drawn from rather simple pharmacological observations.[6]

In our experiments, the effects of SKF 525 A and DMI on the metabolism of OTMN in mice were studied both at room temperature and at an environmental temperature at which no hypothermia developed. The results showed SKF 525 A to be effective at room temperature only and DMI to be ineffective under both experimental conditions. SKF 525 A was less effective in mice than in rats.

In our strain of mice DMI was unable to block TMN or OTMN hypothermia. In some experiments a trivial antagonism was observed. This differs from results obtained in other laboratories in which apparently the same strain of mice was used.[12, 19] The half-life of DMI in our mice is 22 min as compared to more than 5 hr in the rats.[15] Thus in mice effective tissue levels of DMI would be maintained only for a short time while OTMN is eliminated very slowly. For this reason it seems unlikely that DMI should effectively counteract the action of OTMN in mice from this laboratory. Appreciable differences in the metabolic fate of DMI and OTMN as well as in the turnover rates of catecholamines of central adrenergic neurons in the mice of the three laboratories could explain the divergent results. Investigation of these factors seems necessary.

In mice the metabolism of OTMN was inhibited by both Sch 5705 and Sch 5706, but only the latter elicited this effect in rats. Sch 5705 is probably split by an esterase, a process which might be more efficient in rats than in mice. The pharmacological experiments showed that the antagonistic effects of Sch 5705 and Sch 5706 against

TMN (Fig. 11) are predominantly due to a blockade of the bioactivation of TMN. Both drugs have nevertheless been suggested as potentially useful in the treatment of Parkinson's disease in man.[20, 21]

The numerous possibilities for interactions between various drugs and the tremorogenic compounds TMN and OTMN are shown in Fig. 13. Drugs may counteract the TMN syndrome either by decelerating its conversion to OTMN (DMI, SKF 525 A) or by antagonizing the active metabolite at receptor sites (atropine). Drugs may potentiate and prolong the action of the tremorogenic compounds by inhibiting the further metabolism of OTMN (SKF 525 A, DMI). Drugs may also have all these properties and the pharmacological consequences will depend upon the dose used (Sch-compounds). Finally, recent experiments show that drugs may shorten the OTMN-syndrome by accelerating the disappearance of the drug from the brain.[22]

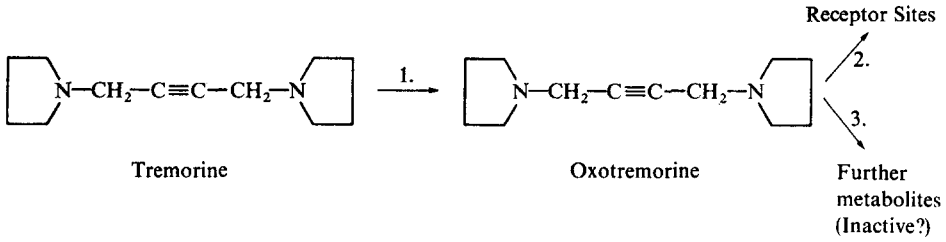

FIG. 13. Possible interactions between various drugs and the tremorogenic compounds TMN and OTMN.
(1) Inhibition of the formation of OTMN from TMN.
(2) Blockade of cholinergic receptor sites of the action of OTMN.
(3) Inhibition of the further metabolism of OTMN to presumably inactive metabolites.

In pharmacological experiments, one drug is often used to elucidate the mechanism of action of another. For example, in screening programs for psychoactive drugs, the effects of the potentially therapeutic drug is often tested solely as to whether it potentiates or antagonizes other compounds. The results of such experiments could be erroneously interpreted when possible alterations in the tissue levels of the "tool drug" are not considered.

It is becoming increasingly evident that a rough evaluation of the effect of new compounds on drug metabolism *in vivo* should precede their introduction in the clinic. In such studies the onset of TMN-tremor combined with the duration of OTMN-tremor (hypothermia) in rats may be used as simple parameters in addition to hexobarbital sleeping time.

As the basic pharmacologist should not be surprised to find it difficult to "confirm" data from other laboratories because of strain differences in drug metabolism, so the clinical pharmacologist should not be disturbed to find that responses to a fixed dose of a drug may vary with the population of humans investigated. In the future drug responses ought to be compared on the basis of the concentration of the drug (or its active metabolites) occurring at specific receptors or in compartments in equilibrium with these sites.

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