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MECHANISM OF POTENTIATION OF BARBITURATES AND MEPBROBAMATE ACTIONS BY IMIPRAMINE

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Abstract—Imipramine (Tofranil) markedly potentiates the actions of hexobarbital, pentobarbital, meprobamate and carisoprodol, but it does not potentiate the action of barbital and chloralhydrate. On the other hand, unlike SKF 525 A, imipramine can re-induce sleep in rats awakening from pentobarbital hypnosis.

Studies of the effect of imipramine on the *in vitro* metabolism of hexobarbital, pentobarbital, meprobamate and carisoprodol indicate that imipramine is an inhibitor of the microsomal drug-metabolizing enzymes. It inhibits especially the enzyme responsible for the metabolism of pentobarbital. Imipramine potentiates the inhibitory actions of SKF 525 A and phenyl*iso*propylhydrazine on the metabolism of hexobarbital and meprobamate.

An inhibition of *in vivo* metabolism of pentobarbital and meprobamate was also demonstrated.

In the imipramine treated rats, carisoprodol, meprobamate hexobarbital and pentobarbital concentrations in the brain, determined on recovery from the loss of the righting reflex, are a little lower than in controls.

The results indicate that imipramine would have to be classified both as a prolonging agent and a potentiating agent by virtue of its inhibitory action on drug metabolism and of its powers of increasing the sensitivity of the central nervous system towards the drugs.

THERE are some drugs which prolong drug action by virtue of their inhibitory effect on drug metabolism; for example, SKF 525 A (β-diethylaminoethyldiphenyl acetate) Lilly 18947 (2:4-dichloro-6-phenyl-phenoxyethyldiethylamine) iproniazid and Sch 5712 (ethyldiethylaminoethyl diester of butylethylmalonic acid) markedly prolong the hexobarbital sleeping time by inhibition of the hexobarbital metabolism in liver microsomes.[1–4]

Imipramine (Tofranil) is one of the most widely used antidepressant agents, but it potentiates the effect of pentobarbital in animal experiments.

The purpose of this study is to investigate the mechanism of the potentiating action of imipramine on the pentobarbital effect. The experiments indicate that imipramine potentiates the effects of pentobarbital, hexobarbital, meprobamate and carisoprodol especially by inhibiting their biotransformation in liver microsomes.

MATERIALS AND METHODS

Female rats of the Sprague–Dawley strain were used throughout the experiments. Carisoprodol and meprobamate were suspended with 1% carboxymethylcellulose solution and all other drugs were dissolved in distilled water; the drugs were all injected intraperitoneally.

The determinations of hexobarbital, pentobarbital, carisoprodol and meprobamate were carried out according to the methods of Axelrod *et al.*, Brodie *et al.*, Kato *et al.*, and Hoffman and Ludwig, respectively.[5–8]

Liver enzyme activities were determined by measuring the metabolized drugs in liver slices or in the microsomal preparation after an incubation of 1 hr. The rats were killed by decapitation and the liver immediately removed and sliced with a microtome. Liver slices (500 mg) were suspended in a Warburg flask which contained 6 ml of Krebs phosphate buffered Ringer (pH 7·4) and 0·2 ml of the substrate (final concentrations of hexobarbital, pentobarbital, meprobamate and carisoprodol were 4×10^{-4} M, 2×10^{-4} M, 3×10^{-4} M and 3×10^{-4} M, respectively), and incubated in an atmosphere of oxygen at 37 °C and shaken.

At the end of the incubation period, the reaction mixture was homogenized and 2 ml of the homogenate were used for the determination of the substrates.

In the experiments with microsomal preparations the liver was homogenized in 3 parts of isotonic KCl (1·15%) with a Potter–Elvehjem type homogenizer. The nuclei and mitochondria were sedimented by centrifugation of the homogenate at $8500 \times g$ for 15 min. The incubation mixture (5·0 ml) contained 2 ml of the microsome-containing supernatant, 0·1 ml of 20 μmole glucose-6-phosphate, 0·4 μmole TPN, 50 μmole nicotinamide, 75 μmole $MgCl_2$, 2M KCl, 2·3 ml of 0·1 M sodium phosphate buffer pH 7·4, and 0·2 ml of the substrate.

The effects of hexobarbital, pentobarbital, meprobamate and carisoprodol were determined by the duration of loss of the righting reflex.

RESULTS

Injection of imipramine (25 mg/kg i.p.) 20 min before the administration of hypnotics markedly potentiates hypnosis by hexobarbital or pentobarbital; on the other hand hypnosis by barbital of chloralhydrate were only slightly potentiated by the administration of imipramine. In fact hypnosis induced by hexobarbital and pentobarbital were prolonged 3·0 times and 4·5 times by the administration of imipramine; however, hypnosis induced by barbital and chloralhydrate were prolonged only 1·2 times and 1·3 times (Table 1).

TABLE 1. EFFECT OF IMIPRAMINE ON SLEEPING-TIME INDUCED BY HEXOBARBITAL, PENTOBARBITAL, BARBITAL OR CHLORALHYDRATE

(Female rats weighing about 180 g used. Imipramine (25 mg/kg) was given intraperitoneally 20 min before injections of hexobarbital (90 mg/kg i.p.), pentobarbital (22 mg/kg i.p.), barbital (215 mg/kg i.p.) or chloralhydrate (200 mg/kg i.p.). The values given represent averages ± standard error.)

	No. of animals	Sleeping time (min)	Variation	*P*
Hexobarbital	12	43 ± 4·3		
Hexobarbital + imipramine	12	128 ± 11·6	×3·0	<0·001
Pentobarbital	12	45 ± 3·8		
Pentobarbital + imipramine	12	205 ± 12·7	×4·5	<0·001
Barbital	8	262 ± 16		
Barbital + imipramine	8	323 ± 21	×1·2	N.S.
Chloralhydrate	8	34 ± 4·6		
Chloralhydrate + imipramine	8	43 ± 6·2	×1·3	N.S.

Hypnosis by meprobamate and paralysis by carisoprodol were also markedly increased by imipramine (Table 2).

The metabolism of hexobarbital, pentobarbital, meprobamate and carisoprodol occur in the liver microsomal fraction and SKF 525 A and Lilly 18947 inhibit their metabolism.[9-12]

TABLE 2. EFFECT OF IMIPRAMINE ON SLEEPING-TIME INDUCED BY MEPROBAMATE AND ON PARALYSIS INDUCED BY CARISOPRODOL

(Female rats, weighing about 180 g were used. Imipramine (25 mg/kg) was given intraperitoneally 20 min before injections of meprobamate (200 mg/kg i.p.) or carisoprodol (160 mg/kg).)

	No. of animals	Duration of action (min)	Variation	*P*
Meprobamate	12	45 ± 8·8		
Meprobamate + imipramine	12	109 ± 15·1	×2·4	<0·001
Carisoprodol	12	44 ± 4·2		
Carisoprodol + imipramine	12	124 ± 10·4	×2·8	<0·001

However, according to Burns *et al.*, barbital is not metabolized to any detectable extent by rats, and according to Friedman and Cooper chloralhydrate is metabolized by the alcohol dehydrogenase. The action of the two drugs is not potentiated by SKF 525 A.[13-15]

These results suggest that imipramine may act like SKF 525 A, on the drug metabolizing enzymes of microsomes and potentiate the action of hexobarbital, pentobarbital, meprobamate and carisoprodol by inhibiting their metabolism.

TABLE 3. BRAIN CONCENTRATION OF DRUGS AT THE RECOVERY OF THE RIGHTING REFLEX

(Female rats, weighing about 160 g were used. Imipramine (25 mg/kg) was given intraperitoneally 20 min before the injections of 175 mg/kg of carisoprodol, 90 mg/kg of hexobarbital or 22 mg/kg of pentobarbital and the rats were killed by blooding at the recovery of the righting reflex. Numbers of the animals used are indicated in the brackets.)

	Duration of loss of righting reflex	*P*	Drug concentration in brain at the recovery of righting reflex	*P*
Carisoprodol	42 ± 5·8 (7)		112 ± 3·1	
Imipramine + carisoprodol	118 ± 12·8 (8)	<0·001	98 ± 3·4	<0·05
Hexobarbital	40 ± 3·3 (7)		49 ± 2·0	
Imipramine + hexobarbital	121 ± 13·4 (8)	<0·001	40 ± 2·3	<0·05
Pentobarbital	49 ± 4·0 (8)		15·5 ± 0·42	
Imipramine + pentobarbital	180 ± 11·3 (8)	<0·001	13·8 ± 0·51	N.S.

The inhibition of *in vivo* metabolism of pentobarbital and meprobamate by imipramine is shown in Figs. 1 and 2.

Part of the potentiating action of imipramine on the drug action is due to its direct action on the central nervous system, since imipramine potentiates, though with less intensity, the effect of barbital and chloralhydrate, and shortens the induction time of the barbital effect.

These facts were confirmed by the experiments demonstrated in Table 3, in which it is observed that carisoprodol, hexobarbital and pentobarbital concentrations in the brain, determined on recovery from loss of the righting reflex, were somewhat lower in the imipramine-treated rats than in controls.

Furthermore, imipramine can reinduce sleep or paralysis in rats awakening or recovered from pentobarbital hypnosis or carisoprodol paralysis, and it also induces sleep when combined with a subhypnotic dose or a subparalytic dose of pentobarbital or carisoprodol.

These results indicate that there is a slight difference in sensitivity towards the drugs between controls and imipramine-treated rats, that is, the sensitivity of the

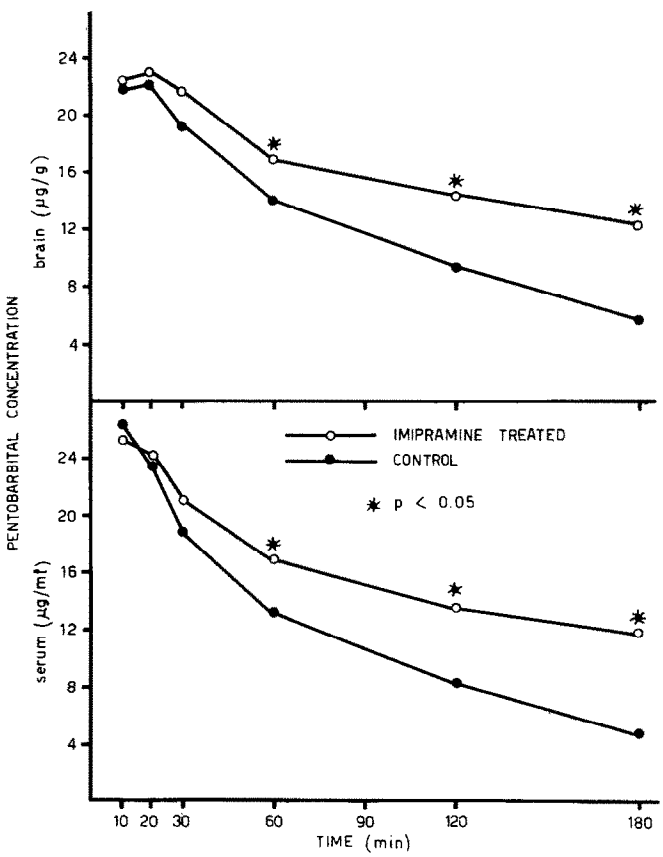

FIG. 1. Inhibition of *in vivo* metabolism of pentobarbital by imipramine. Imipramine (25 mg/kg) was given intraperitoneally 20 min before the injection of pentobarbital (22 mg/kg i.p.). Female rats weighing 100 g were used. The values given represent averages obtained from at least five rats.

central nervous system of imipramine-treated rats was increased but the increased nervous sensitivity contributes only partially to the potentiating effect of imipramine on drug action.

The inhibition of the *in vitro* metabolism of hexobarbital pentobarbital, meprobamate and carisoprodol by imipramine is given in Table 4. The concentrations which produced 50 per cent inhibition of the metabolism of hexobarbital, pentobarbital, meprobamate and carisoprodol by imipramine were $8{\cdot}4 \times 10^{-4}$, $1{\cdot}9 \times 10^{-4}$, $2{\cdot}9 \times 10^{4}$ and $2{\cdot}3 \times 10^{-4}$, respectively, in the liver slices, and $3{\cdot}1 \times 10^{-4}$, $5{\cdot}3 \times 10^{-5}$, $1{\cdot}9 \times 10^{-4}$ and $1{\cdot}3 \times 10^{-4}$, respectively, in the liver microsomal preparation.

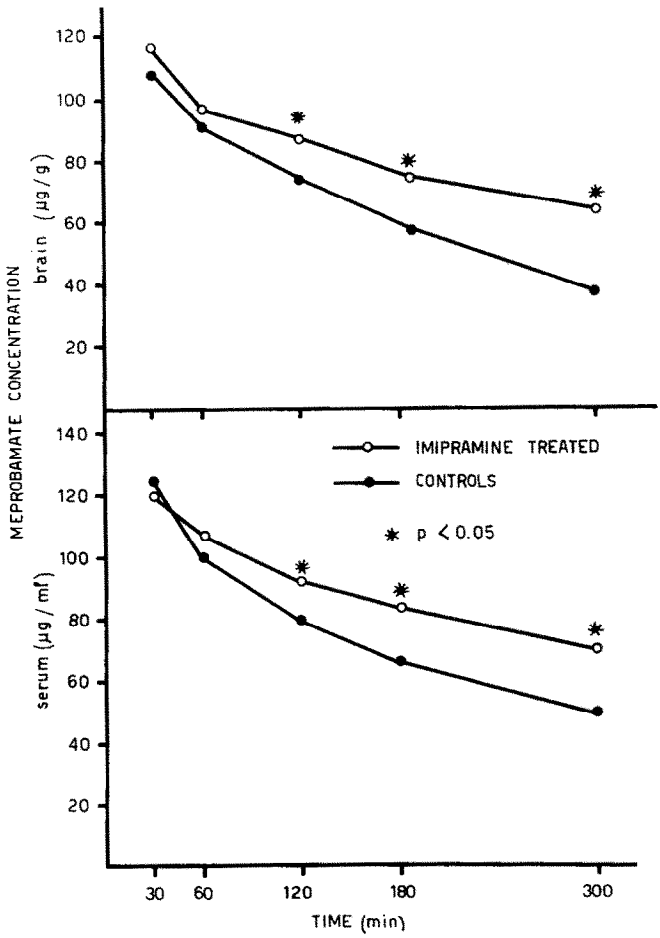

FIG. 2. Inhibition of *in vivo* metabolism of meprobamate by imipramine. Imipramine (25 mg/kg i.p.) was given intraperitoneally 20 min before the injection of meprobamate (150 mg/kg i.p.). Female rats, weighing 100 g were used. The values given represent averages obtained from at least four rats.

It is clearly observed that the enzyme responsible for metabolism of pentobarbital was more sensitive than other enzymes towards imipramine, and the inhibition in the microsomal preparation is somewhat more marked than that in the liver slice preparation.

The inhibitory action of imipramine on drug metabolism is generally one-fifth as potent as that of SKF 525 A, but it is about three times more potent than that of iproniazid.[16]

The nature of the inhibition produced by SKF 525 A is still in question although Brodie[9] has suggested that it is probably a "true enzyme inhibitor", and does not act by means of a physical effect.

A study was therefore carried out to obtain some possible interactions between three different types of drug metabolism inhibitor, i.e. SKF 525 A, imipramine and

TABLE 4. INHIBITORY ACTION OF IMIPRAMINE ON *in vitro* METABOLISM OF HEXOBARBITAL, PENTOBARBITAL, MEPROBAMATE AND CARISOPRODOL

(Female rats weighing about 70 g were used. (SI) = experiments with the liver slices. (M) = experiments with the liver microsomal preparation. Metabolisms of hexobarbital, pentobarbital, meprobamate and carisoprodol in the liver slices were 510 μg/g per 1 hr, 170 μg/g per 1 hr, 182 μg/g per 1 hr and 215 μg/g per 1 hr, respectively. Metabolisms of hexobarbital, pentobarbital, meprobamate and carisoprodol in the microsomal preparation were 302 μg/g per 1 hr, 87 μg/g per 1 hr, 95 μg/g per 1 hr and 118 μg/g per 1 hr, respectively. The values given represent averages obtained at least from three determinations.)

Imipramine concentration		Inhibition of metabolism (%)			
		Hexobarbital	Pentobarbital	Meprobamate	Carisoprodol
2×10^{-5}M	(SI)	11	16	7	12
5×10^{-5}M	(SI)	18	25	16	20
1×10^{-4}M	(SI)	21	34	29	29
2×10^{-4}M	(SI)	30	50	44	49
5×10^{-4}M	(SI)	45	71	60	64
2×10^{-5}M	(M)	13	32	11	17
5×10^{-5}M	(M)	22	50	18	28
1×10^{-4}M	(M)	30	60	34	41
2×10^{-4}M	(M)	42	72	53	64
5×10^{-4}M	(M)	58	93	68	74

TABLE 5. INFLUENCE OF SKF 525 A AND PHENYL*iso*PROPYLHYDRAZINE (PIH) ON THE INHIBITORY ACTION OF IMIPRAMINE

(The values given represent averages obtained at least from three determinations.)

Inhibition	Concentration (moles)	Inhibition of hexobarbital metabolism (%)	Inhibition of meprobamate metabolism (%)
(1) Imipramine	2×10^{-5}	14	13
(2) SKF 525 A	5×10^{-6}	22	24
(3) Imipramine + SKF 525 A		79	80
(4) PIH	2×10^{-5}	22	8
(5) Imipramine + PIH		82	63

phenyl*iso*propylhydrazine (JB 516, PIH), the last being also a potent inhibitor of drug metabolisms because of its hydrazine group.[17, 18] The results are shown in Table 5, where it can be seen that SKF 525 A, imipramine and PIH markedly potentiate the inhibitory action of each other on the drug-metabolizing enzymes.

According to Gillette *et al.*, the antidepressant effect of imipramine is due to its demethylated product, that is, demethylimipramine (DMI) which has a powerful

analeptic effect.[19] The possible inhibitory action of DMI on microsomal drug metabolizing enzymes was therefore-studied, it turned out, however, to be less remarkable than that of imipramine. DMI in the experiment with the microsomal preparation showed an inhibitory action on the metabolism of meprobamate (50 per cent inhibition) and of pentobarbital (50 per cent inhibition) at the concentrations of $3{\cdot}2 \times 10^{-4}$ M and $1{\cdot}2 \times 10^{-4}$ M respectively.

DISCUSSION

Imipramine has a double effect on pentobarbital hypnosis and on carisoprodol paralysis. It induces an increased sensitivity in the c.n.s. towards pentobarbital or carisoprodol; on the other hand, it inhibits the biotransformation of the drugs in the liver microsomes.

Brodie has classified as "true potentiators" compounds such as chlorpromazine and reserpine, which are able to reinduce sleep in animals awakening from hexobarbital hypnosis and which induce sleep when combined with a subhypnotic dose of this barbiturate. The same author also classified compounds such as SKF 525A, Lilly 18947 and iproniazid that act by interfering with the metabolic degradation of hexobarbital as "prolonging agents". By these criteria, imipramine would have to be classified as both a potentiating agent and a prolonging agent since it fulfills the requirements of each category.[1–3, 9]

Indeed, imipramine can prolong pentobarbital hypnosis and carisoprodol paralysis; on the other hand, imipramine can reinduce sleep or paralysis in animals recovering from pentobarbital hypnosis or recovered from carisoprodol paralysis and can also induce sleep or paralysis when combined with a subhypnotic dose of pentobarbital or subparalytic dose of carisoprodol.

Recently Buller *et al.* reported that U-320 (4:5-dihydro-6-methyl-2-(2-(4-pyridyl)-ethyl)-3-pyridazine) would have to be classified as both a potentiating agent and a prolonging agent, but its inhibitory action on the hexobarbital metabolism seems to be one-third or one-quarter than that of imipramine.[16, 20]

The potentiation of barbital and chloralhydrate hypnosis by imipramine indicates that imipramine may increase the sensitivity of the c.n.s. towards the hypnotics. Barbital is not metabolized *in vivo* and chloralhydrate is metabolized by the alcohol dehydrogenase which is not inhibited by SKF 525 A. The potentiating action of imipramine on barbital and chloralhydrate therefore may be due to a direct effect of imipramine on the central nervous system.

Recently we demonstrated that many other drugs, which are considered as hypnotics, depressants or antihistaminics (for example, phenaglycodol, glutethimide, hydroxyzine, azacyclonol and chlorcyclizine) prolong pentobarbital hypnosis, meprobamate hypnosis and carisoprodol paralysis, not only by means of their direct effect on the central nervous system, but also by inhibiting the metabolism of pentobarbital, meprobamate and carisoprodol.

The interaction of simultaneously administered drugs is a common phenomenon in drug metabolism, and therefore this fact must be taken into consideration in the evaluation of a possible influence of certain drugs on the action of other drugs.

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