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INDUCTION OF INCREASED MEPROBAMATE METABOLISM IN RATS PRETREATED WITH SOME NEUROTROPIC DRUGS

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Abstract—Rats pretreated with phenobarbital, phenaglycodol, glutethimide, nikethamide, chlorpromazine, trifluprομazine, meprobamate, carisoprodol, pentobarbital, thiopental, primidone, chloretone, diphenylhydantoine and urethane showed an accelerated metabolism of meprobamate and at the same time, a diminished duration of sleeping time and paralysis due to meprobamate.

These effects developed 24 hr after treatment, the maximum increase in metabolism occurring after about 48 hr. Accelerated metabolism of meprobamate and a diminished duration of sleeping time and paralysis was overshadowed in rats pretreated with SKF 525 A.

Repeated pretreatments enhanced the effect of single injection of phenobarbital and other drugs. Using the liver slice preparation or the liver microsomal preparation, increased meprobamate metabolism was observed *in vitro* under the influence of phenobarbital, etc. In hypophysectomized or adrenalectomized rats similar increase of meprobamate metabolism was observed *in vitro*.

These results suggest a possible role of the metabolic factor in the development of tolerance and cross-tolerance to meprobamate in animals, and in clinical experiments.

IN PREVIOUS work it was observed that rats pretreated with phenobarbital and phenaglycodol, showed remarkable resistance to meprobamate administered 48 hr later. At the same time, an accelerated *in vivo* metabolism of meprobamate was also observed.[1, 2] The accelerated meprobamate metabolism after the pretreatment with phenobarbital or phenaglycodol did not occur if ethionine was injected 30 min before phenobarbital or phenaglycodol, and an induction of increase in the biosynthesis of the enzyme responsible for metabolism of meprobamate was supposed.

It was interesting to see whether or not other neurotropic drugs could also increase the rate of meprobamate metabolism, since the increased metabolism may produce phenomena of cross-tolerance and interactions of two drugs.

In the work reported here, a stimulation of *in vivo* and *in vitro* metabolism of meprobamate and a decrease of its actions by the pretreatment with some neurotropic drugs was demonstrated.

MATERIAL AND METHODS

Experiments were made on female rats of the Sprague–Dawley strain, weighing about 160–180 g. In some *in vitro* experiments, male rats weighing 60 g were used.

(A) Hypnotics and anticonvulsivants: phenobarbital sodium (90 mg/kg), thiopental sodium (30 mg/kg), diphenylhydantoine sodium (100 mg/kg), primidone (200 mg/kg), pentobarbital sodium (25 mg/kg), hexobarbital sodium (100 mg/kg), chloretone (100 mg/kg), chloralhydrate (200 mg/kg), urethane (800 mg/kg), paraldehyde (400 mg/kg), methylpentynol (150 mg/kg), ethyl alcohol 40% (10 ml/kg), glutethimide (Doriden) (80 mg/kg), hydroxydion (Viadril) (50 mg/kg), N-phtalolylglutarimide (500 mg/kg).

(B) Tranquillizers: chlorpromazine (15 mg/kg), promazine (15 mg/kg), triflupromazine (15 mg/kg), perphenazine (15 mg/kg), meprobamate (200 mg/kg), hydroxyzine (150 mg/kg) captodiamine (Covatin) (150 mg/kg), mephenesine dicarbamate (200 mg/kg), methylpentynol carbamate (150 mg/kg), chlordiazepoxide (Librium) (50 mg/kg).

(C) Central myorelaxants: zoxazolamine (100 mg/kg), carisoprodol (150 mg/kg), mephenesine (200 mg/kg).

(D) Central stimulants: megimide (10 mg/kg), nikethamide (200 mg/kg), amphetamine (30 mg/kg), methylphenidate (30 mg/kg).

All these drugs were dissolved in distilled water, with the exception of phenaglycodol, glutethimide, meprobamate, carisoprodol, N-phthaloylglutarimide, mephenesine dicarbamate, methylpentynol carbamate, mephenesine, primidone and zoxazolamine, which were suspended in a 1% carboxymethylcellulose solution. Chloretone was dissolved in arachic oil and ethionine was dissolved in 0·9% sodium chloride solution. All drugs were injected intraperitoneally in volumes of 2 ml/kg of rat bodyweight, with the exception of ethionine which was also injected intraperitoneally but in a volume of 10 ml/kg.

Generally, 48 hr after the pretreatment with the various drugs, the rats were injected intraperitoneally with different doses of meprobamate and the rats were sacrificed 3 hr after the injection.

The determination of serum and brain meprobamate concentrations were carried out following the method of Hoffmann and Ludwig with a slight modification .[3]

Liver enzyme activities were determined by measuring the amount of meprobamate metabolized by liver slices or by the microsomal preparation after an incubation period of 2 hr. The rats were killed by decapitation and the liver immediately removed and sliced with a microtome. The 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 (405 μg, final concentration 3×10^{-4}), and incubated in an atmosphere of oxygen at 37 °C, and at the same time the flask was shaken. At the end of the incubation period, the reaction mixture was homogenized and 2 ml of the homogenate were used for the determination.

In the experiments with microsomal preparations the liver was homogenized in 2 parts of isotonic KCl (1·15 per cent) with a Potter–Elvehjem type homogenizer. The nuclei and mitochondria were sedimented by centrifugation of the homogenated 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, 100 μmole nicotinamide, 75 μmole $MgCl_2$ and 1 M KCl and 2·3 ml of 0·1 M sodium phosphate buffer pH 7·4 and 0·2 ml of the substrate (328 μg, final concentration 3×10^{-4}).

The determination of the duration of sleeping time caused by meprobamate was carried out by measuring the duration of loss of righting reflex.

The paralysis due to meprobamate was determined by the use of an inclined plate (about 40°); rats which could not stay on the plate for at least 10 sec were considered to be paralysed.

RESULTS

Table 1 shows that many neurotropic drugs accelerate the meprobamate metabolism 48 hr after their administration.

The rats pretreated with phenobarbital, phenaglycodol, glutethimide, meprobamate, thiopental, nikethamide, chloretone, chlorpromazine, triflupromazine, primidone, pentobarbital, diphenylhydantoine, urethane, mephenesine dicarbamate, methylpentynol carbamate and carisoprodol showed an accelerated meprobamate metabolism. On the other hand, rats pretreated with paraldehyde, chloralhydrate, ethyl alcohol, methylpentynol, ethyl ether, N-phthaloylglutarimide, chlordiazepoxide, promazine, hydroxyzine, captodiamine, imipramine, megimide, amphetamine, and methylphenidate showed no such change.

These results indicate that the capacity to accelerate meprobamate metabolism is not related to the pharmacological effects of the drugs; for example, many hypnotic drugs and tranquillizers can accelerate the meprobamate metabolisms, but the same effect was observed with nikethamide in spite of its pharmacological action as a central nervous system stimulant.

This capacity also could not be related to the chemical structure of these drugs; for example, glutethimide (α:α-ethylglutarimide) accelerates the meprobamate metabolism, whereas N-phthaloylglutarimide does not do so. Chlorpromazine, triflupromazine, accelerate the meprobamate metabolism but promazine does not give the same effect.

The concentration curves of meprobamate in serum and brain in rats pretreated with phenobarbital or glutethimide (Doriden) are shown in Figs. 1–2. The rats pretreated with phenobarbital or glutethimide have a more rapid decrease of meprobamate concentrations in serum and brain than the control rats. Similar results were obtained with phenaglycodol, meprobamate and nikethamide pretreated rats. The biological half-lifes (B.H.L.) of meprobamate, which were injected intraperitoneally at doses of 150 mg/kg, are calculated in Figs. 1 and 2 and the other similar figures, are as follows:

(1) B.H.L. 200 min in the control rats.

(2) B.H.L. 55 min in the phenobarbital-pretreated rats.

(3) B.H.L. 78 min in the phenaglycodol-pretreated rats.

(4) B.H.L. 106 min in the glutethimide-pretreated rats.

(5) B.H.L. 121 min in the meprobamate-pretreated rats.

(6) B.H.L. 122 min in the nikethamide-pretreated rats.

Three hours after the injection of 100 mg/kg of meprobamate the serum and brain concentrations in control rats are 43 mg/kg and 32 mg/kg, respectively; on the other hand, in order to obtain the same concentrations of meprobamate 3 hr after the

TABLE 1. EFFECT OF PRETREATMENT WITH VARIOUS NEUROTROPIC DRUGS ON METABOLISM OF MEPROBAMATE

Pretreatments	Doses (mg/kg)	Meprobamate concentration			
		Serum (μg/ml)	Variations (%)	Brain (μg/g)	Variations (%)
(1) Controls		73 ± 2·4 (103)		62 ± 2·3 (108)	
(2) Phenobarbital	90	13 ± 1·4 (27)	−82 (°°°)	11 ± 1·8 (28)	−77 (°°°)
(3) Phenaglycodol	130	26 ± 2·3 (18)	−65 (°°°)	19 ± 2·1 (19)	−69 (°°°)
(4) Glutethimide	80	44 ± 2·4 (14)	−40 (°°°)	34 ± 2·1 (14)	−45 (°°°)
(5) Meprobamate	200	48 ± 2·4 (10)	−34 (°°°)	36 ± 2·4 (10)	−42 (°°°)
(6) Thiopental	30	44 ± 2·5 (12)	−40 (°°°)	34 ± 2·5 (12)	−45 (°°°)
(7) Nikethamide	200	47 ± 2·7 (15)	−36 (°°°)	40 ± 2·8 (15)	−36 (°°°)
(8) Chloretone	110	45 ± 2·9 (16)	−38 (°°°)	40 ± 3·0 (16)	−36 (°°°)
(9) Chlorpromazine	15	53 ± 4·1 (19)	−27 (°°)	49 ± 3·4 (20)	−21 (°)
(10) Triflupromazine	15	46 ± 4·3 (12)	−37 (°°°)	43 ± 3·9 (12)	−30 (°°°)
(11) Primidone	200	49 ± 3·5 (8)	−33 (°°°)	44 ± 3·2 (8)	−27 (°°)
(12) Pentobarbital	25	53 ± 2·8 (8)	−27 (°°)	45 ± 3·3 (8)	−27 (°°)
(13) Diphenylhydantoine	100	57 ± 4·4 (7)	−22 (°°)	51 ± 3·9 (7)	−18
(14) Urethane	700	63 ± 5·3 (8)	−16	51 ± 3·0 (8)	−18 (°)
(15) Mephenesine dicarbamate	200	57 ± 2·9 (8)	−22 (°°)	47 ± 2·7 (8)	−23 (°°)
(16) Methylpentynol carbamate	150	52 ± 3·5 (8)	−29 (°°°)	46 ± 3·9 (8)	−35 (°°)
(17) Carisoprodol	150	58 ± 4·4 (8)	−21 (°°)	50 ± 3·8 (8)	−20 (°°)

All drugs were injected 48 hr before the injection of meprobamate (150 mg/kg).
The rats were killed 3 hr after the injection of meprobamate.
The values are means ± standard error. The numbers in the brackets indicate the numbers of animals used.
Significance: (°°°) = $P < 0{\cdot}001$; (°°) = $P < 0{\cdot}01$; (°) = $P < 0{\cdot}05$.

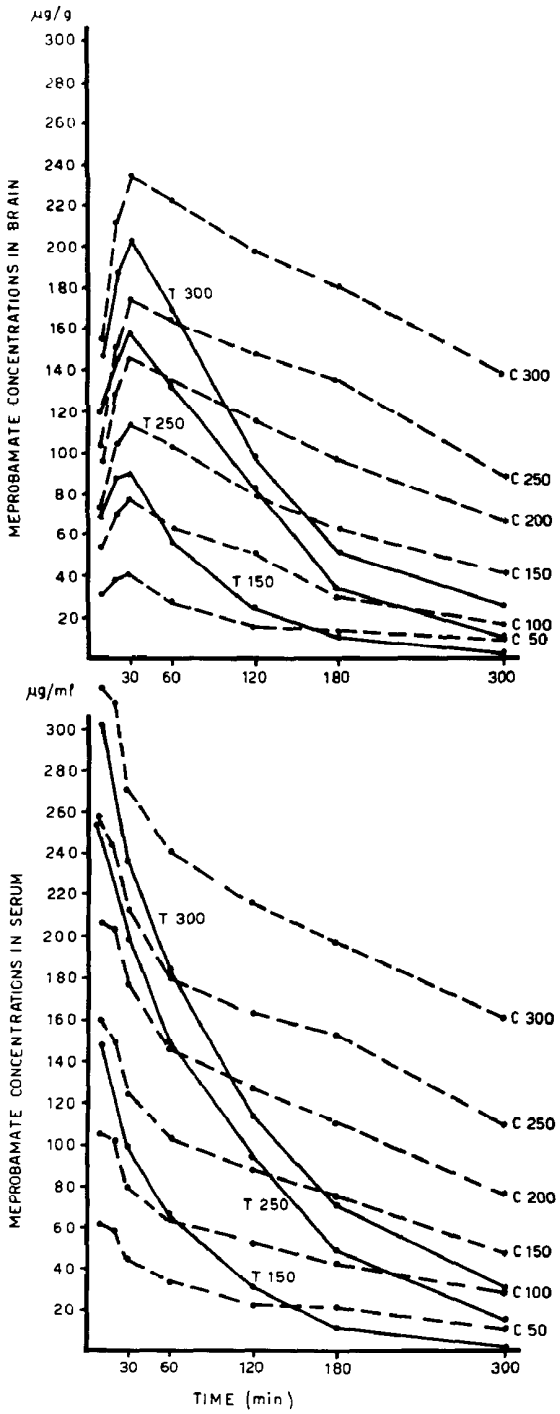

FIG. 1. Serum and brain meprobamate concentrations in controls and phenobarbital-pretreated rats. Rats were injected intraperitoneally with different doses of meprobamate 48 hr after pretreatment with phenobarbital (90 mg/kg i.p.). Determination of meprobamate concentrations was carried out at various time intervals after the injection. C. 300, C. 250, C. 200, C. 150, C. 100, C. 50: control injected 300 mg/kg, 250 mg/kg, 200 mg/kg, 150 mg/kg, 100 mg/kg or 50 mg/kg of meprobamate. T. 300, T. 250, T. 150 pretreated rats injected 300, 250, 150, mg/kg of meprobamate.

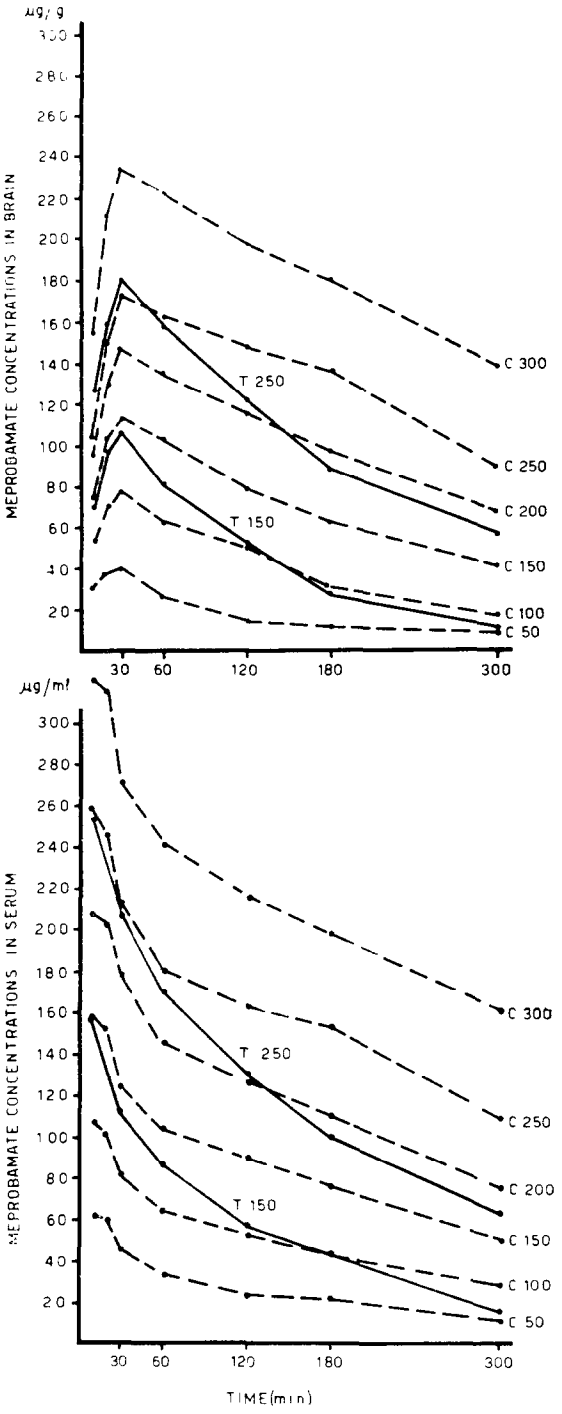

FIG. 2. Serum and brain meprobamate concentrations in controls and glutethimide (Doriden)—pretreated rats. Rats were injected intraperitoneally with different doses of meprobamate 48 hr after pretreatment with gluthethimide (80 mg/kg i.p.).

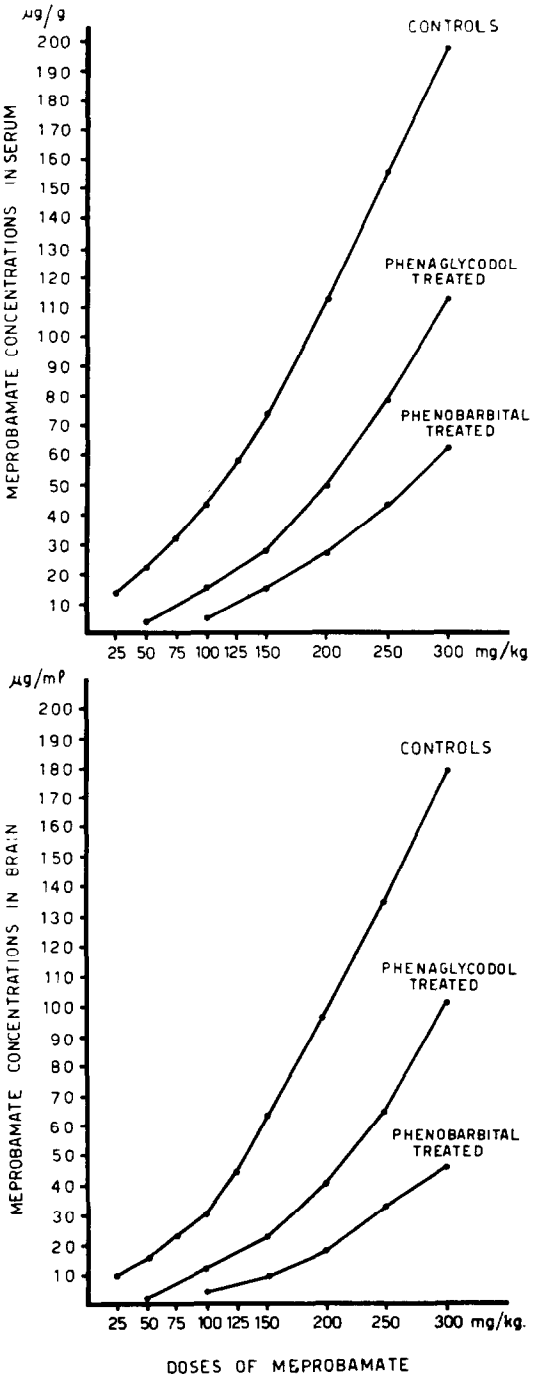

FIG. 3. Relationship between injected dose and meprobamate concentrations in serum or brain in controls and phenobarbital- or phenaglycodol-pretreated rats. Rats were injected intraperitoneally with different doses of meprobamate 48 hr after pretreatment with phenobarbital (90 mg/kg i.p.) or phenaglycodol (130 mg/kg i.p.). Determination of meprobamate concentration was carried out 3 hr after the injection.

injection, it was necessary to inject 248 mg/kg to the phenobarbital pretreated rats and 181 mg/kg to the phenaglycodol pretreated rats (Fig. 3).

But this accelerated meprobamate metabolism is not observed immediately after injection of the drugs, but the onset of the acceleration of metabolism appeared between 12 and 24 hr, after the injection of the drugs (inducers) (Fig. 4). The maximal accelerated metabolism of meprobamate occurred about 48 hr after the injection, and could still be clearly observed even after 4 days.

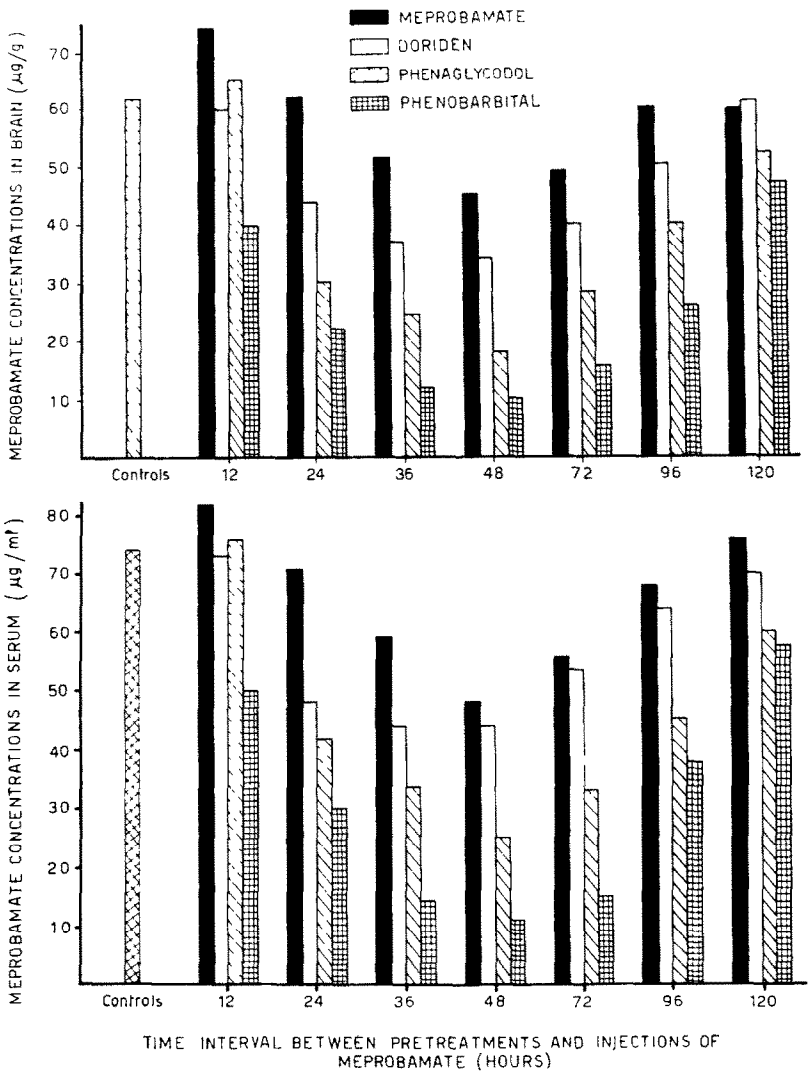

FIG. 4. Serum and brain meprobamate concentrations (3 hr after injection) in controls and phenobarbital, phenaglycodol, glutethimide (Doriden) or meprobamate-pretreated rats, at different intervals between pretreatments and injection of meprobamate. Rats were injected intraperitoneally 150 mg/kg of meprobamate 12, 24, 36, 48, 72, 96, 120 hr after pretreatments with phenobarbital (90 mg/kg, i.p.), phenaglycodol (130 mg/kg, i.p.), gluthethimide (80 mg/kg, i.p.) or meprobamate (200 mg/kg, i.p.). Determination of meprobamate concentrations was carried out 3 hr after the injection.

Fig. 5 shows the relationship between intensity of accelerated meprobamate metabolism and doses of the inducers. Phenobarbital even at a small dose, showed a very clear effect on meprobamate metabolism. The diminished pharmacological effects of meprobamate are accompanied with accelerated meprobamate metabolism,

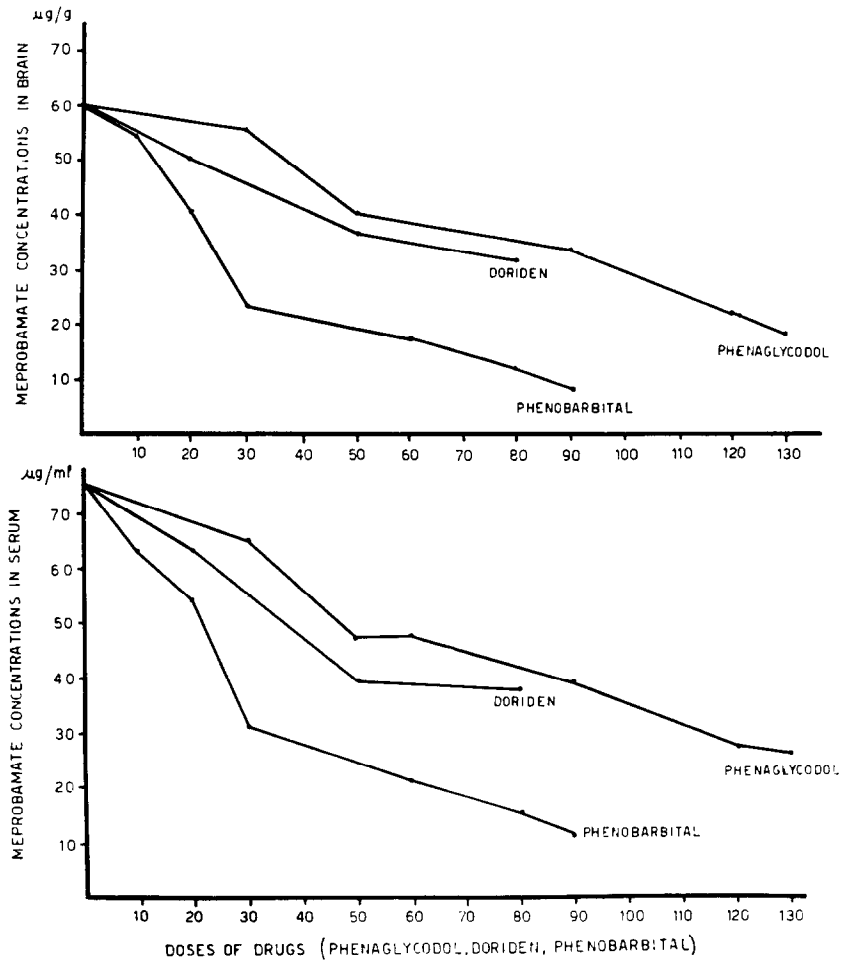

FIG. 5. Relationship between intensity of accelerated meprobamate metabolism and doses of the inducers. Ordinate: serum and brain meprobamate concentrations 3 hr after injection of meprobamate (150 mg/kg i.p.). Abscissa: Doses of phenobarbital, phenaglycodol and glutethimide. Rats pretreated with phenobarbital (i.p.) phenaglycodol (i.p.) or glutethimide (Doriden) (i.p.) 48 hr before injection of meprobamate.

and the drugs which did not induce the accelerated metabolism had no such effect (Table 2).

It was also observed that the increased rates of meprobamate metabolism are far more remarkable by repeated pretreatments with the inducers, than by single injections; however, under these experimental conditions there was no marked difference between, treatments of 4 days and of 7 days (Table 3).

As previously reported, the induced acceleration of meprobamate metabolism is antagonized by 100–250 mg/kg ethionine administered intraperitoneally 30 min before the injection of phenobarbital or phenaglycodol, though the above-given doses of the ethionine do not modify the meprobamate metabolism of normal rats. In this work, the same effect was observed by the use of glutethimide or niketamide.

TABLE 2. EFFECT OF PRETREATMENT WITH SOME NEUROTROPIC DRUGS ON SLEEPING TIME AND PARALYSIS DUE TO MEPROBAMATE

Pretreatment	No. of animals	Sleeping time	Variation (%)	*P*	Paralysis % after 3 hr
Controls	32	143 ± 5·7			80
Phenobarbital	24	88 ± 7·3	−38	<0·001	35
Phenaglycodol	24	101 ± 6·1	−29	<0·001	55
Glutethimide	16	91 ± 6·0	−36	<0·001	50
Nikethamide	23	115 ± 9·3	−20	<0·05	70
Meprobamate	14	101 ± 5·3	−29	<0·001	55
Thiopental	24	107 ± 7·8	−25	<0·05	60
Chlorpromazine	24	110 ± 7·5	−23	<0·01	60

300 mg/kg and 200 mg/kg meprobamate were respectively injected intraperitoneally for the determination of sleeping time and percentage of paralysis. Percentage of paralysis was measured 3 hr after the injection. Each group consisted of at least twenty rats.

TABLE 3. EFFECT OF REPEATED PRETREATMENT WITH THE INDUCING DRUGS ON THE METABOLISM OF MEPROBAMATE

Pretreatment	Doses (mg/kg)	Days of pre-treatment	Meprobamate concentration: Serum (μg/ml	Variation (%)	Brain (μg/g)	Variation (%)
(1) —	—	—	73 ± 3·4 (19)		62 ± 2·9 (19)	
(2) Phenobarbital	50	1	22 ± 2·3 (16)	−70	17 ± 1·8 (16)	−73
(3) Phenobarbital	50	4	8 ± 2·1 (13)	−89	5 ± 1·3 (12)	−92
(4) Phenobarbital	50	7	3 ± 1·4 (8)	−96	5 ± 1·5 (8)	−92
(5) Phenaglycodol	90	1	38 ± 2·6 (8)	−49	29 ± 1·9 (8)	−53
(6) Phenaglycodol	90	4	17 ± 2·3 (11)	−78	13 ± 2·1 (12)	−79
(7) Phenaglycodol	90	7	18 ± 3·0 (8)	−76	10 ± 2·3 (8)	−84
(8) Glutethimide	50	1	38 ± 2·4 (8)	−49	32 ± 2·4 (8)	−48
(9) Glutethimide	50	4	30 ± 2·3 (8)	−60	19 ± 1·8 (7)	−69
(10) Niketamide	130	1	54 ± 3·3 (7)	−27	41 ± 2·3 (8)	−34
(11) Niketamide	130	4	43 ± 2·9 (8)	−42	34 ± 2·0 (8)	−45
(12) Niketamide	130	7	45 ± 3·1 (8)	−39	34 ± 1·3 (8)	−45
(13) Chlorpromazine	10	1	58 ± 2·9 (8)	−22	48 ± 1·9 (8)	−23
(14) Chlorpromazine	10	4	45 ± 3·1 (8)	−39	35 ± 2·3 (8)	−44
(15) Chlorpromazine	10	7	42 ± 3·0 (5)	−43	34 ± 2·4 (5)	−45
(16) Triflupromazine	10	1	55 ± 2·4 (8)	−26	45 ± 1·7 (8)	−28
(17) Triflupromazine	10	4	40 ± 2·1 (8)	−46	31 ± 1·8 (8)	−50

150 mg/kg of meprobamate were injected 48 hr after the single pretreatment and 24 hr after the last injection of the repeated pretreatment. Rats were sacrificed 3 hr after the injection.

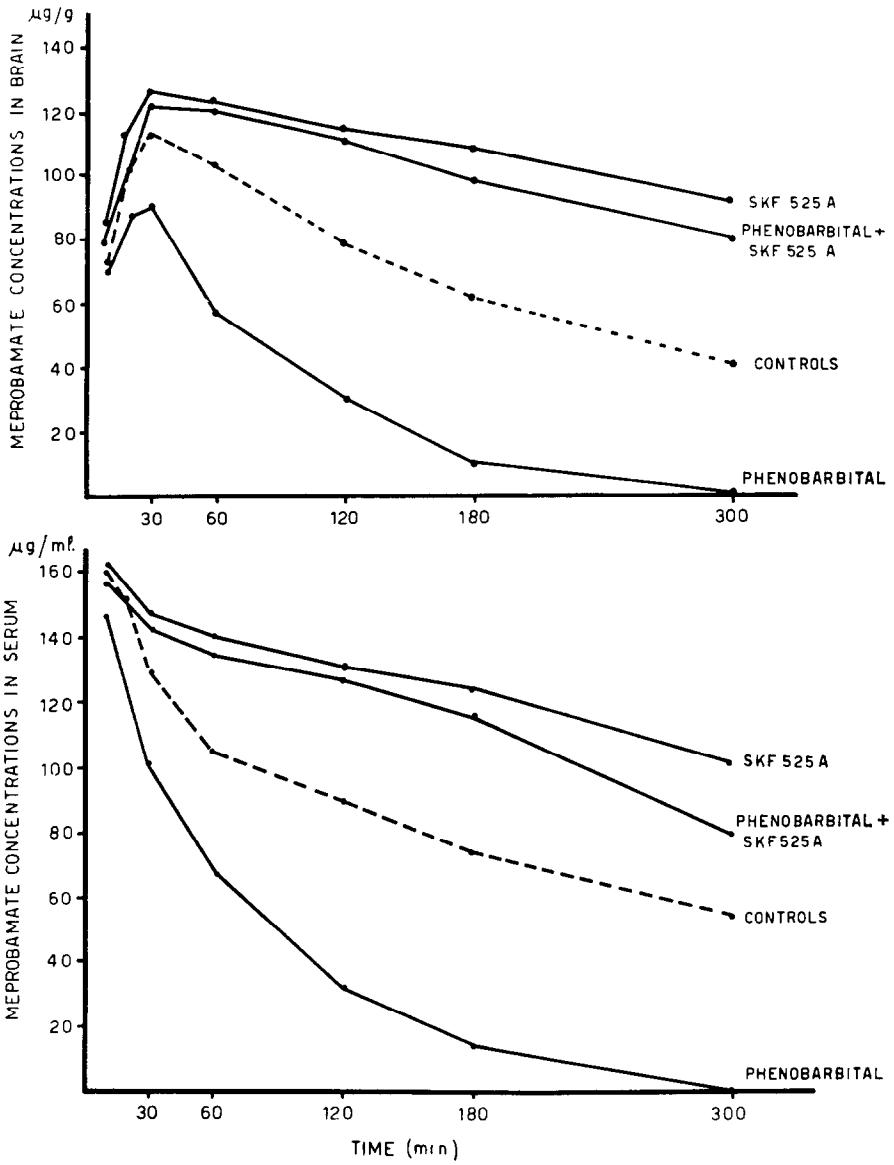

FIG. 6. Effect of SKF 525 A on the phenobarbital induced acceleration of meprobamate metabolism. Rats pretreated with phenobarbital (90 mg/kg i.p.) 48 hr before injection (i.p.) of 150 mg/kg of meprobamate. SKF 525 A (50 mg/kg) was injected intraperitoneally 30 min before the injection of meprobamate. Determination of meprobamate concentration was carried out 3 hr after the injection.

During this work it was also found that the meprobamate metabolism was inhibited by the administration of SKF 525 A,* which is a metabolic inhibitor of the barbiturates and some other drugs.

Fig. 6 shows that the accelerated meprobamate metabolism is almost completely nullified by SKF 525 A (50 mg/kg, i.p.) administered 30 min before the injection of

* SKF 525 A was kindly supplied by Dr. H. E. Duell (Smith Klein & French Laboratories, Philadelphia).

meprobamate. The same antagonistic phenomena was also observed as regards to the pharmacological effect of meprobamate (Table 4, and Fig. 7).

As demonstrated in Table 5, the drugs which stimulated the *in vivo* metabolism of meprobamate, induced an increase of activity of the meprobamate metabolizing enzyme of the liver and the drugs which did not stimulate the *in vivo* metabolism, did not increase the enzyme activity.

TABLE 4. EFFECT OF SKF 525 A ON SLEEPING TIME AFTER MEPROBAMATE IN NORMAL RATS AND IN THE PHENOBARBITAL OR PHENAGLYCODOL PRETREATED RATS

Pretreatment		sleeping time (min)	Variation (%)	*P*	Paralysis (%) after 5 hr
I	II				
(1) —	—	148 ± 3·9 (40)	—	—	45
(2) Phenobarbital	—	85 ± 6·5 (24)	−43	(1)–(2) < 0·001	0
(3) Phenaglycodol	—	97 ± 5·9 (24)	−35	(1)–(3) < 0·001	15
(4) —	SKF 525 A	656 ± 29·5 (21)	+343	(1)–(4) < 0·001	100
(5) Phenobarbital	SKF 525 A	483 ± 40·4 (13)	+226	(2)–(5) < 0·001	95
(6) Phenaglycodol	SKF 525 A	503 ± 34 (15)	+239	(3)–(6) < 0·001	90

50 mg/kg SKF 525 A was injected 30 min before the injection of the meprobamate. 300 mg/kg and 200 mg/kg; of meprobamate were injected respectively for determination of the sleeping time and percentage of paralysis: percentage of paralysis was measured 5 hr after the injection. Each group consisted of twenty rats.

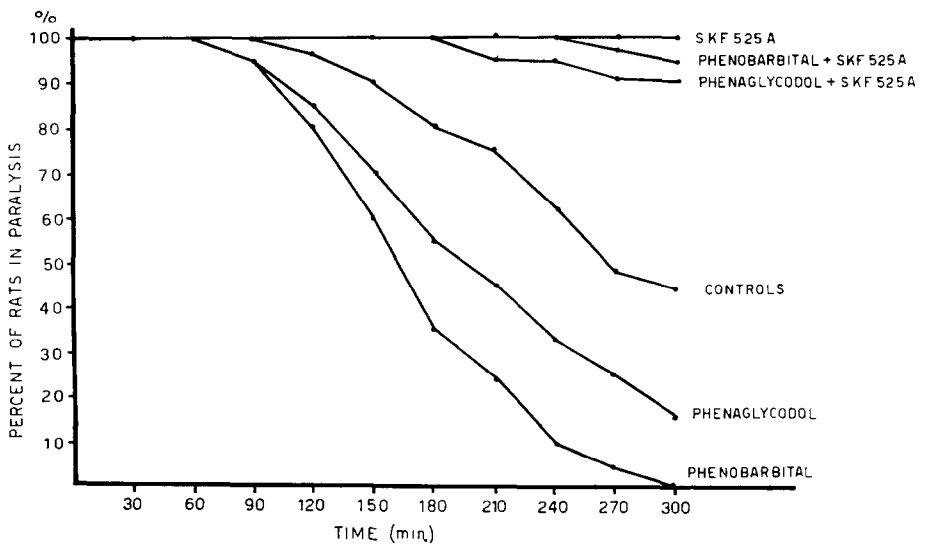

FIG. 7. Effect of SKF 525 A on the decreased paralytic effect of meprobamate induced by pretreatment with phenobarbital or phenaglycodol. Rats were pretreated with phenobarbital (90 mg/kg, i.p.) or phenaglycodol (130 mg/kg) 48 hr before injection of 200 mg/kg of meprobamate. SKF 525 A (50 mg/kg) was injected intraperitoneally 30 min before the injection of meprobamate.

Increases of the enzyme activities began 12–24 hr after the administration of the drugs and the maximum increases were found 48 hr after, and from this point the activity began to decrease gradually, and the variation of *in vitro* metabolisms were in accord with *in vivo* metabolisms as observed in the preceding experiments.

TABLE 5. STIMULATION OF *in vitro* METABOLISM OF MEPROBAMATE BY THE PRETREATMENT OF SOME NEUROTROPIC DRUGS

Pretreatment	Doses (mg/kg)	No. of animals	Enzyme activity (μg/2 hr)	Variation	*P*
Controls	—	12	218 ± 7·2	—	—
Phenobarbital	70	10	513 ± 13·9	+135	<0·001
Phenaglycodol	100	6	401 ± 12·8	+84	<0·001
Glutethimide	60	6	349 ± 9·2	+60	<0·001
Primidone	160	4	332 ± 7·3	+53	<0·001
Thiopental	27	5	328 ± 7·0	+51	<0·001
Nikethamide	160	3	315 ± 8·5	+45	<0·001
Chlorpromazine	12	6	299 ± 13·4	+37	<0·001
Meprobamate	160	4	295 ± 8·7	+35	<0·001
Pentobarbital	23	4	283 ± 10·2	+29	<0·001
Carisoprodol	130	4	275 ± 8·8	+27	<0·001

Male rats of weighing 60 g were used.
The all drugs were given 48 hr before the sacrifice.
The enzyme activity was determined by the liver slices.

The inducing drugs used above do have some action on the hypothalamus–hypophysis–adrenal gland axis. Therefore we tried to find out if the inducing drugs act through the axis or whether they act directly on the liver. Table 5 shows that the inducing drugs may act directly and not through hypophysis or adrenal gland.

The administration of adrenaline (50 mg/kg, i.p.), noradrenaline (1 mg/kg, i.p.), serotonine (2 mg/kg, i.p.), histamine (10 mg/kg, i.p.), acetylcholine (7 mg/kg, i.p.) and aspirin (500 mg/kg, i.p.) and also electroshock (90 V, 1 sec) treatment did not significantly modify the meprobamate metabolism 48 hr later.

DISCUSSION

It is a well known fact that the drugs gradually become less effective when they were administered repeatedly over a long period. This phenomenon has often been observed in the experimental animal as well as in man. There are some reports on the developments of tolerance to the meprobamate.[4–6]

The animals which have gained resistance to a drug sometimes show resistance to other drugs also (cross tolerance). It is also a well known fact that many drugs which show tolerance and cross tolerance belong to the neurotropic class of drugs.

Recently Remmer observed that rats pretreated with phenobarbital become resistant to hexobarbital and at the same time an increased breakdown of hexobarbital by liver microsomes was observed.[7] On the other hand, the increased drug metabolism caused by pretreatment with drugs was first observed by Conney *et al.*[8, 9], and has recently been extensively studied by Conney *et al.*[10, 11]

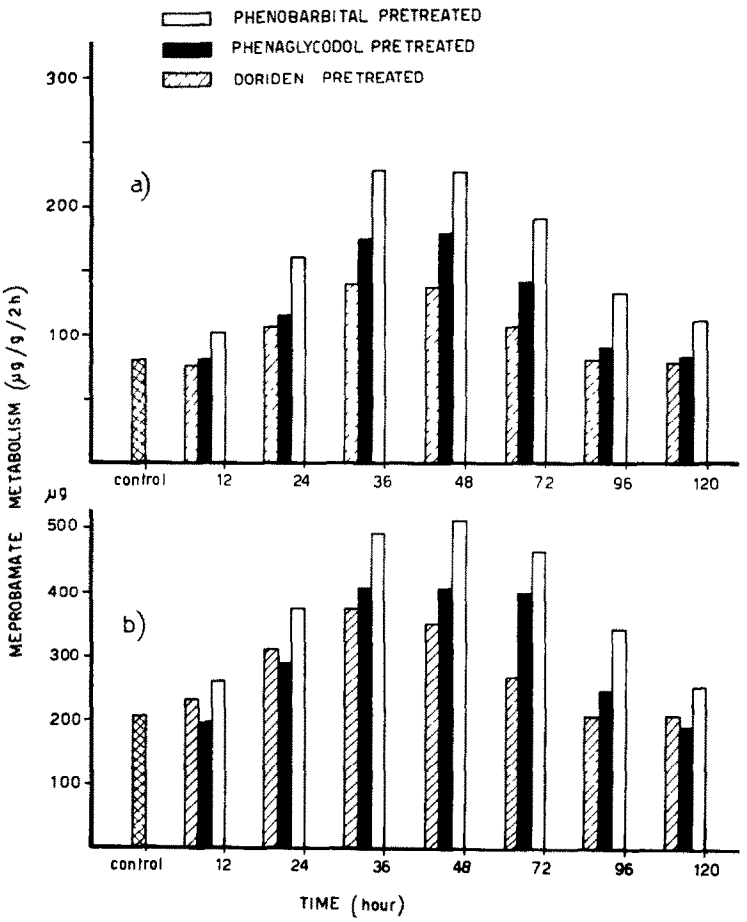

FIG. 8. Induction of meprobamate metabolizing enzyme after pretreatment with phenobarbital, phenaglycodol or glutethimide. 60 mg/kg of phenobarbital, 90 mg/kg of phenaglycodol or 60 mg/kg of glutethimide (Doriden) were injected intraperitoneally. The values given represent averages obtained at least six rats. Abscissa: Interval between the pretreatments and sacrifice. (a) Experiment with the microsomal preparation; (b) experiment with liver slices.

The observation reported here allows some interpretation on the tolerance and especially on the cross tolerance of neurotropic drugs, and these results are in accord with those previously obtained on the increased metabolism of pentobarbital.

The induction mechanism which causes the increases of drug metabolism is not yet clear, but evidence has been given that an increased microsomal enzyme activity due to *de novo* biosynthesis of enzymes in the liver is responsible for this increase.[7–15]

According to our results the capacity of the induction is related to neither the chemical structure, nor to the pharmacological effect of the drugs. It is probable that liposolubility and the type of the metabolism of the inducers are important factors; most of the inducers are liposoluble and themselves are metabolized by microsomal enzymes of liver which required TPNH and oxygen, and their metabolisms are inhibited by SKF 525 A, Lilly 18947, iproniazid and isoniazid.[16–18]

TABLE 6. METABOLISM OF MEPROBAMATE IN HYPOPHYSECTOMIZED OR ADRENALECTOMIZED RATS AFTER PRETREATMENT WITH PHENOBARBITAL

	Pretreatment	Enzyme activity (μg/g)
Hypophysectomized rat	—	148 ± 7·8 (4)
Hypophysectomized rat	+	398 ± 7·8 (4)
Adrenalectomized rat	—	129 ± 8·9 (4)
Adrenalectomized rat	+	343 ± 13·8 (4)

Female rats of Sprague–Dawley strain, weighing 140 g were used. The rats were hypophysectomized 10 days before of adrenalectomized 6 days before sacrifice. 60 mg/kg phenobarbital were injected intraperitoneally 36 hr before sacrifice. The enzyme activity was determined by the liver slices.
The number of animals is given in the brackets.

A further study on the mechanism, and especially on the role of metabolic factors in the development of tolerance, is necessary.

We have also observed phenomena similar to that of meprobamate metabolism, in the metabolisms of pentobarbital, carisoprodol and strychnine.[14, 15, 19] This observation is of practical importance for the evaluation of interaction of drugs in animals and also probably in clinical experiments.

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