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INCREASED ACTIVITY OF MICROSOMAL STRYCHNINE-METABOLIZING ENZYME INDUCED BY PHENOBARBITAL AND OTHER DRUGS

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Abstract—This report deals with induction of increased activity of microsomal strychnine-metabolizing enzyme and of decreased toxicity of strychnine by phenobarbital, phenaglycodol, glutethimide, thiopental, nikethamide, primidone, diphenylhydantoine, urethane, meprobamate and carisoprodol (inducing drugs). The increased activity of microsomal strychnine metabolizing enzyme and the decreased toxicity developed 12 hr after the injection of the inducing drugs, the maximal increase occurred after about 36–48 hr. The development of these two effects was inhibited by ethionine injected shortly before the inducers.

In the case of intravenous injection of strychnine in rats pretreated 48 hr before with the inducing drugs, the decreased toxicity was not observed. SKF 525 A injected 30 min before strychnine also overshadowed the decreased toxicity in the pretreated rats. The increased activity of strychnine-metabolizing enzyme accounted for the decreased strychnine toxicity. A decreased picrotoxin toxicity similar to that observed with strychnine was also demonstrated.

IN PREVIOUS papers, Kato reported the decrease of strychnine toxicity induced by the pretreatment (48 hr before) with phenaglycodol and thiopental.[1, 2] The administration of ethionine, 30 min before the injection of the inducing drugs, completely inhibited the induced decrease of strychnine toxicity, and an induction of strychnine-metabolizing enzyme by phenaglycodol or thiopental was postulated.[2]

According to Adamson and Fouts strychnine was metabolized in liver microsomes in the presence of TPNH and oxygen.[3] The microsomal strychnine-metabolizing enzyme was similar to the enzymes responsible for breakdown of hexobarbital, pentobarbital, meprobamate, carisoprodol, zoxazolamine and aminopyrine, which have been considered as inducible drug-metabolizing enzymes.[2–13]

In the present study, the induction of a decrease in strychnine toxicity by several drugs was demonstrated and an increase of hepatic microsomal drug-metabolizing enzyme accounted for the responsible factor in the decrease of strychnine toxicity.

MATERIALS AND METHODS

Female rats of the Sprague–Dawley strain, weighing about 180 g and 80 g were used for the determination of the strychnine toxicity and of the strychnine metabolism, respectively.

Doses of the drugs used in the determination of strychnine toxicity are as follows: phenobarbital sodium (90 mg/kg), phenaglycodol (130 mg/kg), glutethimide (80 mg/kg), thiopental sodium (30 mg/kg), nikethamide (150 mg/kg), primidone (150

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mg/kg), diphenylhydantoine (120 mg/kg), urethane (500 mg/kg), meprobamate (200 mg/kg), carisoprodol (150 mg/kg) and pentobarbital sodium (25 mg/kg). For the determination of strychnine metabolism 60 mg/kg of phenobarbital sodium, 90 mg/kg of phenaglycodol, 60 mg/kg of glutethimide, 27 mg/kg of thiopental sodium, 100 mg/kg of nikethamide and 150 mg/kg of meprobamate were used.

Phenaglycodol, glutethimide, meprobamate and carisoprodol were suspended in a 1% carboxymethylcellulose solution and given intraperitoneally and the other drugs were given intraperitoneally after dissolving in distilled water. Generally the pretreatments with the inducing drugs were carried out 48 hr before the determination of strychnine toxicity and strychnine metabolism. Ethionine was dissolved in 0·9% sodium chloride solution and given intraperitoneally at doses of 250 mg/kg and 200 mg/kg in the determination of the toxicity and of the metabolisms respectively.

Strychnine toxicity was evaluated by the determination of 50 per cent convulsions doses (CD_{50}) and 50 per cent lethal doses (LD_{50}) according to the method of Litchfield and Wilcoxon. Strychnine sulphate was given intraperitoneally at a volume of 2 ml/kg after dissolving in distilled water. The intravenous injection was carried out in the left femoral venous.

The *in vitro* metabolism of strychnine was determined by measuring the metabolized strychnine in liver slices or in the microsomal-contaning supernatant after an incubation. Generally pooled livers obtained from two or three rats were used in the determination of the enzyme activity.

The rats were killed by decapitation and the livers immediately removed and sliced with a microtome or homogenized with a Potter–Elvehjem type homogenizer adding 3 vols. of 1·15% KCl. The liver slices (500 mg) were suspended in a Warburg flask which contained 6 ml of Krebs phosphate buffered Ringer (pH 8·2) and 0·2 ml of 532 μg of strychnine sulphate, final concentration 2×10^{-4} M, and incubated in an atmosphere of oxygen at 37 °C for 1 hr with shaking. 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 strychnine concentration.

In the experiments with the microsomal preparation, the nuclei and mitochondria were sedimented by centrifugation of the homogenate at $8{\cdot}500 \times g$ for 15 min and the microsomal preparation (5·0 ml) was made up adding the following to 3 ml of the microsomal-containing supernatant: 0·1 ml of 20 μmole glucose-6-phosphate, 0·4 mole TPN, 50 μmole nicotinamide, 75 μmole $MgCl_2$ and 1 M KCl, and more 1·3 ml of 0·1 M phosphate buffer (pH 8·2)and 0·2 ml of the substrate (final concentration 2×10^{-4} M). The microsomal preparation was incubated in 25 ml Erlenmeyer flasks which were shaken for 1 hr in the presence of air at 37 °C, and at the end of the incubation 2 ml of the reaction mixture were used for the determination.

The remaining strychnine concentration was determined by the ultra-violet absorption in a spectrophotometer as previously described.[15]

RESULTS

Effect of the pretreatment with various drugs on the strychnine toxicity

The strychnine toxicity was examined 48 hr after the pretreatment with various drugs. Phenobarbital, phenaglycodol, glutethimide, thiopental, nikethamide, primidone, diphenylhydantoine, urethane, meprobamate, carisoprodol and pentobarbital were found to be effective drugs as inducers (Table 1).

TABLE 1. EFFECT OF PRETREATMENT WITH VARIOUS DRUGS IN THE STRYCHNINE TOXICITY

Pretreatment	No. of animals	No. of rats fallen in convulsion	No. of rats died	Mortality (%)
1. Controls	45	36	34	76
2. Phenobarbital	24	1	0	0
3. Phenaglycodol	23	3	2	9
4. Glutethimide	24	4	2	8
5. Thiopental	16	5	3	18
6. Nikethamide	24	8	7	19
7. Primidone	16	6	4	25
8. Diphenylhydantoine	16	7	5	31
9. Urethane	16	9	7	44
10. Meprobamate	16	9	8	50
11. Carisoprodol	16	11	9	55
12. Pentobarbital	16	11	10	62

Rats were pretreated with various drugs 48 hr before injection of strychnine (1·8 mg/kg i.p.).

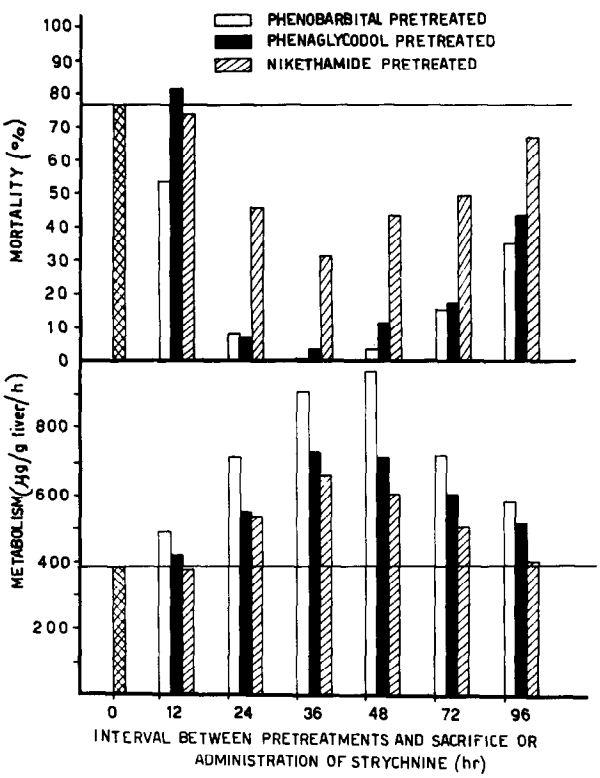

FIG. 1. Effect of pretreatments with phenobarbital, phenaglycodol or nikethamide on mortality by strychnine and on the strychnine metabolism. Rats were pretreated with phenobarbital, phenaglycodol or nikethamide at different time intervals before the injection of strychnine (1·8 mg/kg) or the determination of strychnine metabolism.

Phenobarbital, phenaglycodol and glutethimide were more potent inducers, especially after phenobarbital pretreatment the mortality due to 1·8 mg/kg of strychnine (i.p.) was 0 per cent.

As reported in a previous publication the decrease of strychnine toxicity was not due to a direct effect of the drugs. The inducing drugs were not limited only to the depressant drugs, but nikethamide could also decrease strychnine toxicity. The results were in accord with a previous work on the sleeping-time of pentobarbital after the pretreatment with various drugs.[12]

Effect of pretreatment with phenobarbital, phenaglycodol or nikethamide on mortality by strychnine and on strychnine metabolism

Variations of mortality by strychnine and the strychnine metabolism at different time intervals after the pretreatments with phenobarbital, phenaglycodol and nikethamide were summarized in Fig. 1.

A decrease of the mortality and an increase of the metabolism markedly occurred 24 hr after the pretreatments but 12 hr after the pretreatments marked changes could not be observed.

After 48 hr the LD_{50} (50 per cent lethal doses) was increased about 2·4, 2·0 and 1·5 times and the metabolisms were increased about 2·4, 1·9 and 1·4 times in rats pretreated with phenobarbital, phenaglycodol and nikethamide respectively (Table 2). Fig. 2 demonstrated the remarkable metabolic difference also in an early stage of the incubation.

TABLE 2. EFFECT OF ETHIONINE ON THE MODIFICATIONS OF STRYCHNINE TOXICITY AND METABOLISM INDUCED BY THE PRETREATMENTS WITH SOME DRUGS

Treatments	No. of rats	Toxicity		Metabolism (μg/g per hr)	
		CD_{50} (mg/kg)	LD_{50} (mg/kg)	In slices	In microsomal preparation
Controls	143	1·50 (1·36–1·65)	1·62 (1·48–1·78)	384 ± 10 (14)	248 ± 7 (12)
Phenobarbital	61	3·69 (3·32–4·12)	3·89 (3·50–4·34)	928 ± 25 (16)	593 ± 20 (12)
Phenaglycodol	70	3·12 (2·84–3·45)	3·27 (2·95–3·62)	728 ± 28 (8)	435 ± 23 (12)
Glutethidmide	48	2·95 (2·60–3·31)	3·13 (2·80–3·50)	640 ± 19 (6)	383 ± 14 (6)
Thiopental	52	2·85 (2·50–3·25)	2·98 (2·69–3·30)	598 ± 23 (6)	—
Nikethamide	48	2·45 (2·15–2·79)	2·58 (2·29–2·93)	525 ± 16 (6)	315 ± 11 (4)
Meprobamate	47	2·15 (1·94–2·39)	2·25 (2·04–2·49)	492 ± 18 (6)	—
Ethionine	52	1·54 (1·35–1·76)	1·63 (1·38–1·90)	366 ± 17 (8)	233 ± 14 (8)
Phenobarbital + ethionine	44	1·59 (1·40–1·80)	1·79 (1·53–2·09)	413 ± 21 (8)	271 ± 17 (8)
Phenaglycodol + ethionine	44	1·59 (1·38–1·82)	1·72 (1·43–2·06)	370 ± 25 (8)	245 ± 19 (6)
Glutethimide + ethionine	40	1·63 (1·36–1·96)	1·73 (1·37–2·17)	385 ± 19 (6)	250 ± 12 (4)
Nikethamide + ethionine	36	1·48 (1·28–1·72)	1·65 (1·36–2·00)	378 ± 15 (4)	263 ± 13 (4)

Ethionine was given 30 min before the injection of the inducers.
In the brackets after CD_{50} and LD_{50} were given 95 per cent fiducial limits.
In the brackets after standard error were given number of the determination.

Effect of ethionine on the induced modifications of strychnine toxicity and metabolism

Strychnine metabolism in the slices and in the microsomal preparations increased after the pretreatment with the drugs which can increase CD_{50} (50 per cent convulsion dose) and LD_{50} (50 per cent lethal dose) of strychnine (Table 2).

It is observable that there are strict relationships between the increase of metabolisms and the increase of CD_{50} and LD_{50} induced by the pretreatment with the different drugs. All drugs, which failed to increase the LD_{50} of strychnine, failed to increase its metabolisms.

Ethionine, a potent inhibitor of protein biosynthesis and of induction of some enzymes, can antagonize the effect of phenobarbital, phenaglycodol and nikethamide on metabolisms and toxicity of strychnine.[2, 8–11, 13, 16–19]

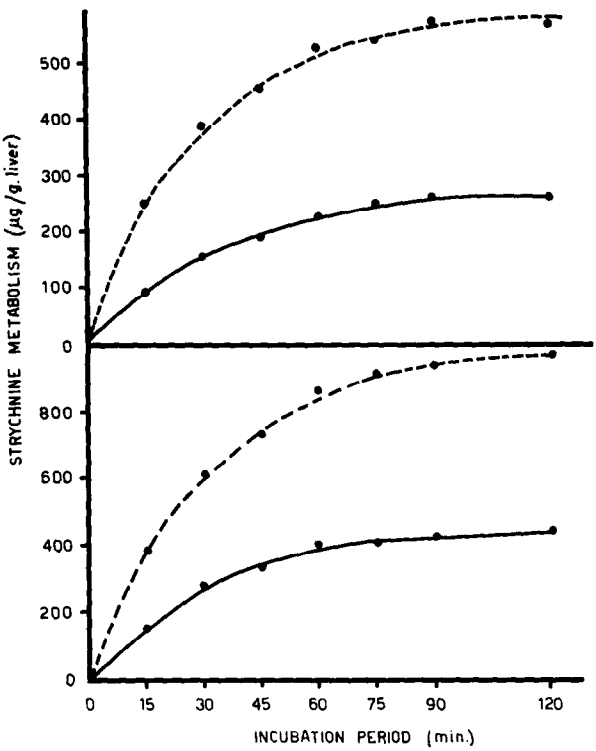

FIG. 2. Metabolism of strychnine in the different incubation periods. (a) Strychnine metabolism in the liver microsomal preparation (upper figure). (b) Strychnine metabolism in the liver slices (lower figure). Phenobarbital (60 mg/kg) was given intraperitoneally 48 hr before the experiments. ———— controls; - - - - - pretreated rats

Effect of SKF 525 A on the modifications of strychnine toxicity and metabolism induced by phenobarbital or phenaglycodol*

SKF 525 A (β-dimethylaminoethyldiphenylpropylacetate) is a potent inhibitor of the metabolism of some drugs and an inhibitory action of SKF 525 A on the strychnine metabolism was also observed.[1, 3, 15, 20, 21] In this work a possible annulment of the decreased strychnine toxicity induced by pretreatment with phenobarbital or phenaglycodol was investigated.

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

As shown in Table 3, pretreatments with SKF 525 A overshadowed the effect of phenobarbital or phenaglycodol, in fact LD_{50} of normal rat increased from 1·62 mg/kg to 3·27 mg/kg or 3·89 mg/kg in phenobarbital or phenaglycodol pretreated rats, but after the injection of SKF 525 A the LD_{50} were 1·33 mg/kg, 1·45 mg/kg, 1·43 mg/kg, respectively, in control, phenobarbital or phenaglycodol pretreated rats.

In agreement with the modification of toxicity, similar results could be observed in the strychnine metabolisms.

TABLE 3. EFFECT OF SKF 525 A ON THE MODIFICATION OF STRYCHNINE TOXICITY AND METABOLISM INDUCED BY PHENOBARBITAL OR PHENAGLYCODOL

Treatments	No. of animals	Strychnine toxicity		Strychnine metabolism (μg/g per hr)	
		CD_{50} (mg/kg)	LD_{50} (mg/kg)	In slices	In microsomal preparation
1. Controls	143	1·50 (1·36–1·65)	1.62 (1·48–1·78)	385 ± 12 (12)	238 ± 18 (10)
2. Phenobarbital	61	3·69 (3·32–4·12)	3·89 (3·50–4·34)	949 ± 30 (8)	582 ± 45 (8)
3. Phenaglycodol	70	3·12 (2·84–3·45)	3·27 (2·95–3·62)	736 ± 28 (8)	409 ± 31 (8)
4. SKF 525 A	48	1·30 (1·21–1·40)	1·33 (1·25–1·42)	42 ± 6 (6)	24 ± 8 (6)
5. Phenobarbital + SKF 525 A	28	1·36 (1·25–1·49)	1·45 (1·35–1·58)	53 ± 8 (4)	30 ± 10 (4)
6. Phenaglycodol + SKF 525 A	32	1·35 (1·28–1·45)	1·43 (1·34–1·54)	58 ± 9 (3)	24 ± 8 (4)

SKF 525 A (50 mg/kg) was given 30 min before the injection of strychnine.
In the strychnine metabolism the concentration of SKF 525 A was 2×10^{-4}.

Strychnine toxicity after intravenous injection in rats pretreated with phenobarbital, phenaglycodol or SKF 525 A

Strychnine convulsion occurs in rats 5–8 sec after the intravenous injection of the drug. Therefore if the decreasing effect of phenobarbital, or phenaglycodol and the increasing effect of SKF 525 A on strychnine toxicity after intraperitoneal injection were due only to a modification of metabolic rate of the drugs, the modification of the strychnine toxicity should not appear after the intravenous injection.

Table 4 shows that there is no modification of strychnine toxicity after intravenous injections, and the LD_{50} of controls and the treated animals is about 0·56–0·63 mg/kg.

TABLE 4. STRYCHNINE TOXICITY AFTER INTRAVENOUS INJECTION IN RATS PRETREATED BY PHENOBARBITAL, PHENAGLYCODOL AND SKF 525 A

Treatments	No. of rats	CD_{50} (mg/kg)	LD_{50} (mg/kg)
1. Controls	58	0·50 (0·45–0·56)	0·56 (0·51–0·64)
2. Phenobarbital	42	0·54 (1·57–0·63)	0·60 (0·44–0·68)
3. Phenaglycodol	38	0·50 (0·44–0·59)	0·57 (0·50–0·65)
4. SKF 525 A	32	0·54 (0·47–0·62)	0·60 (0·52–0·70)
5. Phenobarbital + SKF 525 A	27	0·58 (0·49–0·68)	0·63 (0·53–0·74)
6. Phenaglycodol + SKF 525 A	26	0·55 (0·48–0·65)	0·63 (0·55–0·72)

Phenobarbital and phenaglycodol were given 48 hr before and SKF 525 A given 30 min before the injection of the strychnine.

Effect of phenobarbital on strychnine metabolism in hypophysectomized or adrenalectomized rats

Studies were carried out to determine whether the induction of strychnine-metabolizing enzyme by phenobarbital or phenaglycodol was mediated through endocrine glands.

The results in Table 5 show that removal of the pituitary gland or adrenal glands did not prevent the induction of strychnine-metabolizing enzyme by phenobarbital or phenaglycodol. These results are similar to those of a previous study and to the results of Conney *et al.*[6, 12]

TABLE 5. EFFECT OF PHENOBARBITAL AND PHENAGLYCODOL ON STRYCHNINE METABOLISM IN HYPOPHYSECTOMIZED RATS OR ADRENALECTOMIZED RATS

Treatments	No. of rats	Strychnine metabolism (μg/g per hr)	*P*
Hypex	4	268 ± 23	
Hypex + phenobarbital	3	703 ± 59	<0·001
Hypex + phenaglycodol	3	429 ± 35	<0·01
Adrex	4	238 ± 27	
Adrex + phenobarbital	4	611 ± 46	<0·001
Adrex + phenaglycodol	4	436 ± 38	<0·001

Female rats, weighing about 120 g were used.
Hypophysectomy and adrenalectomy were carried out 10 days and 6 days respectively before the determination of the strychnine metabolism in the liver slices.

Effect of phenobarbital and phenaglycodol on toxicity and metabolism of strychnine in adult female and male rats

In previous experiments, we observed a marked sex difference in the toxicity and metabolism of strychnine.[13, 15] The effect of phenobarbital and phenaglycodol on toxicity and metabolism of strychnine in adult male rats was therefore examined in comparison with female rats.

Male rats have high metabolic activity in strychnine metabolisms and high LD_{50}, and the effects of phenobarbital and phenaglycodol were also observed, but the increase of the metabolism and LD_{50} were less marked than in female rats (Table 6).

TABLE 6. EFFECT OF PHENOBARBITAL AND PHENAGLYCODOL ON TOXICITY AND METABOLISM OF STRYCHNINE IN ADULT FEMALE AND MALE RATS

Sex	Treatment	No. of rats	Toxicity LD_{50} (mg/kg)	Metabolism (μg/g per hr)
Female	—	72	1·63 (1·46–1·83)	198 ± 12 (6)
Female	Phenobarbital	48	3·78 (3·39–4·19)	431 ± 29 (6)
Female	Phenaglycodol	42	3·22 (2·86–3·61)	362 ± 27 (6)
Male	—	61	2·96 (2·64–3·33)	402 ± 18 (6)
Male	Phenobarbital	38	4.02 (3·61–4·49)	502 ± 32 (6)
Male	Phenaglycodol	35	3·62 (3·24–4·05)	463 ± 38 (6)

Female and male rats weighing about 180 g and 250 g were used. The metabolism was determined by the use of liver slices.

Effect of phenobarbital, phenaglycodol, glutethimide, SKF 525 A and ethionine on the toxicity of picrotoxin

No modification of the pentamethylentetrazol toxicity was observed by pretreatment (48 hr before) with phenobarbital or phenaglycodol, but a marked modification of the picrotoxin toxicity was observed after the pretreatment with phenobarbital, phenaglycodol or glutethimide. Table 7 shows results similar to those obtained with strychnine as described above.

TABLE 7. EFFECT OF PHENOBARBITAL, PHENAGLYCODOL, GLUTETHIMIDE, SKF 525 A AND ETHIONINE ON THE TOXICITY OF PICROTOXIN

Pretreatment	Picrotoxin toxicity			
	No. of rats	Subcutaneous injection LD_{50} (mg/kg)	No. of rats	Intravenous injection LD_{50} (mg/kg)
1. Female controls	82	3·3 (3·1–3·6)	46	2·2 (2·0–2·5)
2. Phenobarbital	48	6·9 (5·9–8·1)	32	2·7 (2·2–3·4)
3. Phenaglycodol	47	5·6 (4·9–6·5)	—	—
4. Glutethimide	42	5·1 (4·7–5·7)	—	—
5. SKF 525 A	24	2·0 (1·8–2·3)	24	1·8 (1·5–2·3)
6. Phenobarbital + SKF 525 A	36	2·4 (2·1–2·8)	32	2·0 (18–2·3)
7. Ethionine	24	3·2 (2·7–3·8)	24	2·2 (1·9–2·6)
8. Ethionine + Phenobarbital	36	3·8 (3·2–4·6)	32	2·5 (2·2–2·9)
9. Male controls	48	5·5 (4·8–6·4)	36	2·6 (2·3–3·0)
10. Phenobarbital	39	7·9 (6·5–9·6)	24	2·8 (2·3–3·4)
11. SKF 525 A	24	2·3 (1·9–2·9)	24	1·9 (1·6–2·3)

Female and male rats, weighing about 200 g and 300 g were used.
Phenobarbital, phenaglycodol and glutethimide were given 48 hr before and SKF 525 A was given 30 min before the injection of strychnine. Ethionine was given 30 min before the administration of phenobarbital.

Since convulsion and death after the intravenous injection of picrotoxin occur about 12 min and 40–50 min, respectively, there are some differences in LD_{50} among the different pretreated rats.

A sex difference as in the case of strychnine, was also observed in picrotoxin toxicity. These results suggest a presence of a similar stimulation of picrotoxin metabolism in the rats pretreated with phenobarbital, phenaglycodol and glutethimide and of a sex difference in picrotoxin metabolism like in strychnine metabolism.

DISCUSSION

In recent years, there has been much evidence to show that many drugs can induce an increase of metabolism of many pharmacologically interesting drugs. Phenobarbital, phenaglycodol, glutethimide, phenylbutazone, orphenadrine, tolbutamide, niketamide, urethane, chloretone, primidone, meprobamate, chlorcyclizine, chlorpromazine were reported as the inducing drugs, and on the other hand, pentobarbital, hexobarbital, phenylbutazone, aminopyrine, meprobamate, carisoprodol, zoxazolamine and OMPA (octamethylpyrophosphoramide) were reported as the induced drugs.[1, 2, 4–13, 22, 23]

In the work reported here, the induction of increase in strychnine metabolism was confirmed as a responsible factor for the increased tolerance in the rats pretreated with phenaglycodol or thiopental, as reported in the previous work. After the pretreatments with phenobarbital, glutethimide, nikethamide primidone, diphenylhydantoin, urethane or meprobamate an increased strychnine metabolism and an increased tolerance were observed; phenobarbital was found to be the most active compound. These results are well in accord with the results obtained in studies on pentobarbital and meprobamate metabolisms and on their hypnotic effect as previously reported.

It might seem impossible that only the metabolic difference produce such a remarkable difference in the strychnine toxicity, since strychnine convulsion occurs 7–8 min after the intraperitoneal injection, and it might seem more probable that an increased resistance of the central nervous system to strychnine by the pretreatment with phenobarbital or phenaglycodol is a more important factor. But the increased tolerance could not be observed in the cases of intravenous injection, administration of SKF 525 A and jointly pretreatment with ethionine. Therefore, if there were some modifications of resistance of the central nervous system to strychnine they would have only negligible influence on the toxicity.

These results therefore give evidence that the metabolic factor is the most important, at least in rats, in the determination of strychnine toxicity. The pharmacological experiments obtained with picrotoxin were similar to those obtained with strychnine. These results suggest that the metabolism of picrotoxin might take place in microsomes requiring TPNH and oxygen and its metabolism is stimulated by pretreatment with phenobarbital, phenaglycodol or glutethimide.

The mechanism of the induction of strychnine metabolizing enzyme is not yet known, but it seems to be a similar mechanism to that of the induction of pentobarbital or meprobamate metabolizing enzymes.

Recently we observed that many inducing drugs, for example, chlorpromazine, chlorcyclizine, pentobarbital, phenobarbital, phenaglycodol, chloretone, glutethimide and nikethamide can inhibit the microsomal drug metabolizing enzymes, and on the other hand, inhibitors of microsomal drug-metabolizing enzymes, for example SKF 535 A, Lilly 18947, Lilly 32391 and MG 3062, can induce an increase of the microsomal drug-metabolizing enzymes activity.[24–27]

It is of interest to note that all the induced drugs and most of the inducers were metabolized in the microsomes in the presence of TPNH and oxygen and some of the inducing drugs also have inhibitory action on the microsomal drug-metabolizing enzymes.[27]

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