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THE EFFECT OF CERTAIN INHIBTORS IN PRODUCING SHORTENING OF HEXOBARBITAL ACTION*

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Abstract—Agents that initially prolong the duration of hexobarbital action (phase A) by decreasing its rate of biotransformation seem to have a subsequent shortening effect (phase B) on hexobarbital action. By inferences based on studies using ethionine and methionine, the phase B effects of SKF 525-A, EPB, nikethamide, and iproniazid appeai to involve adaptive increases in activity of the enzyme system of the liver that metabolizes hexobarbital.

PIH was one agent tested that did not produce the diphasic effect. It produced the phase A effect, but under the present conditions the phase B effect was not present. The phase B effect of PIH may be masked by a central component; however, no direct time relationship between the central anticonvulsant activity and effect on hexobarbital action was found.

IN A previous paper Serrone and Fujimoto[1] have reported on the diphasic effect which N-methyl-3-piperidyl-N′,N′-diphenyl carbamate hydrochloride (MPDC) has in mice on the duration of action of hexobarbital. If the interval between administration of the compound and the hexobarbital were short (less than 12 hr), prolongation of narcosis occurred, owing to inhibition of metabolism. If the interval were long (24–72 hr), shortening of narcosis and stimulation of metabolism of hexobarbital occurred. The question arises as to whether this diphasic effect is a phenomenon of more general occurrence. That is, would any compound that inhibits hexobarbital metabolism also show a subsequent phase of stimulation of hexobarbital metabolism? The purpose of this study was to investigate this question.

METHODS

The drugs taken for study were N-ethyl-3-piperidylbenzilate hydrochloride (EPB, JB 318); β-diethylaminoethyl diphenylpropylacetate hydrochloride (SKF 525-A); iproniazid phosphate; and β-phenylisopropyl hydrazine hydrochloride (PIH, JB 516). These drugs are known to inhibit hexobarbital metabolism.[2] An additional drug, N,N-diethylnicotinamide (nikethamide) was included.

The possible effects of these agents in altering the duration of action of hexobarbital were studied in male Swiss albino mice (19 to 50 g). The mice were maintained *ad libitum* on Purina laboratory chow pellets and water throughout the experiments. Drugs were made up in distilled water in concentrations such that a volume of 0·1

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ml of solution was given per 10 g body weight. Groups of mice were pretreated at various times with a single dose of the drug, after which hexobarbital sleeping times were determined on them. For each drug the pretreatment time, dose, route, and number of mice per group are indicated in Results. The sleeping time to hexobarbital Na, 150 mg/kg, i.p., was measured as previously described.[3] Significance of the difference between the mean sleeping times of the experimental group(s) versus the control group was assessed by the *t*-test, and $P = 0{\cdot}05$ or less was taken as the level of significance. The control group received no pretreatment, since significant differences in sleeping time do not occur between such a control group and groups pretreated with distilled water at various times.[1] Since all sleeping times for a given experiment were performed simultaneously, the single untreated control group was used rather than the water-pretreated groups. Running parallel groups of the latter along with drug-pretreated groups would have been difficult technically owing to the large numbers of mice involved. In later experiments DL-ethionine (200 mg/kg) was administered orally in combination with the drugs. Also, a combination of ethionine, the drug, and DL-methionine (200 mg/kg) was used. The effects of these combinations on hexobarbital sleeping time were measured.

RESULTS

Fig. 1 (nikethamide) shows a typical curve of the diphasic effects produced by these agents on hexobarbital sleeping time in mice. The results for SKF 525-A, EPB, and iproniazid are given in Table 1. If the pretreatment interval between the administra-

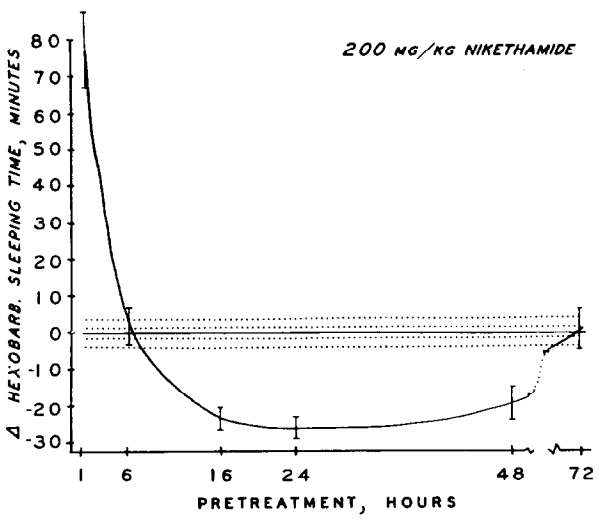

FIG. 1. Relationship between hexobarbital sleeping time in mice and the period of pretreatment with nikethamide. The abscissa gives the interval in hours between the administration of the agent and the hexobarbital sodium, 150 mg/kg, i.p. The ordinate gives the change in mean hexobarbital sleeping time compared with the control. Each value represents 8–12 mice, and the vertical line represents the standard error. The control sleeping time is indicated by the horizontal line at zero; the actual control sleeping time was 63·8 min. The dotted area is the standard error for each control group.

tion of the agents and the hexobarbital was short, prolongation of sleeping time occurred; this phase hereafter will be referred to as phase A. If the interval was long, a shortening of sleeping time was found; this will be designated as phase B.

The phase A effects may be attributed to inhibition of hexobarbital metabolism. Fouts and Brodie[4, 5] have shown that SKF 525-A and iproniazid inhibit hexobarbital metabolism. Fujimoto *et al.*[3] and Serrone and Fujimoto[2] found that EPB also inhibits hexobarbital metabolism.

The finding of prolongation of sleeping time by nikethamide was surprising, since nikethamide is generally believed to be an analeptic agent. Because of the strong phase

TABLE 1. THE EFFECT OF SKF 525-A, EPB AND IPRONIAZID ON HEXOBARBITAL SLEEPING TIME (S.T.) IN MICE

No. of mice	Group (Dose, mg/kg)	Pretreatment time (hr)	S.T. (min)	s.e.	P*
10	Control		78·1	4·1	
10	SKF 525-A (20, p.o.)	1	190·7	10·5	0·001
10		6	175·2	8·3	0·001
10		16	58·2	3·9	0·01
10		24	55·5	5·0	0·01
10		48	51·8	4·3	0·001
10		72	68·2	4·9	0·1
10	Control		63·2	3·2	
9	EPB (20, p.o.)	1	83·2	6·3	0·01
10		6	70·7	6·9	0·9
10		16	63·6	12·6	0·9
9		24	44·5	3·3	0·001
9		48	50·5	3·8	0·02
10		72	55·8	4·8	0·3
10	Control		53·6	3·3	
5	EPB (50, p.o.)	1	157·0	13·0	0·001
6		6	121·5	7·1	0·001
7		24	56·8	7·3	0·7
11		48	48·4	3·7	0·3
6		72	46·3	6·2	0·2
10	Control		42·8	3·3	
10	EPB (50, p.o.)	96	36·3	4·7	0·2
10		120	29·3	6·8	0·05
10		144	33·6	4·7	0·1
10	Control		62·4	3·8	
10	Iproniazid (200, s.c.)	6	129·0	9·5	0·001
10		16	63·5	6·5	0·9
10		24	50·2	3·3	0·05
10		48	48·2	3·2	0·01
10		72	49·0	3·5	0·02

Hexobarbital, Na = 150 mg/kg, i.p.

* Experimental group vs. control group.

A effect that was obtained, we anticipated that nikethamide was inhibiting hexobarbital metabolism. *In vitro*-experiments on rat liver homogenates were performed; 50 per cent inhibition of hexobarbital metabolism was obtained at concentrations of $9{\cdot}8 \times 10^{-4}$ nikethamide. This concentration is about 20 times greater than that for SKF 525-A added *in vitro*.[2] A similar order of magnitude was found *in vivo* between doses of these two agents in producing phase A effects. Therefore a major factor in-

volved in prolongation of hexobarbital action by nikethamide appears to be inhibition of hexobarbital metabolism.

With regard to phase B effects, one way in which hexobarbital sleeping time may be shortened is by increasing the rate of metabolism of hexobarbital through adaptive increases in microsomal enzyme activity.[6] DL-Ethionine blocks this adaptive process and DL-methionine reverses the action of ethionine. Results indicating the probable nature of the phase B effects of SKF 525-A, EPB, nikethamide, and iproniazid are given in Table 2. The shortening of sleeping time produced by the 24-hr pretreatment

TABLE 2. EFFECT OF ETHIONINE AND METHIONINE ON THE SHORTENING OF HEXOBARBITAL SLEEPING TIME IN MICE PRODUCED BY SKF 525-A, EPB, NIKETHAMIDE AND IPRONIAZID*

No. of mice	Group (dose, mg/kg)	Pretreatment time (hr)	S.T. (min)	s.e.	*P*
9	Control		95·1	4·0	
10	SKF 525-A (20, p.o.)	24	52·8	3·8	0·001
10	SKF 525-A Ethionine (200, p.o.)	24	87·1	4·9	0·2
10	SKF 525-A Ethionine Methionine (200, p.o.)	24	74·0	2·6	0·001
10	SKF 525-A	48	45·1	3·2	0·001
10	SKF 525-A Ethionine	48 24	60·2	2·8	0·001
10	Control		96·7	6·5	
11	EPB (20, p.o.)	24	76·9	4·2	0·02
11	EPB Ethionine (200 ,p.o.)	24	96·7	5·9	0·9
9	EPB Ethionine Methionine (200, p.o.)	24	63·9	4·4	0·01
10	EPB	48	76·3	6·1	0·05
9	EPB Ethionine	48 24	75·3	5·0	0·02
8	Control		67·5	8·9	
6	Nikethamide (200, p.o.)	24	45·3	2·4	0·001
8	Nikethamide Ethionine (200, p.o.)	24	63·1	3·6	0·3
8	Nikethamide Ethionine Methionine (200, p.o.)	24	46·4	2·6	0·001
6	Nikethamide	48	47·1	2·9	0·001
9	Nikethamide Ethionine	48 24	54·1	2·9	0·01
9	Control		69·7	6·2	
10	Iproniazid (200, s.c.)	24	40·6	3·0	0·001
10	Iproniazid Ethionine (200, p.o.)	24	73·3	3·8	0·7
11	Control		82·7	8·3	
9	Iproniazid (200, s.c.) Ethionine (200, p.o.) Methionine (200, p.o.)	24	47·1	2·3	0·01
10	Control		56·3	5·8	
10	Iproniazid (200, s.c.)	48	36·2	2·9	0·01
10	Iproniazid Ethionine (200, p.o.)	48 24	40·6	3·3	0·05

* See notes to Table 1.

with the agents (the phase B effect expected) was blocked by giving ethionine simultaneously with the agents. Ethionine itself at the dose level of 200 mg/kg had no effect on control hexobarbital sleeping time.[6] On the other hand, if the agents were given 48 hr instead of 24 hr before administration of the hexobarbital, and the time of ethionine administration remained the same (24 hr), the ethionine was no longer effective, presumably because the adaptive process had already taken place. That is, agent (at 48 hr) plus ethionine (24 hr) produced a shortening of hexobarbital sleeping time. Also, if methionine, ethionine, and the agent were given together at 24 hr, the blocking effect of ethionine was antagonized by the methionine, and the shortening of sleeping time resulted. Thus, these data as a whole were interpreted as indirectly indicating that the phase B effects of these agents were due to adaptive increases in activity of the enzyme system that metabolizes hexobarbital.

The results with PIH deserve special mention (Fig. 2, Table 3). As expected from earlier work,[2, 3, 7] PIH gave what corresponded to the phase A effects; that is, prolongation of sleeping time owing to inhibition of metabolism. Effects corresponding to

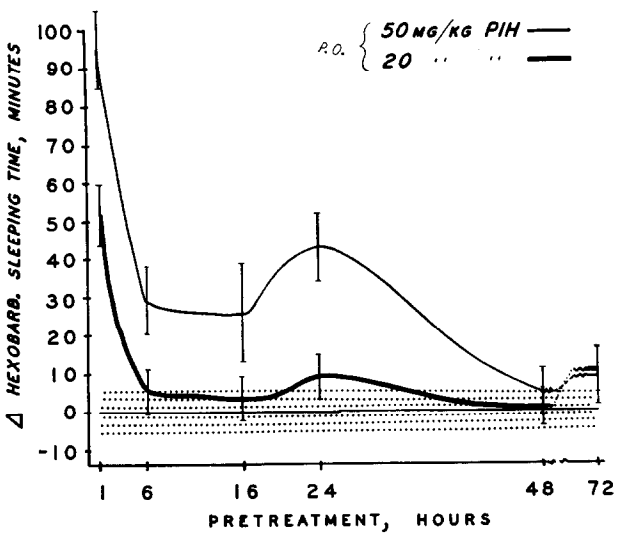

FIG. 2. Relationship between hexobarbital sleeping time in mice and period of pretreatment with PIH administered orally. Each value represents 9–12 mice. Legend as in Fig. 1. Control sleeping time was 61·3 min.

phase B were not obtained. Instead of shortening the sleeping time, another phase of prolongation was observed. Since Horita[8] had shown that the magnitude of the inhibitory effect of PIH on liver monoamine oxidase was dependent on the routes of administration, both the oral and subcutaneous routes were tried. The magnitude of the phase A effects was greater with the subcutaneous route of administration, but the second phase of prolongation was still present. The data in Table 4 on the anticonvulsant action of PIH indicated that there was little correspondence in time between the central anticonvulsant action of PIH and the prolongation of hexobarbital sleeping times. The presence of the second phase of prolongation precluded demonstration of shortening of sleeping time (corresponding to phase B).

DISCUSSION

It has been shown in the present study that SKF 525-A, EPB, nikethamide, and iproniazid, compounds that inhibit the metabolism of hexobarbital and thereby prolong hexobarbital action, cause a subsequent shortening of hexobarbital action. While this study was in progress[9, 10] others have reported similar findings. Rümke and Bout[11] reported the shortening of hexobarbital sleeping time in mice by SKF

TABLE 3. EFFECT OF SUBCUTANEOUSLY ADMINISTERED PIH ON HEXOBARBITAL SLEEPING TIME IN MICE*

No. of mice	Group (dose, mg/kg)	Pretreatment time (hr)	S.T. (min)	s.e.	P
9	Control		50·5	3·1	
11		1	160·5	9·3	0·001
11		6	123·9	10·8	0·001
10	PIH (50, s.c.)	16	112·5	9·5	0·001
11		24	96·0	7·8	0·001
9		48	58·0	3·1	0·1
9		72	53·7	6·1	0·6
9	Control		50·3	3·0	
10		1	92·8	3·1	0·001
10		6	61·7	2·6	0·02
9	PIH (20, s.c.)	16	65·0	2·6	0·01
9		24	67·2	4·5	0·01
9		48	66·3	3·6	0·01
9		72	53·9	5·4	0·50

* See note to Table 1.

TABLE 4. RELATIONSHIP BETWEEN THE ANTICONVULSANT ACTIVITY IN MICE AND PERIOD OF PRETREATMENT WITH PIH

No. of mice	Group (dose, mg/kg)	Pretreatment time (hr)	Extensor phase (sec)	s.e.	P
12	Control		13·2	0·8	
12		1	13·3	0·8	0·9
11		6	4·7	1·5	0·001
11	PIH (50, p.o.)	16	8·3	1·6	0·05
10		24	11·7	1·5	0·3
9		48	13·0	0·2	0·8

* See note to Table 1.

525-A and iproniazid, but no explanation was given for the observed effects. Holtz *et al.*[12] reported the shortening effect of iproniazid. Their explanation was that iproniazid produced changes in the amine levels in brain, and this change was responsible for the shortening of hexobarbital action. Our results with ethionine and methionine indicate that the effect of iproniazid during phase B was on the liver. Unfortunately, neither Holtz's nor our experiments were mutually exclusive, so that both occurrences are possible.

Because Brazda and Baucum[13] reported that nikethamide stimulates pentobarbital metabolism in rats, our findings regarding the phase B effect of nikethamide on hexobarbital action were as expected. That is, nikethamide produced an adaptive increase

in hexobarbital metabolism. However, our discovery that nikethamide inhibits hexobarbital metabolism adds to the general proposition of this paper. Incidentally, Brazda and Baucum found prolongation of pentobarbital sleeping time in rats given nikethamide which corresponds to our phase A effect, but they did not postulate a mechanism for this particular effect.

Although the compounds we have discussed fit the general proposition, the fact that PIH under the present conditions did not have a phase B effect leads to the following conclusion. A high probability exists that inhibitors of hexobarbital metabolism will produce a subsequent increase in hexobarbital metabolism, but there are exceptions. Of course, the converse statement is not true, that compounds producing adaptive increases in hexobarbital metabolism are initially inhibitors. Conney *et al.*,[14] Kato,[15, 16] and Rümke *et al.*[11] have reported on many agents that evidently are not inhibitors of hexobarbital metabolism but that do produce increases in hexobarbital metabolism.

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