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RELATIONS BETWEEN BARBITURATE BRAIN LEVELS AND SLEEPING TIME IN VARIOUS EXPERIMENTAL CONDITIONS

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Abstract—A linear correlation has been obtained between sleeping time and brain pentobarbital concentration 90 min after treatment of rats with 30 mg/kg i.p. of pentobarbital. Pretreatment with various drugs abolished or modified this correlation. The effect of these drugs is analysed in an attempt to identify the mechanism responsible for the modification of the pharmacological response. SKF-525A, DDT and low doses of chlorpromazine seem to act on pentobarbital sleeping time only by modifying its metabolism. Amphetamine, on the contrary, reduces the pentobarbital sleeping time by a mechanism which is not of metabolic origin. With phenobarbital pretreatment there is a modification of pentobarbital sleeping time which is the resultant of two different mechanisms: increased metabolism and increased sensitivity. Chlorpromazine at high doses, as well as diazepam, affect pentobarbital sleeping time without affecting pentobarbital metabolism.

ENHANCEMENT of barbiturate narcosis has been currently used[1–4] as a method for screening central depressant drugs. In the last few years, however, it has been reported that drugs which are devoided of any apparent central activity may affect barbiturate sleeping time. In fact compounds, such as SKF-525A, which block metabolism of barbiturates[5, 6] or compounds, such as DDT, which increase metabolism of barbiturates[7, 8] are known to increase or decrease, respectively, barbiturate sleeping time.

The aim of this study is to analyse the effect of some drugs on pentobarbital narcosis. By correlating a biochemical measurement (pentobarbital brain level) with a pharmacological determination (duration of the sleep) in rats it seems possible to obtain useful information about the mechanisms involved in the interaction between drugs and pentobarbital.

MATERIALS AND METHODS

Female Sprague–Dawley rats (150 g $\pm$ 10) were injected intraperitoneally with sodium pentobarbital (30 mg/kg). For each animal the sleeping time and the pentobarbital concentration in the brain were determined 90 min after treatment with the barbiturate. Other groups of animals were sacrificed at the time of awakening and their brain pentobarbital determined.

Pentobarbital sleeping time was evaluated as the time elapsed between loss and regaining of the righting reflex. Pentobarbital brain concentration was determined by the method of Brodie *et al.*[9] with minor modifications.

The regression lines were obtained by plotting the sleeping time of each rat against its pentobarbital brain concentration and statistical analysis was conducted by the method of Moore and Edwards.[10]

The following drugs were administered: sodium pentobarbital 30 mg/kg i.p.; sodium phenobarbital 10 mg/kg i.p. (Abbott Lab., Chicago); DDT (Schuchard, München) 1 mg/kg i.p. × 2 days; SKF-525A (beta-diethylaminoethyl-diphenyl-*n*-propyl acetate) (Smith, Kline & French Lab., Philadelphia) 15 mg/kg per os; *d*-Amphetamine sulphate (Recordati, Milan) 0·5 mg/kg or 2·5 mg/kg i.p.; Chlorpromazine (Farmitalia, Milan) 2·5 mg/kg or 4 mg/kg i.p.; Diazepam (Ravizza, Milan) 25 mg/kg per os.

RESULTS

In Fig. 1 are reported the regression lines obtained by plotting the sleeping time of rats treated with pentobarbital against the corresponding pentobarbital level found in the brain 90 min after barbiturate administration. Each regression line is obtained from groups of rats belonging to different groups of experiments.

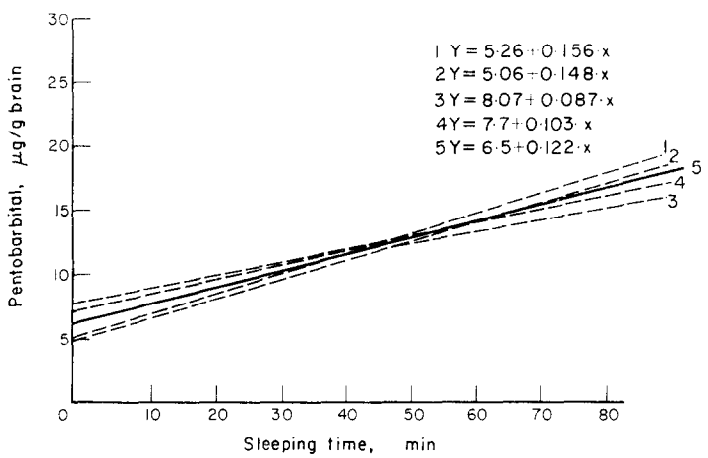

FIG. 1. Regression lines obtained by plotting the sleeping times of rats against their brain pento barbital concentration 90 min after barbiturate administration.

Each curve was obtained from group of 36 rats treated in different period of the year.

The curve No. 5 represents the mean regression line for all the animals.

The analysis of the regression shows no statistically significant difference between any one of the regression lines. (Deviation from parallelism $P > 0{\cdot}05$; coincident lines $P < 0{\cdot}05$.)

A significant correlation between sleeping time and brain pentobarbital exists for all the groups of animals and a good reproducibility is obtained because no statistically significant difference exists between any one of these regression lines (deviation from parallelism: $P > 0{\cdot}05$; coincident lines: $P < 0{\cdot}05$). The solid line represents the mean regression line for all the animals.

In Table 1 the brain pentobarbital concentration of rats sacrificed at the time of awakening after narcosis is compared with the brain levels of animals sacrificed 90 min after barbiturate administration. The rats are divided in four groups according to their sleeping time. Pentobarbital brain level at the time of awakening is constant

and independent of the duration of sleep whereas brain levels 90 min after pentobarbital administration are significantly more elevated in "long" than in "short" sleepers.

Table 2 shows the effect of a pretreatment with various drugs on sleeping time and brain concentration of pentobarbital. The drugs were administered at various times before pentobarbital injection. Pretreatment by SKF-525A, a blocker of drug metabolism, is able to increase both sleeping time and pentobarbital brain concentration,

TABLE 1. PENTOBARBITAL BRAIN CONCENTRATION IN RATS WITH DIFFERENT SENSITIVITY TO PENTOBARBITAL (30 mg/kg i.p.) INDUCED SLEEPING TIME

Group	Sleeping time (min)	Pentobarbital concentration (μg/g + S.E.) at awakening	Pentobarbital concentration (μg/g + S.E.) 90 min after administration
I	40–50	(7) 15·4 ± 1·5	(29) 12·05 ± 0·5
II	51–60	(13) 15·6 ± 0·7	(36) 11·55 ± 0 4
III	61–70	(11) 15·2 ± 1·3	(36) 13·8 ± 0·7*
IV	> 70	(12) 14·1 ± 0·4	(30) 15·7 ± 0 6†

The number of animals is given in brackets.
* $P < 0.05$ in respect to the groups II and I.
† $P < 0.001$ in respect to the groups II and I.

TABLE 2. EFFECT OF VARIOUS DRUGS ON THE SLEEPING TIME INDUCED BY PENTOBARBITAL (30 mg/kg i.p.) AND ON BRAIN PENTOBARBITAL CONCENTRATION MEASURED 90 min AFTER PENTOBARBITAL ADMINISTRATION

No. of rats	Treatment (mg/kg)	Time between treatment and pentobarbital (hr)	Sleeping time (min ± S.E.)	Pentobarbital concentration (μg/g brain ± S.E.)
166	Saline	—	54 ± 0·5	14·1 ± 0·2
14	SKF-525A 15 per os	1	73 ± 2·5*	17·9 ± 0·9*
11		2	73 ± 2·3*	16·9 ± 0·8*
4		48	60 + 7	13·9 ± 1·1
4		72	67 ± 7·7	14·6 ± 1·1
21	Chlorpromazine 2·5 i.p.	1	74 ± 3*	18·4 ± 1·2*
23		2	54 ± 4	15·2 ± 0·4
18	4 i.p.	1	83 ± 3*	16·6 ± 0·6
20		2	76 ± 3*	14·9 ± 0·9
8	Diazepam 25 per os	2	68 ± 5*	13·2 ± 1·3
6		4	72 ± 10*	15·8 ± 1·3
8		24	49 ± 5	14·0 ± 0·4
30	DDT 1 i.p. × 2 days	24	35 ± 3*	12·7 ± 0·6*
58	Phenobarbital 10 i.p.	24	33 ± 2*	8·4 ± 0·4*
38	Amphetamine 0·5 i.p.	1	44 ± 2*	15·3 ± 0·6

* $P < 0.01$ vs. the group of saline of each experiment.

when administered 1 or 2 hr before the barbiturate. On the contrary, DDT, as well as phenobarbital, which are typical inducers of drug metabolism, decrease both sleeping time and pentobarbital brain levels. With amphetamine pretreatment the sleeping time of rats is decreased although pentobarbital levels are unchanged. Moreover when determinations were performed at the awakening, amphetamine-treated rats showed

higher pentobarbital concentrations than control rats (Table 3). The effect of chlorpromazine is different according to the dose administered. At a dose of 2·5 mg/kg the levels are higher and the sleeping time prolonged 1 hr after treatment. However, at a dose of 4 mg/kg chlorpromazine produces a marked increase in narcosis without any increase in brain pentobarbital levels. Similar results are obtained with 25 mg/kg of diazepam. All the data of each treatment presented in Table 2 are then plotted to obtain regression lines showing correlations between brain pentobarbital concentration and sleeping time.

TABLE 3. EFFECT OF *d*-AMPHETAMINE ON PENTOBARBITAL SLEEPING TIME AND PENTOBARBITAL BRAIN CONCENTRATION AT THE AWAKENING

No. of rats	Treatment (mg/kg i.p.)	Sleeping time (min ± S.E.)	Pentobarbital brain concentration (μg/g ± S.E.)
14	Saline	60 ± 4	15·8 ± 1
25	*d*-amphetamine sulphate 2·5	41 ± 3†	19·5 ± 0·7*

Pentobarbital (30 mg/kg i.p.) was given 1 hr after amphetamine or saline.
* $P < 0.05$
† $P < 0.01$
} vs. saline.

No correlation between pentobarbital levels and sleeping times was found in diazepam and chlorpromazine (4 mg/kg) treated rats. For the other treatments, the statistical analysis of regression lines indicates that SKF-525A, chlorpromazine (2·5 mg/kg) and DDT lines are not different from the corresponding control lines. However, for SKF-525A and CPZ treatments the points are shifted up along the line towards longer sleeping times and corresponding higher brain pentobarbital levels, whereas for DDT treatment, which decreases the sleeping time and pentobarbital levels, the points are shifted down along the line. In the case of phenobarbital pretreatment the data indicate that the levels of brain pentobarbital correspond to higher duration of sleep than for controls. The curve is parallel but not coincident ($P < 0.05$) with the control line. When phenobarbital was given 4 hr before pentobarbital, sleeping time (S.T.) and pentobarbital concentration in brain were not modified in respect to the controls (controls, S.T. 56′ ± 3 and pentobarbital 16·2 ± 1 μg/g, phenobarbital pretreated rats S.T. 57′ ± 1, pentobarbital 17·3 ± 1 μg/g). The line concerning the treatment amphetamine-pentobarbital though it has the slope not statistically different from that of controls, is not coincident with them (deviation from parallelism—$P > 0.05$; effect of treatments $P < 0.01$).

DISCUSSION

A dose of pentobarbital (30 mg/kg i.p.) given to female Sprague–Dawley rats induces a narcosis which is variable in time. This variability may be probably accounted by different pentobarbital brain levels because: (a) linear correlation is obtained

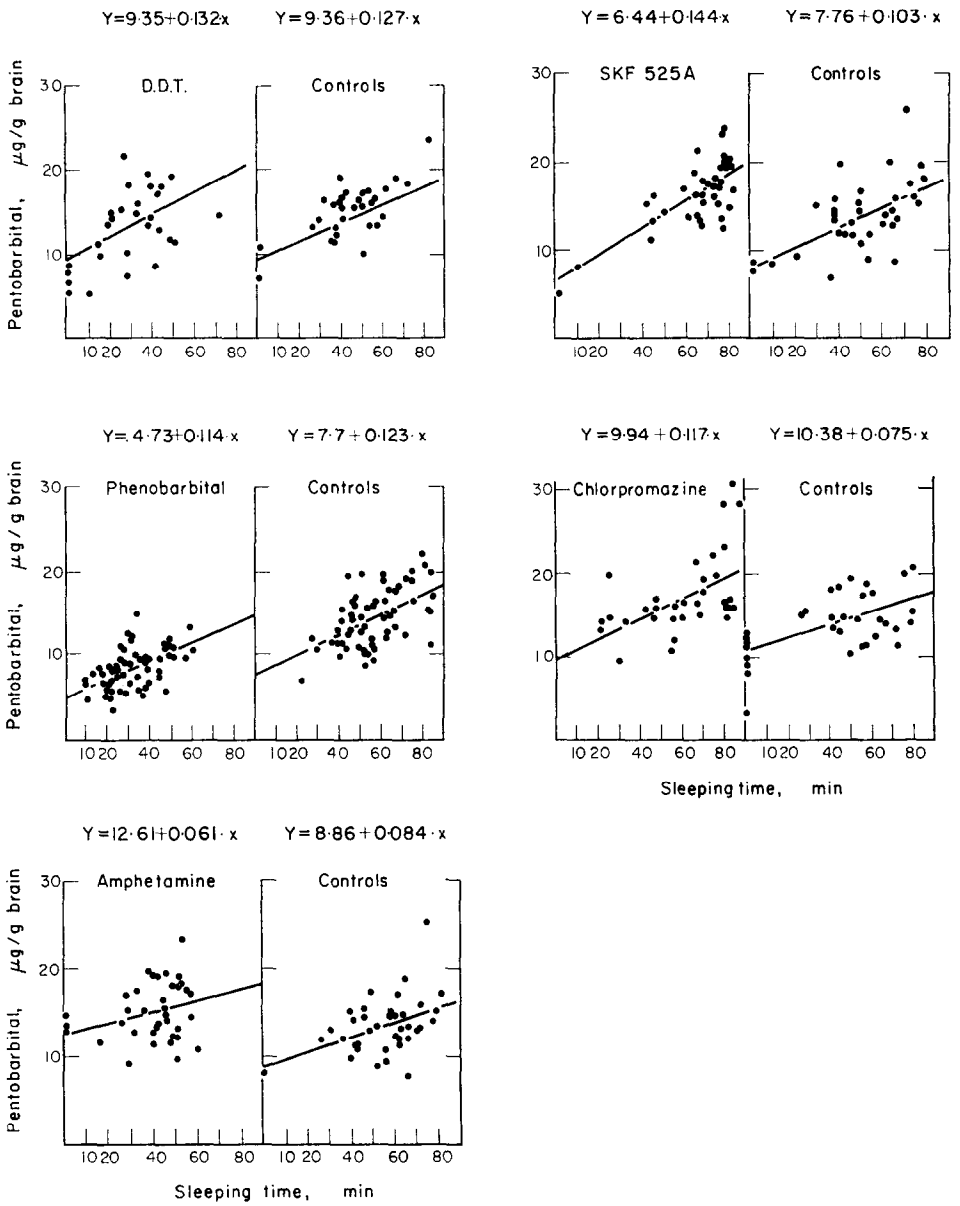

FIG. 2. Regression lines obtained by plotting the sleeping times of rats against their brain pentobarbital concentration 90 min after barbiturate administration.

The data obtained from DDT (1 mg/kg i.p. 2 days), SKF 525A (15 mg/kg per os), phenobarbital (10 mg/kg i.p.) chlorpromazine (2·5 mg/kg i.p. 2 days) and *d*-amphetamine sulphate (500 μg/kg i.p.) pretreated rats are those reported in Table 2.

between sleeping time of rats and its barbiturate brain concentration 90 min after pentobarbital administration; (b) all the rats, independently from the duration of sleeping time, awake with the same pentobarbital brain levels. The importance of barbiturate brain levels to determine the pharmacological activity has been supported by several authors. Grieg[11] found that anesthesia is better correlated with the brain than with plasma concentrations of barbiturates. Noordhoek[12] demonstrated that the decay curve of hexobarbital from brain is monophasic in type and a good correlation was found between barbiturate brain levels and behaviour of animals. Kato[13] has recently found that individual differences in the effect of pentobarbital narcosis in rats are due to differences in the brain concentrations of the drug, probably dependent on the individual rate of metabolism. Also according to the data reported by Mitoma[14] there is a clear correlation between the duration of hexobarbital narcosis and the intensity of hexobarbital metabolism by liver microsomal enzymes. In our experiments constant concentrations of brain pentobarbital at awakening indicate a constant brain sensitivity to the barbiturate according to Bianchine and Ferguso[16] Noordhoek[12] and Winnie.[15] On the other hand, several conditions such as pretreatment with drugs, room temperature or painful stimulation may be responsible for awakening at different brain barbiturate concentrations.[17] Even the dose of barbiturate administered may be responsible for this effect. In fact, Palumbi *et al.*[18] found an increase in barbiturate brain levels at awakening depending on the dose of the drug. However, Noordhoek[12] found that this is evident only for high differences in the dose of hexobarbital in mice. This suggests that side-effects such as hypothermia or hypotension may be responsible for these different results.[19]

The effects of pretreatment with some drugs on the brain levels and on sleeping time after pentobarbital administration has been studied. With SKF-525A or DDT pretreatment, respectively, a blocker and an inducer of pentobarbital metabolism,[20] a linear correlation is still maintained, and the regression line is not different from that of controls. This is not surprising because under our experimental conditions a drug which modifies only barbiturate brain level without exerting other pharmacological actions, is expected to change the duration of sleeping time according to a relation similar to that of controls.

For analysing the effect of some centrally acting drugs on this test chlorpromazine, diazepam, amphetamine and phenobarbital were selected.

The action of drugs on this test may be different depending on the dose administered, as with chlorpromazine pretreatment. The correlation between brain levels and sleeping time is not present when 4 mg/kg of chlorpromazine, as well as 25 mg/kg diazepam are given to rats. With lower doses of chlorpromazine (2·5 mg/kg) however, the correlation is still obtained. It is difficult to explain this difference although with the higher dose of chlorpromazine some pharmacological effects, such as a drop in body temperature, may appear and interfere with this test.[21]

With the low dose of chlorpromazine both sleeping time and pentobarbital brain levels are increased. The increase in level alone may explain sleeping time potentiation because no modification in regression line occurs. Accordingly chlorpromazine has been reported to block pentobarbital metabolism either *in vitro* or *in vivo*, when given shortly before the barbiturate.[22]

Amphetamine is able to reduce barbiturate sleeping time without lowering pentobarbital brain level. With this pretreatment the regression line, though having the same

slope, is shifted to the left in respect to that of controls. This may indicate that the effect of the pentobarbital present in the brain is lowered in amphetamine treated rats. The higher pentobarbital concentration at awakening in amphetamine treated rats (see Table 3) than in controls is conceivable with this interpretation. These results are in agreement with the known CNS stimulant activity of amphetamine. Phenobarbital decreases brain pentobarbital concentration and it shortens the sleeping time. The reduced levels in brain pentobarbital is considered the result of the known inducing action of phenobarbital on the hepatic microsomal enzymes responsible for the metabolism of pentobarbital.[23] However, a shift to the right of the regression line occurs, suggesting that another effect in addition to that on metabolism is present. In fact pentobarbital present in the brain seems to be more effective as a narcotic agent in phenobarbital pretreated than in control rats. A direct effect of phenobarbital, present in the brain, on the narcotic activity of pentobarbital may be excluded because phenobarbital does not modify the two parameters considered when given 4 hr before the pentobarbital administration.

The phenomenon observed may be therefore explained as an increased sensitivity of the CNS to pentobarbital. Aston[24] found in fact that pentobarbital treatment produces a latent hypersensitivity to pentobarbital itself that develops a long time after withdrawal and that does not depend on pentobarbital metabolism.[25]

With the reported elaboration of the data it is possible to evidentiate this hypersensitivity also during the first day after treatment, when the "inducing" activity of phenobarbital is present.

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