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PHENOBARBITONE ENZYME INDUCING EFFECT AND THE ACTION OF METHYSERGIDE ON THE BARBITURATE SLEEPING TIME IN GAMMA-IRRADIATED RATS

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Abstract—Both phenobarbitone and methysergide produce a decrease of the thialbarbitone sleeping time in gamma-irradiated rats. Phenobarbitone was injected 2 and 48 hr after irradiation with 600 r and 800 r. The effect of phenobarbitone was found to be maximal 48 hr after its injection, and it was evident only in rats irradiated with 600 r. Methysergide was also injected 2 and 48 hr after irradiation and this procedure was found not to affect the thialbarbitone sleeping time. On the contrary, methysergide significantly depressed the thialbarbitone sleeping time if injected 15–30 min before thialbarbitone. This effect of methysergide is present only in irradiated animals. Ethionine, if injected 15 min before methysergide, inhibited the action of methysergide on the thialbarbitone sleeping time. This effect of ethionine was not blocked by methionine. It is suggested that phenobarbitone and methysergide affect the thialbarbitone sleeping time either by different mechanisms, or by similar mechanisms in which time factor is different.

INTRODUCTION

IT WAS reported in a previous work that the whole-body X-irradiation with 600 r and 800 r prolongs the barbiturate sleeping time in rats. This effect was observed both after head shielded and whole-body X-irradiation, but it was absent in head irradiated animals.[1] It was also found that methysergide, a highly potent and specific 5-hydroxytryptamine antagonist, if injected after irradiation and 15 min before thialbarbitone, significantly depressed the prolonging effect of X-irradiation on the thialbarbitone sleeping time[2], whereas lysergic acid diethylamide did not show this type of activity. The suggestion was therefore made that the action of methysergide on the thialbarbitone sleeping time in X-irradiated rats might be due to its anti-5-hydroxytryptamine activity, although the changes in the detoxication processes of the barbiturates are known to occur after X-irradiation[3] and thereby influence the barbiturate sleeping time.

The content of 5-hydroxytryptamine in the gut in the early phase after irradiation could be changed[4], but these changes do not seem to be highly significant 24 and 48 hr after irradiation.[5] The changes in 5-hydroxytryptamine content of the brain occur only after very high doses of X-rays.[6] On the other hand, many substances are known to be able to induce the microsomal enzymes of the liver[7–9] and thus to influence drug metabolism and to affect in this way e.g. the barbiturate sleeping time. It was therefore decided to compare the enzyme inducing effect of phenobarbitone and the

action of methysergide on the thialbarbitone sleeping time in gamma-irradiated rats in order to get more data on the enzyme induction in irradiated animals and on the mechanism of methysergide action.

MATERIALS AND METHODS

Only male rats weighing from 195 to 215 g were used in these experiments. The animals were irradiated from a cobalt-60 source with an average of 44 r/min and then kept under ordinary laboratory conditions.

Thialbarbitone Sodium (Kemithal-Sodium) was injected intravenously in a dose of 50 mg/kg. The sleeping time (in this paper called thialbarbitone or barbiturate sleeping time) was measured from the end of injection until the righting reflex was restored. The control experiments with irradiated non-treated animals were carried out in almost each case side by side with phenobarbitone and methysergide treated irradiated animals.

Methysergide (Deseril*) was injected intraperitoneally in a dose of 1 mg/kg, 2 and 48 hr after irradiation, and also 15 min before injection of thialbarbitone in various periods of time after irradiation.

DL-Ethionine was dissolved in 10 % hydrochloric acid and neutralized by sodium hydroxyde to pH 6·4. This substance was also injected intraperitoneally in doses ranging from 50 to 250 mg/kg, 15 min before injection of methysergide and 30 min before injection of thialbarbitone. These doses of DL-ethionine produced 24 hr after injection a mortality rate which ranged from 20 to 100 per cent. DL-Methionine was dissolved and neutralized in the same way as DL-ethionine; this substance was also injected intraperitoneally in a dose of 250 mg/kg.

Phenobarbitone–sodium was injected intraperitoneally in a dose of 90 mg/kg, 2 and 48 hr after irradiation.

RESULTS

The effect of phenobarbitone and methysergide in animals irradiated with 600 r

It has been found that many drugs, such as phenobarbitone, glutethimide, phenaglycodol, chlorcyclizine, phenylbutazone, aminopyrine, zoxazolamine, meprobamate, chlorpromazine, nikethamide and chloretone, if injected 12–24 hr in advance, induce an increased activity of some microsomal drug-metabolizing enzymes.[10–13] We have used phenobarbitone in the present experiments. This substance was injected intraperitoneally, 2 and 48 hours after irradiation. In a separate series of animals methysergide was injected in the same way and in the same periods of time after irradiation as phenobarbitone. In both groups then the thialbarbitone sleeping time was measured in various periods of time after irradiation. The results are compared and shown in Table 1.

It can be observed that pretreatment of the irradiated animals with phenobarbitone significantly shortened the thialbarbitone sleeping time, both when injected 2 hr and 48 hr after irradiation. This effect of phenobarbitone is most pronounced 48 hr after its injection, whereas 24 hr after injection of phenobarbitone the thialbarbitone sleeping

* Kindly supplied by Sandoz, Basle.

time is significantly prolonged. The effect of phenobarbitone on the thialbarbitone sleeping time in irradiated animals almost disappears after 96 hr. On the other hand, methysergide both when injected 2 hr and 48 hr after irradiation did not change the thialbarbitone sleeping time 24 hr, 48 hr, 96 hr and 5 days after irradiation.

TABLE 1. THE EFFECT OF PHENOBARBITONE AND METHYSERGIDE ON THE THIALBARBITONE SLEEPING TIME IN RATS IRRADIATED WITH 600 r(MEAN ± S.E.). THE NUMBER OF ANIMALS IS INDICATED IN BRACKETS

Time after irradiation	1. Irradiated controls	2. Irradiated animals treated with phenobarbitone-Na, 2 hr after irradiation	3. Irradiated animals treated with merthysergide, 2 hr after irradiation	4. Irradiated animals treated with phenobarbitone-Na, 48 hr after irradiation	5. Irradiated animals treated with methysergide, 48 hr after irradiation
24 hr	11·5 ± 0·16 (19)	14·4 ± 0·64 (20)	12·1 ± 0·29 (20)		
48 hr	13·5 ± 0·29 (20)	11·2 ± 0·22 (20)	13·7 ± 0·27 (20)		
96 hr	13·9 ± 0·30 (20)	12·8 ± 0·32 (19)	14·4 ± 0·38 (20)		
5 days	14·5 ± 0·47 (20)			10·9 ± 0·19 (19)	14·3 ± 0·34 (10)
8 days	14·8 ± 0·33 (28)	15·7 ± 0·53 (20)	15·1 ± 0·45 (19)	14·7 ± 0·49 (10)	15·3 ± 0·52 (9)

24 hr	48 hr	96 hr	5 days
P(1:2) < 0·001	P(1:2) < 0·001	P(1:2) < 0·025	P(1:4) < 0·001
P(2:3) < 0·005	P(2:3) < 0·001	P(2:3) < 0·005	P(4:5) < 0·001
P(1:3) not sign	P(1:3) not sign	P(1:3) not sign	P(1:5) not sign

The effect of phenobarbitone and methysergide in animals irradiated with 800 r

Both phenobarbitone and methysergide were injected intraperitoneally in animals irradiated with 800 r, 2 hr and 48 hr after irradiation. The thialbarbitone sleeping time was then measured in various periods of time after irradiation. The results of these experiments are shown in Table 2.

TABLE 2. THE EFFECT OF PHENOBARBITONE AND METHYSERGIDE ON THE THIALBARBITONE SLEEPING TIME IN RATS IRRADIATED WITH 800 r (MEAN ± S.E.). THE NUMBER OF ANIMALS IS INDICATED IN BRACKETS

Time after irradiation	1. Irradiated controls	2. Irradiated animals treated with phenobarbitone-Na, 2 hr after irradiation	3. Irradiated animals treated with methysergide, 2 hr after irradiation	4. Irradiated animals treated with phenobarbitone-Na, 48 hr after irradiation	5. Irradiated animals treated with methysergide, 48 hr after irradiation
24 hr	12·1 ± 0·18 (20)	12·2 ± 0·31 (20)	11·8 ± 0·19 (20)		
48 hr	13·0 ± 0·26 (10)	12·5 ± 0·33 (10)	13·3 ± 0·24 (10)		
96 hr	18·9 ± 0·64 (19)	17·1 ± 0·69 (14)	20·6 ± 0·84 (16)		
5 days	18·3 ± 0·86 (9)			18·1 ± 1·1 (7)	18·5 ± 0·95 (8)
8 days	19·3 ± 0·97 (17)	17·4 ± 0·76 (10)	20·0 ± 0·87 (10)	18·2 ± 1·01 (4)	19·7 ± 1·76 (8)

It can be seen that neither phenobarbitone nor methysergide do not significantly change the thialbarbitone sleeping time in rats irradiated with 800 r.

The effect of methysergide and ethionine

Ethionine has been known to be an amino acid antagonist which is able to inhibit the incorporation of methionine and glycine into liver protein.[14] This substance also

completely prevented induction of microsomal enzyme activity by polycyclic hydrocarbons and by drugs.[8, 9, 15] Thus, for example, the inducing effect of phenobarbitone on the microsomal enzymes could be prevented by pretreatment of the animal with ethionine.[12] If the action of methysergide in shortening the action of thialbarbitone in irradiated rats is due to induction of microsomal drug metabolizing enzymes, then the pretreatment of the irradiated animal with ethionine should abolish the effect of methysergide. The results of this series of experiments are shown in Table 3.

TABLE 3. THE EFFECT OF METHYSERGIDE AND ETHIONINE ON THE THIALBARBITONE SLEEPING TIME IN RATS IRRADIATED WITH 600 r(MEAN ± S.E.). THE NUMBER OF ANIMALS IS INDICATED IN BRACKETS.

Time after irradiation	1. Irradiated controls	2. Irradiated animals treated with methysergide, 15 min before thialbarbitone	3. Irradiated animals treated with ethionine, 30 min before thialbarbitone	4. Irradiated animals treated both with methysergide and ethionine
24 hr	11·9 ± 0·14 (19)	10·4 ± 0·15 (20)	12·8 ± 0·27 (10)	18·3 ± 0·80 (10)
48 hr	13·2 ± 0·65 (30)	10·6 ± 0·10 (20)	13·2 ± 0·37 (10)	14·2 ± 0·50 (10)
5 days	14·2 ± 0·24 (28)	10·8 ± 0·14 (20)	14·4 ± 0·46 (30)	16·4 ± 0·70 (29)
8 days	16·4 ± 0·97 (17)	11·2 ± 0·27 (10)	17·0 ± 1·05 (10)	20·7 ± 0·97 (9)
	24 hr	48 hr	5 days	8 days
	P(1:2) < 0·001	P(1:2) < 0·005	P(1:2) < 0·001	P(1:2) < 0·001
	P(1:3) < 0·01	P(1:3) not sign.	P(1:3) not sign.	P(1:3) not sign.
	P(1:4) < 0·001	P(1:4) not sign	P(1:4) not sign	P(1:4) < 0·02
	P(3:4) < 0·001	P(3:4) not sign	P(3:4) < 0·02	P(3:4) < 0·05
	P(2:3) < 0·001	P(2:3) < 0·001	P(2:3) < 0·001	P(2:3) < 0·001

It can be observed from the Table 3 that pretreatment with ethionine prevented the action of methysergide in irradiated rats. The effect of ethionine itself on the thialbarbitone sleeping time varied in various experimental groups. It was observed occasionally that ethionine prolonged the thialbarbitone sleeping time in irradiated animals, whereas in other groups this effect was absent.

Methionine has been already found to block the action of ethionine in preventing protein synthesis.[15, 16] In the present experiments methionine was found not to block the action of ethionine. Thus, in a separate group of irradiated animals with 600 r the thialbarbitone sleeping time in animals treated with methysergide was 11·1 ± 0·11 (10 animals), whereas in rats treated both with methysergide and ethionine this time was 14·8 ± 0·31 (10 animals). In the group of rats treated with methysergide, ethionine and methionine the thialbarbitone sleeping time was 18·2 ± 0·47 (10 animals, mean ± S.E.). It can be even observed that the thialbarbitone sleeping time in animals treated with methysergide, ethionine and methionine is significantly longer ($P < 0·001$) than in animals treated only with methysergide and ethionine.

The effect of methysergide in non-irradiated rats

It was previously shown that the potentiating action of chlorpromazine on the thiopental narcotic action is not inhibited by methysergide, whereas the potentiating action of 5-hydroxytryptamine on the thiopental effect is blocked.[20] It would therefore mean that methysergide is not a general anti-narcotic substance. In the present experiments methysergide was found not to affect the thialbarbitone sleeping time in

normal non-irradiated rats. Thus, in a control group of 10 animals the thialbarbitone sleeping time was 10·9 ± 0·16 min, whereas in methysergide pretreated group of 10 animals this time was 10·4 ± 0·43 min (mean ± S.E.).

DISCUSSION

It was found in the present experiments that phenobarbitone produced a decrease in duration of thialbarbitone sleeping time in animals irradiated with 600 r. This effect of phenobarbitone was probably due to microsomal drug metabolizing enzyme inducing effect. Such an effect of phenobarbitone in normal non-irradiated animals has already been described.[12] In animals irradiated with 800 r phenobarbitone did not affect the thialbarbitone sleeping time. The barbiturates are mainly metabolised through oxydations in the sidechain,[21] although other processes also take place, e.g. desulfuration of the thiobarbiturates, loss of N-alkyl radicals and probably hydrolytic opening of the barbiturate ring. It seems therefore possible that irradiation with 800 r affects some of these processes in such a way that enzyme induction is no more possible. Irradiation has already been found to slow the processes of oxydation and oxydative phosphorylation.[22, 23] Our experiments also suggest that the phenobarbitone enzyme inducing effect in the irradiated animals probably might be used as an indicator of the received dose of irradiation.

On the other hand, methysergide if injected in the same way and in the same periods of time after irradiation (2 and 48 hr) as phenobarbitone, did not affect the thialbarbitone sleeping time both in animals irradiated with 600 r and 800 r. On the contrary, methysergide significantly reduced the duration of the thialbarbitone sleeping time in irradiated animals if injected 15–30 min before thialbarbitone.[2] This suggests that the action of phenobarbitone in influencing the thialbarbitone sleeping time is substantially different from the effect of methysergide.

Ethionine has been found to inhibit the induction of tryptophan-peroxidase, of the demethylase of dimethylaminoazobenzene and of glucose-6-phosphatase.[17–19] In the present experiments ethionine, if injected 15 min before methysergide, was found to inhibit the action of methysergide on the thialbarbitone sleeping time in animals irradiated with 600 r. On the other hand, this effect of ethionine was not blocked by methionine. Ethionine itself was found in these experiments to act on the thialbarbitone sleeping time in various groups of animals in two different ways: to prolong it, or to leave it unchanged. It would therefore mean that ethionine might influence the action of methysergide by some other effect which is not necessarily connected with inhibitory action of ethionine on biosynthesis of enzyme protein.

The evidence obtained in these experiments indicates the clear difference between the phenobarbitone microsomal drug metabolizing enzyme inducing action and the effect of methysergide. The effect of phenobarbitone was found to be maximal 48 hr after its injection, which is in accordance with the results of other authors obtained on non-irradiated animals.[12] Meanwhile, the effect of methysergide in irradiated animals is evident 15–30 min after its injection. It is also characteristic that methysergide affects the thialbarbitone sleeping time only in irradiated and not in normal non-irradiated animals. It is therefore concluded that phenobarbitone and methysergide either affect the thialbarbitone sleeping time in gamma-irradiated rats by different mechanisms, or by similar mechanisms in which time factor is quite different.

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