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The effect of methysergide and X-irradiation on the barbiturate sleeping-time in rats

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Methysergide (1-methyl-D-lysergic acid butanolamide bimaleate), a highly potent and specific 5-hydroxytryptamine antagonist, if injected after irradiation, significantly depresses the prolonging effect of x-irradiation on the barbiturate sleeping-time in rats. Lysergic acid diethylamide did not show this type of activity.

5-Hydroxytryptamine, when injected before irradiation, did not depress the typical response of the irradiated animals to barbiturates. When used after irradiation, 5-hydroxytryptamine caused a further prolongation of the barbiturate sleeping-time.

Cysteamine, when injected before irradiation, depressed or blocked the prolonging effect of x-irradiation on the barbiturate sleeping-time. When injected after irradiation, cysteamine was found to cause a further prolongation of the barbiturate sleeping-time. The mechanisms of action of methysergide, 5-hydroxytryptamine and cysteamine are briefly discussed.

1. Introduction

Evidence was presented in a previous work that whole-body x-irradiation with 600 r and 800 r prolongs the barbiturate sleeping-time in rats and that in this effect there was a dose–response relationship. In the head-irradiated animals no prolongation of the barbiturate sleeping-time was observed, whereas irradiation of the animal with the head shielded produced the same effect as whole-body irradiation (Varagić, Stepanović and Hajduković 1962). Chernov and Raushenbakh (1960) found in the early phase after irradiation a sharp reduction in the 5-hydroxytryptamine content of the intestine and an increase of the same substance in the brain of rats and guinea-pigs affected by radiation sickness, and several investigators have shown that 5-hydroxytryptamine prolongs the barbiturate sleeping-time (Shore, Silver and Brodie 1955, Fornarolli and Koller 1955, Fastier 1956). The subject of this work was to investigate further the action of x-irradiation in prolonging the barbiturate sleeping-time. If the liberation of 5-hydroxytryptamine by irradiation were the cause of the altered reactivity to barbiturates in x-irradiated animals, then 5-hydroxytryptamine antagonists should antagonize or block the prolonged effect of barbiturates in x-irradiated animals. The effect of 5-hydroxytryptamine antagonists is compared with the action of cysteamine and 5-hydroxytryptamine itself, which are known to be protective agents against x-irradiation.

2. Methods

Male rats weighing from 190 to 215 g were used in these experiments. All were given x-irradiation to the whole body with 800 r (16 mA; 200 kV; 0·5 mm Cu filter) and then kept under ordinary laboratory conditions.

Thialbarbitone sodium was injected intravenously in a dose of 50 mg/kg. The sleeping-time was measured until the righting reflex was restored. The waking-time was taken as that at which the rat righted and began to move away from its sleeping position.

Cysteamine (Lambratene) was injected intraperitoneally in a dose of 100 mg/kg, 10 min before irradiation, and after irradiation, 30 min. before thialbarbitone was injected.

Methysergide (Deseril, supplied by Sandoz, Basel) was also injected intraperitoneally after irradiation in a dose of 1 mg/kg, 30 min before injection of thialbarbitone. In a separate series of animals methysergide was injected in the same dose and in the same way, but 30 min before irradiation. Lysergic acid diethylamide was injected intraperitoneally in two doses: 50 μg/kg and 10 mg/kg, after irradiation 30 min before thialbarbitone was injected. 5-Hydroxytryptamine was injected in the same way both before and after irradiation in doses of 20 mg/kg and 10 mg/kg.

3. Results

3.1. *5-Hydroxytryptamine antagonists and barbiturate sleeping time in X-irradiated animals*

In a series of 10 animals lysergic acid diethylamide (LSD) was used as 5-hydroxytryptamine antagonist. This substance has been known to be a very potent 5-hydroxytryptamine antagonist (Erspamer 1954) and to be able to block the prolonging action of 5-hydroxytryptamine on the barbiturate sleeping-time (Shore *et al.* 1955). In this series of animals lysergic acid diethylamide was injected intraperitoneally after irradiation, 30 min before injection of thialbarbitone. The doses were 50 μg/kg and 10 mg/kg, and the results are presented in table 1.

Time after irradiation	1. Controls	2. Irradiated animals	3. Irradiated animals treated with lysergic acid diethylamide	
4 days	8·6 ± 0·27(10)	17·0 ± 0·65(8)	14·8 ± 0·97(10)	50 μg/kg LSD
7 days	8·7 ± 0·24(10)	14·0 ± 1·09(8)	14·8 ± 1·4 (10)	
14 days	8·5 ± 0·3 (10)	14·0 ± 1·1 (6)	15·6 ± 2·03 (5)	10 mg/kg LSD

Table 1. The effect of lysergic acid diethylamide, when injected after irradiation, on the barbiturate sleeping-time (in min ± SE) in irradiated animals. The number of animals is indicated in brackets.

It may be seen that lysergic acid diethylamide did not significantly affect the barbiturate sleeping-time in x-irradiated rats.

In a separate group of experiments the x-irradiated animals were treated with methysergide (1-methyl-D-lysergic acid butanolamide bimaleate) in a dose of 1 mg/kg, 30 min before the barbiturate was injected. Methysergide was used

because of its highly specific anti-5-hydroxytryptamine activity and lack of other pharmacodynamic properties (Fanchamps, Doepfner, Weidmann and Cerletti 1960). The results are presented in table 2.

Time after irradiation	1. Controls	2. Irradiated animals	3. Irradiated animals treated with methysergide	*P*
24 hours	9·2 ± 0·22(10)	12·7 ± 0·5 (20)	11·5 ± 0·42(20)	$P(1:2) < 0{\cdot}001$ $P(1:3) < 0{\cdot}01$ $P(2:3)$ not signif.
48 hours	9·3 ± 0·19(20)	14·7 ± 0·47(30)	12·6 ± 0·33(29)	$P(1:2) < 0{\cdot}001$ $P(1:3) < 0{\cdot}001$ $P(2:3) < 0{\cdot}001$
5 days	9·2 ± 0·14(30)	21·6 ± 1·23(25)	13·7 ± 0·78(25)	$P(1:2) < 0{\cdot}001$ $P(1:3) < 0{\cdot}001$ $P(2:3) < 0{\cdot}001$
8 days	9·0 ± 0·2 (18)	26·7 ± 1·43(22)	17·4 ± 0·86(24)	$P(1:2) < 0{\cdot}001$ $P(1:3) < 0{\cdot}001$ $P(2:3) < 0{\cdot}001$

Table 2. The effect of x-irradiation (800 r) and methysergide, when injected after irradiation, on the barbiturate sleeping-time (in min ± SE). Number of animals is indicated in brackets.

The barbiturate sleeping-time in non-irradiated animals treated with methysergide was 10·15 ± 0·23 min (15 animals), whereas the sleeping-time in the control group of 10 animals was 10·1 ± 0·3 min.

It can be seen that methysergide significantly shortened the barbiturate sleeping-time in x-irradiated animals. This effect was observed 2 days, 5 days and 8 days after irradiation. Nevertheless, the barbiturate sleeping-time in x-irradiated animals treated with methysergide was still significantly longer than in normal controls.

3.2. *The effect of methysergide when injected before irradiation*

In a group of 10 animals methysergide was injected intraperitoneally before irradiation in a dose of 1 mg/kg. There was no difference in barbiturate sleeping-time between these animals and control non-treated irradiated animals. Twenty-four hours, 2 days, 5 days and 8 days after irradiation the barbiturate sleeping-time in these animals was 11·6 ± 0·44 min, 15·1 ± 0·81 min, 30·7 ± 2·4 min and 34·0 ± 1·6 min, whereas in control non-treated irradiated animals this time was 13·5 ± 0·52 min, 16·2 ± 0·84 min, 24·5 ± 1·3 min and 27·0 ± 2·5 min (mean ± SE).

3.3. 5-*Hydroxtryptamine and barbiturate sleeping-time in X-irradiated animals*

5-Hydroxytryptamine has been known to have a protective action in x-irradiated rats (Dukor and Schuppli 1961, Supek, Randić and Lovašen 1961).

Cysteamine, another protective agent, was found in a previous work to depress or even abolish the prolongation of the barbiturate sleeping-time in x-irradiated rats when injected before irradiation (Varagić *et al.* 1962). In the present experiments 5-hydroxytryptamine was injected both before and after irradiation. When used before irradiation, 5-hydroxytryptamine was injected intraperitoneally in a dose of 20 mg/kg, 5 min before irradiation. When used after irradiation, 5-hydroxytryptamine was also injected intraperitoneally in a dose of 10 mg/kg, 4 days after irradiation and 30 min before injection of thialbarbitone.

It was found that in irradiated animals 5-hydroxytryptamine, when injected after irradiation, causes a further significant prolongation of the barbiturate sleeping-time, as shown in table 3.

Time after irradiation	1. Controls	2. Irradiated animals	3. Irradiated animals treated with 5-HT (10 mg/kg, 30 min before barbiturate)	*P*
4 days	9·6 ± 0·3(9)	22·3 ± 1·04(7)	30·7 ± 0·9(7)	$P(1:2) < 0{\cdot}001$ $P(2:3) < 0{\cdot}001$ $P(1:3) < 0{\cdot}001$

Table 3. The effect of 5-hydroxytryptamine, when injected after irradiation, on the barbiturate sleeping-time in x-irradiated rats (in min ± SE). The number of animals is indicated in brackets.

The results in table 3 can be compared with the response of the normal non-irradiated animals to 5-hydroxytryptamine and methysergide. The same dose of 5-hydroxytryptamine caused a statistically-significant prolongation of the barbiturate sleeping-time from 9·3 ± 0·16 min in controls to 14·5 ± 0·5 min ($P < 0{\cdot}001$). In animals treated both with 5-hydroxytryptamine and methysergide the barbiturate sleeping-time was 9·2 ± 0·2 min. These numbers are the mean figures for the results obtained in 3 groups of 10 animals each.

It was also found that the treatment of the rats with 5-hydroxytryptamine before irradiation did not depress the typical pattern of response of the irradiated animals to thialbarbitone. The results from table 4 show that pre-treatment with 5-hydroxytryptamine can even prolong the effect of the barbiturate in irradiated animals.

3.4. *Comparison of the effects of cysteamine and methysergide*

In one group of 10 irradiated animals cysteamine was injected after irradiation intraperitoneally in a dose of 100 mg/kg, 30 min before thialbarbitone was injected. In the second group of 10 animals cysteamine was injected in the same dose and in the same way, but before irradiation. The results of these experiments are shown in table 5.

In a separate group of 10 irradiated rats both methysergide and cysteamine were injected after irradiation, 30 min before thialbarbitone was injected. The barbiturate sleeping-time in these animals (24 hours, 2 days and 5 days after irradiation) was 22·4 ± 1·5 min, 24·4 ± 2·4 min and 32·8 ± 2·03 min.

In a group of 10 non-irradiated animals cysteamine was injected intraperitoneally in a dose of 100 mg/kg, and 24 hours later the barbiturate sleeping-time was measured. Mean sleeping-time in these animals was 11·6 min, whereas the control animals slept 10·2 min.

Time after irradiation	1. Controls	2. Irradiated animals	3. Irradiated animals treated with 5-HT (20 mg/kg, 5 min before irradiation)	*P*
24 hours	9·6 ± 0·3 (10)	12·9 ± 0·45(10)	12·5 ± 0·6(10)	$P(1:2) < 0{\cdot}001$ $P(1:3) < 0{\cdot}01$
48 hours	9·6 ± 0·23(10)	13·7 ± 0·4 (10)	15·5 ± 0·8(10)	$P(1:2) < 0{\cdot}001$ $P(1:3) < 0{\cdot}001$
5 days	9·9 ± 0·25(10)	21·7 ± 1·6 (4)	33·0 ± 1·3 (5)	$P(1:2) < 0{\cdot}001$ $P(1:3) < 0{\cdot}001$

Table 4. The effect of 5-hydroxytryptamine, when injected before irradiation, on the barbiturate sleeping-time in x-irradiated rats (in min ± SE). The number of animals is indicated in brackets.

Time after irradiation	1. Controls	2. Irradiated animals	3. Irradiated animals treated with cysteamine *before* irradiation	4. Irradiated animals treated with cysteamine *after* irradiation	*P*
24 hours	9·3 ± 0·25(20)	14·9 ± 0·9 (10)	11·4 ± 0·72(10)	21·2 ± 1·7(10)	$P(2:3) > 0{\cdot}01$ $P(2:4) > 0{\cdot}01$ $P(3:4) < 0{\cdot}001$
48 hours	9·5 ± 0·16(20)	16·2 ± 1·09(10)	12·5 ± 0·61(10)	26·3 ± 1·3(10)	$P(2:3) > 0{\cdot}01$ $P(2:4) < 0{\cdot}001$ $P(3{\cdot}4) < 0{\cdot}001$
5 days	9·3 ± 0·2 (17)	26·2 ± 3·7 (8)	16·0 ± 0·9 (9)	35·0 ± 3·5** (7)	$P(2:3) < 0{\cdot}02$ $P(2:4)$ not signif. $P(3:4) < 0{\cdot}001$

Table 5. Comparison of the effect of cysteamine on the barbiturate sleeping-time (in min ± SE) when injected before and after irradiation. The number of animals is indicated in brackets.

4. Discussion

It was found in the present experiments that methysergide (1-methyl-D-lysergic acid butanolamide bimaleate) significantly depressed the effect of x-irradiation in prolonging the barbiturate sleeping-time in rats. The suggestion was made in a previous work (Varagić *et al.* 1962) that some humoral factor might be released by x-irradiation, which contributes to the prolongation of the barbiturate hypnotic action in irradiated rats. Methysergide has been found to be a potent and highly specific 5-hydroxytryptamine antagonist which possesses practically no other pharmacodynamic properties (Fanchamps *et al.* 1960). It is therefore possible that the action of methysergide on the barbiturate sleeping-time in x-irradiated animals is due to its anti-5-hydroxytryptamine activity. On the other hand, lysergic acid diethylamide, which has been known to block the prolonging effect of 5-hydroxytryptamine on the barbiturate sleeping-time (Shore *et al.* 1955), did not in our experiments significantly alter the response of the irradiated rats to thialbarbitone. A similar discrepancy was found by Halbert (1961) in studies of the protective effect of methysergide on the streptolysin-toxicity. In rabbits and mice methysergide prolonged life when given before a lethal dose of streptolysin, whereas other 5-hydroxytryptamine antagonists had a less pronounced effect.

5-Hydroxytryptamine has been known to have a protective action against x-irradiation (Dukor and Schuppli 1961, Supek *et al.* 1961). In our experiments 5-hydroxytryptamine, when injected before irradiation, did not depress the typical response of the irradiated rats to thialbarbitone; a prolongation on the fifth day after injection was even observed (table 4). In studies of 5-hydroxytryptamine metabolism, Titus and Udenfriend (1954) found that 40 per cent of the injected amine could be found in urine in the form of 5-hydroxy-indolacetic acid within 24 hours. This might indicate a change in the degradation process of 5-hydroxytryptamine in x-irradiated rats. When used after irradiation, 5-hydroxytryptamine prolonged the barbiturate sleeping-time as well as in the control animals.

Cysteamine, when used before irradiation, was found to depress or abolish the prolonging effect of x-irradiation on the hypnotic action of thialbarbitone (Varagić, *et al.* 1962). If injected after irradiation, as in the present experiments, cysteamine was found to cause a further prolongation of the barbiturate sleeping-time. The striking difference between methysergide and cysteamine is that cysteamine depresses or blocks the prolonging action of x-irradiation on the barbiturate sleeping-time only if injected before irradiation, whereas methysergide acts in this way only if injected after irradiation. These findings also suggest that the mechanisms of the protective action of cysteamine and 5-hydroxytryptamine in improving the overall survival of the irradiated rats are different.

In the animals treated with methysergide, the barbiturate sleeping-time was significantly shorter than in non-treated irradiated animals, but still significantly longer than in normal non-irradiated control animals. This indicates that after x-irradiation more factors might be involved in altering the response to barbiturates. X-irradiation was found to produce some change in the detoxication process of barbiturates. Grossman (1961) found that a statistically-insignificant fall in the blood level of thiopental sodium, a slight increase in its excretion in the urine, and a prolongation of its presence in the brain occurred in rats irradiated with 600 r.

Le méthysergide (bimaléate de butanolamide de l'acide 1-méthyl-D-lisergique), un antagoniste puissant et spécifique de la 5-hydroxytryptamine, administrée après l'irradiation, diminue d'une façon significative le pouvoir de l'irradiation de prolonger la durée du sommeil provoquée chez les rats par les barbituriques. La diéthylamide lisergique ne donne pas lieu à la même activité.

La 5-hydroxytryptamine, appliquée avant l'irradiation, ne déprime pas l'effet dormitif des barbituriques. La même substance, administrée après l'irradiation, augmente la durée du sommeil dû aux barbituriques.

La cystéamine, si elle a été administrée avant l'irradiation, déprime ou bloque le pouvoir de l'irradiation de prolonger la durée du sommeil provoquée par les barbituriques. Si elle a été appliquée après l'irradiation, la cystéamine provoque une prolongation additionée d'effet du barbiturique. Le mécanisme d'action du méthysergide, 5-hydroxytryptamine et de la cystéamine a été discuté.

Die nach Röntgen-bestrahlung bei Ratten auftretende Verlängerung der Barbituratnarkose wird durch Methysergid (1-methyl-D-Lysergsäure-butanolamid bimaleat), ein spezifischer Hemmstoff des Serotonins, signifikant aufgehoben wenn es nach der Bestrahlung injiziert wird. Lysergsäure-amid hat keine verhindernde Wirkung.

Bei Ratten die vor der Bestrahlung Serotonin erhalten hatten, war die Dauer der Barbituratnarkose nicht verkürzt. Injiziert man jedoch Serotonin nach der Bestrahlung, so kommt es zu einer deutlichen Verlängerung der Narkosedauer.

Cysteamin, vor der Bestrahlung injiziert, deprimiert oder blockiert die Verlängerung der Barbituratnarkose nach Röntgen Bestrahlung der Ratten. Wenn man nach der Bestrahlung injiziert, verursacht Cysteamin weitere Verlängerung der Narkosedauer. Der Mechanismus der Methysergid-, 5-Hydroxytryptamine-, und Cysteamin-Wirkung wird diskutiert.

References

Chernov, G., and Raushenbakh, M., 1960, *Probl. Gematol.*, **5**, 3.
Dukor, P., and Schuppli, R., 1961, *Experientia*, **17**, 257.
Erspamer, V., 1954, *Pharmacol. Rev.*, **6**, 425.
Fastier, F. N., 1956, *Experientia*, **12**, 241.
Fanchamps, A., Doepfner, W., Weidmann, H., and Cerletti, A., 1960, *Schweiz. med. Wschr.*, **90**, 1040.
Fornarolli, R., and Koller, M., 1955, *Farmaco*, **9**, 546.
Grossman, V., 1961, *Biochem. Pharmacol.*, **8**, 52.
Halbert, S. P., 1961, *Biochem. Pharmacol.*, **8**, 75.
Shore, P. A., Silver, S. L., and Brodie, B. B., 1955, *Experientia*, **11**, 272.
Supek, Z., Randić, M., and Lovašen, Z., 1961, *Int. J. Rad. Biol.*, **4**, 111.
Varagić, V., Stepanović, S., and Hajduković, S., 1962, *Arch. int. Pharmacodyn.*, **138**, 113.
Titus, E., and Udenfriend, S., 1954, *Fed. Proc.*, **13**, 411.