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METABOLISM AND ACTIVITY OF ZOXAZOLAMINE IN WHITE RATS DURING FORCED IMMOBILIZATION WITH AND WITHOUT HYPERTHERMIA

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I. INTRODUCTION

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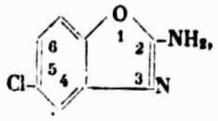
The activity and metabolism of medicinal substances can be modified in animals which have been subjected to stress. The pharmacological and biochemical consequences are functions of the nature and intensity of the aggression, the nature of the medication and the species and even the strain of the animal (1, 3, 12, 13, 14, 20, 21, 22, 23, 24, 25, 26, 27).

In an earlier study [9] we showed that in the rat, forced immobilization brings about a potentialization of the hypnotic effects of hexobarbital and of barbital by distinct mechanisms: inhibition of catabolism for hexobarbital, better penetration of the blood-brain barrier for barbital. Adding a further stress to simple restraint, /38 such as an abrupt lowering of the ambient temperature which produces a state of hypothermia, magnifies the observed potentialization by producing a very marked increase in reactivity vis-a-vis these effectors [19].

Does forced immobilization of the rat, whether or not accompanied by hypothermia, lead to modification of the response to the action of other central depressants which are not hypnotics? To contribute to this question, in the present paper we study the influence of restraint of rats held either at normal body temperature or at lowered body temperature, on the effects of a muscle relaxant, zoxazolamine, whose action is exercised through the central nervous system and which is easily metabolized by the organism [11]. Having found an exaggeration of the myorelaxant activity under our experimental conditions, we performed determinations of zoxazolamine in the blood, brain and liver which provide some data on the mechanism of this phenomenon.

II. TECHNIQUES

Zoxazolamine, 2-amino-5-chlorobenzoxazole, 

was used after solubilization as the hydrochloride, as a 1% aqueous

*Numbers in margin indicate foreign pagination.

solution. It was administrated intraperitoneally (i.p.) at a volume ranging from 0.2 to 0.6 ml per 100 gm body weight.

The experimental animals were female rats--the action of zoxazolamine is independent of sex [15,5]--of the Wistar strain, aged 8 to 10 weeks and weighing 135 to 187 gm. They were given water but no food for 24 hours before experimentation, which does not, however, modify the activity of zoxazolamine (personal verification and [16]).

Restraint was accomplished by immobilization inside a metal mesh by the technique we have described previously [6]. Hypothermia developed during restraint when it was imposed at ambient temperatures below that of thermal neutrality for the rat [2,4,7,18]. To maintain the animals at normal body temperature during restraint, they were placed in a chamber held at a temperature of 27 ± 0.5 °C. To create more or less deep hypothermia during restraint, the animals were placed for variable lengths of time in an environment held at 10 ± 0.5 °C [18].

At the end of restraint, which lasted from 30 minutes to 18 hours, and no matter what the ambient temperature was during restraint, the animals were placed at the usual laboratory temperature of 22 ± 1 °C, treated immediately with zoxazolamine, and held in this environment /39 for the duration of the experiment. More rarely, zoxazolamine was administered to restrained or free rats held at 27 ± 0.5 °C.

The experiments were carried out on groups of at least 10 rats; for each of them, unrestrained control rats were tested on the same day under the same conditions of fasting and ambient temperature as the restrained rats.

The action of zoxazolamine is manifested by a flaccid paralysis characterized by its latency period and its duration. The latency time (TL) corresponds to the interval between administration of zoxazolamine and loss of postural reflexes. The duration of paralysis (DP) was determined by measuring the time necessary for the rats, placed in

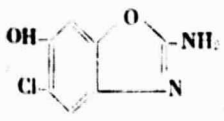
lateral decubitus, to recover reflexes of posture and begin to move about. Although the animals were tested under conditions as rigorously identical as possible, individual differences, often large, were obtained for durations of paralysis; they would be a function of the concentration of the active substance at the site of action, the concentration itself depending in particular on the metabolic rate, which varies from one animal to another [17]. This is why tests must be performed on a large enough number of animals that the results can be analyzed statistically.

The body temperature (TR) was determined by means of a thermocouple introduced into the sigmoid.

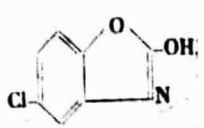
The presence of gastric ulcers was noted on sacrificing the animals by decapitation, at the end of the test; the stomachs removed were opened by an incision along the large curvature and examined after gentle rinsing with distilled water. The percentage of rats with ulcers was noted. The severity of the gastric attack was evaluated with the aid of a notation system which we have adopted in earlier work [6,8]: 0 : normal stomach; 1 : one ulcer; 2 : two ulcers; 3 : three ulcers; 4 : four ulcers or more; the average index was calculated for each group of rats.

Determination of zoxazolamine levels in the blood, brain and liver was carried out according to the spectrophotometric technique of Burns, Yu, Berger and Gutman [10]. In preliminary testing we confirmed that quantities of zoxazolamine ranging from 30 to 50 µg added to the blood and to cerebral and hepatic tissue are recovered with 92% efficiency.

The presence of metabolites of zoxazolamine, 6-hydroxy-zoxazolamine,



and



which respectively represent about 50% and 2% of the dose administered, does not interfere with quantitation of zoxazolamine [11].

The statistical significance of the pharmacological and biochemical results was established by using the Student t test.

III. EXPERIMENTAL RESULTS /40

We shall compare the pharmacological and biochemical results obtained for rats held either at normal body temperature or subjected to hypothermia and restrained for a variable period of time, with results for free (unrestrained) control rats held in the same ambient temperature conditions as the restrained animals.

A. Rats restrained at normal body temperature.

To take into account the influence of restraint by itself, we eliminated the hypothermia which it engenders by subjecting the animals to an ambient temperature of 27 ± 0.5 °C while they were immobilized. Treatment with zoxazolamine was applied at the conclusion of restraint and the animals were then held at an ambient temperature of 22 °C or 27 °C.

1. Pharmacological results

a. Ambient temperature of 22 °C.

The latency period and the duration of paralysis produced by doses of zoxazolamine ranging from 40 to 60 mg/kg were determined on rats which had just undergone restraint for 135 minutes and on free control rats (Table I).

The 40-mg/kg dose, inactive in the free rats, became effective in 50% of the animals which had been immobilized. The 50- and 60-mg/kg active doses produced significantly longer durations of paralysis ($P < 0.001$) in the previously restrained rats than in the free controls. The potentialization observed did not intensify with the duration of restraint. Thus, the 50-mg/kg dose of zoxazolamine, which produced 26 ± 1.5 min of paralysis after 2 hr. 15 min. of res-

traint (Table I), produced 30 ± 1.7 min and 24.5 ± 2.6 min of paralysis in groups of 10 rats to which restraints of 4 hr. 30 min. and 18 hr., respectively, were applied.

We point out that under our experimental conditions the temperature-lowering effects of zoxazolamine are identical in previously-restrained animals and in free animals (Table I).

To determine the persistence of the potentializing effect of restraint, we administered the 50-mg/kg dose of zoxazolamine to groups of 10 rats at variable times after conclusion of the aggression /41
(Table II). The potentialization ($P < 0.001$) observed in animals tested immediately after cessation of the stress, was still manifested, though less strongly($0.01 < P < 0.05$), for 4 hours after conclusion of restraint. 24 and 48 hrs. after conclusion of restraint, the duration of paralysis was not significantly different from that of the free controls.

b. Ambient temperature of 27 °C.

Elevating the temperature of the environment shortens the myorelaxant activity of zoxazolamine [5]. Free and restrained rats subjected to the action of zoxazolamine, no longer in the usual 22 °C environment, but at the 27 °C ambient temperature, showed shorter durations of paralysis but potentialization ($0.001 < P < 0.01$) under the influence of restraint was always evident (Table I).

2. Biochemical results

The levels of zoxazolamine in the blood, brain and liver were /42
determined for rats in which the duration of paralysis produced by the 50-mg/kg dose was potentialized by restraint for 135 min. The quantitations were made either 3.7 min (end of latency period), or 10, 30, 60, 90 and 240 min. after administration of the myorelaxant. The results were compared to those obtained for free control rats sacrificed at the same intervals. All these results appear in Table III; several observations can be made from them:

- at the end of the latency period of the restrained animals (3.7 minutes), shorter than that of the free animals (5.5 minutes), the blood and brain levels for the two groups of rats can be superposed, although the zoxazolamine content of hepatic tissue is higher ($0.01 < P < 0.05$) in the stressed rats;
- 10 to 60 minutes after administration of zoxazolamine, the blood and cerebral levels of the free and the restrained rats were not significantly different; the hepatic level, originally higher in the restrained animals (to 10 min) then became equal to (at 30 min) or even less than (at 60 min) that of the free controls; /43
- 90 to 240 minutes after administration of the myorelaxant, the blood and cerebral levels of the restrained rats were less ($0.001 < P < 0.05$) than those of the free controls while the hepatic levels were not significantly different for the two groups of animals.

B. Rats restrained with hypothermia

The animals were subjected to a two-fold stress: restraint and lowering of the temperature of their surroundings. The restrained animals held at an ambient temperature of 10 °C developed a hypothermia which depended on the duration of restraint; under our test conditions, this lay between 33.7 and 24.2 °C. During their stay at 10 °C, the free control animals showed only a very slight lowering (1 °C) of their body temperature. /44

1. Pharmacological results

Two series of experiments were carried out to determine the intensity and duration of the potentialization phenomenon observed in the stressed animals.

a. Potentialization of the action of zoxazolamine /45

At the end of the aggression, which lasted for 30 to 135 min., the animals were replaced in their usual thermal environment (22 ± 1 °C) and immediately treated with zoxazolamine at doses ranging from

10 to 50 mg/kg. The results obtained are shown in Table IV.

The 50-mg/kg zoxazolamine dose which produced paralysis of free rats which lasted 24 ± 1.4 min, had a significantly longer effect, 57 ± 2.7 min ($P < 0.001$) even after a short restraint of 30 min. accompanied by a 3.6 °C lowering of the rectal temperature. The potentialization is intensified with prolongation of restraint (30 to 135 min) which in turn produces greater and greater hypothermia (33.1 and 24.2 °C). After 135 minutes of restraint, the paralysis can last 9.7 times longer than that of free control animals.

Normally inactive doses of zoxazolamine--20, 15 and even 10 mg/kg--become effective after restraint for 90 to 120 min, which produces hypothermia of 27.8 and 25 °C, respectively.

We also find that in stressed rats the temperature-lowering action of zoxazolamine itself does not appear: the rectal temperature at the end of paralysis is not significantly lower than that recorded at the moment the myorelaxant is administered; in these animals a certain degree of rewarming is even observed when the duration of paralysis is long enough (Table IV).

b. Duration of potentialization.

After restraint for 90 to 135 mins. at an ambient temperature of 10 °C, the rats were held at the ambient temperature of the laboratory (22 ± °C) and treated with zoxazolamine (20 and 50 mg/kg) at intervals ranging from 1 hr to 48 hrs. after cessation of stress, during which intervals the hypothermia progressively disappeared. Free control rats held under identical thermal conditions were tested simultaneously.

The results of table V show that the 20-mg/kg dose of zoxazolamine, ineffective in the controls and which produced paralysis in rats which had undergone restraint for 120 minutes accompanied by hypothermia to 25 °C, still showed an action when it was administered 3 hours after the end of stress. Four hours after the end of stress the paralysing

action of the effector was no longer detectable.

The 50-mg/kg dose, normally effective, still had a potentialized action ($P < 0.001$) 18 hr. after cessation of stress although the animals no longer showed any hypothermia; 24 hrs. after restraint, the animals were still paralyzed longer than the controls tested simultaneously, but in view of the scatter of the results, the difference between the two groups is no longer significant ($P > 0.05$). /46 Finally, 48 hrs. after restraint for 135 mins., the duration of paralysis was comparable to that for the controls ($P = 0.10$).

2. Biochemical results

The levels in the blood, brain and liver were determined comparatively for rats which had been immobilized for 120 mins. and had hypothermia to 25 to 27 °C and for free control rats. Two series of experiments were carried out, using: a. the 20-mg/kg dose of zoxazolamine, ineffective in the controls but producing in the stressed animals a duration of paralysis varying from 32 ± 2.3, depending on the group of rats, to 42 ± 4 mins.; b. the 50-mg/kg dose of zoxazolamine, which produced a duration of paralysis of 24 ± 1.4 min. in the controls and 155 ± 16 min. in the previously stressed animals /48 (Table IV).

a. Administration of 20 mg/kg of zoxazolamine (Table VI).

At the end of the latency period of the stressed animals (4.5 minutes), the levels of zoxazolamine in the blood and liver were higher ($0.005 < P < 0.01$ and $P < 0.001$ respectively) than in controls sacrificed at the same time, while the levels in the brain did not differ significantly for the two groups.

Ten minutes and 35 minutes (end of paralysis of stressed animals) after administration, the blood, brain and liver zoxazolamine levels were respectively higher ($P < 0.001$ and $0.001 < P < 0.01$) in the hyperthermal restrained animals than in the control animals.

b. Administration of 50 mg/kg of zoxazolamine (Table VI).

The animals were sacrificed 10, 30, 90 and 240 mins. after administration of the myorelaxant. The zoxazolamine levels in the blood of animals restrained hypothermally remained higher ($0.001 < P < 0.01$) than those of the control animals even after 4 hr; the cerebral levels of the two groups of animals superposable after intervals of 10 and 30 mins., were significantly higher ($0.001 < P < 0.01$) in the stressed animals 90 and 240 mins. after administration of zoxazolamine; the hepatic level, higher ($0.001 < P < 0.01$) in restrained animals 10 min. after administration of zoxazolamine, was not significantly different from that of the controls after 30 and 90 mins. and became higher than the latter after 240 mins.

IV. RESULTS AND DISCUSSION

This work has revealed the impact on the activity of zoxazolamine of an aggression consisting of forced immobilization for a more or less long time, whether or not accompanied by hypothermia.

Potentialization of the myorelaxant effects of zoxazolamine was found in all stressed animals. We have established the characteristics of this potentialization and attempted to elucidate its mechanism by means of parmocological and biochemical testing.

We note that our results contradict those of Bousquet, Rupe and Miya [3], who observed a shortening of the action of zoxazolamine in animals stressed by ligature of the paw. It is probable that this disagreement can be attributed to the difference in the nature and intensity of the aggression to which one can also add the difference in the rat strains tested.

a. Characteristics of the potentialization /49

1. Immobilization

To evaluate the effects produced by forced immobilization alone, we eliminated the hypothermia which it produces by placing the test animals and the controls in a chamber maintained at 27 ± 0.5 °C.

Restraint for 135 mins. brought about an increase in the myorelaxant power of zoxazolamine ($P < 0.001$), with the ratio of durations of paralysis for restrained and free rats ranging from 1.7 to 2.2 (Table I). In addition, a dose of zoxazolamine inactive in all free animals became active in 50% of the animals subjected to immobilization: the ratio of the limiting effective doses in restrained and in free animals was 0.8 (Table I).

Potentialization did not intensify with length or restraint, even when it reached 18 hrs. It persisted for at least 4 hrs. after cessation of stress (Table II).

2. Immobilization and hypothermia

To determine the influence of immobilization accompanied by hypothermia, we maintained the restrained animals and the controls in a chamber at 10 ± 0.5 °C. Even short restraint, 30 min., which resulted in a lowering of body temperature by 3.6 °C, significantly ($P < 0.001$) prolonged the duration of paralysis produced by zoxazolamine (Table IV).

The potentialization intensified with duration of restraint and degree of hypothermia. For 135 mins. restraint, which was accompanied by a 13 °C lowering of body temperature, the ratio of the durations of paralysis of restrained and of free rats was 9.7. The potentializing effect persisted for at least 18 hr. after cessation of stress (Table V).

A dose of zoxazolamine inactive in normal animals became active in stressed animals, the ratio of the limiting effective doses for animals restrained 120 mins. with hypothermia and for control animals was about 0.2 (Table IV).

b. Mechanism of potentialization.

Our testing has been oriented toward examining two possibilities which could account for an increase in pharmacological activity: slowing of the catabolism of the active substance, or elevation of the sensitivity threshold of the animal toward the drug. /50

1. Immobilization

Under the influence of restraint, the catabolism of zoxazolamine is not modified, at least for the duration of paralysis (Table III). In contrast, the reactivity of the animals toward the myorelaxant is increased. Thus, a normally ineffective dose of zoxazolamine can produce paralysis in animals which have previously been restrained (Table I). Furthermore, determination of cerebral zoxazolamine levels at the end of the latency period for restrained animals showed that at identical cerebral levels, although all restrained rats were paralyzed, 90% of the free control animals still were not (Table III).

2. Immobilization and hypothermia

Under the influence of restraint accompanied by hypothermia, catabolism of zoxazolamine is slowed, especially at the cerebral level. This slowing appears at the onset of paralysis for the 20-mg/kg dose (Table VI), although it appears only belatedly with the 50-mg/kg dose--around 90 mins. after administration of the myorelaxant (Table VI).

The reactivity of the restrained and hypothermic animals was considerably increased over that of normal rats. A zoxazolamine dose only 20% of the limiting effective dose for the controls produced paralysis in rats which had been restrained with hypothermia (Table VI).

A biochemical proof of this hypersensitivity was furnished in particular by examination of the cerebral zoxazolamine levels after

administration of a dose (20 mg/kg) which was ineffective in the controls. In previously stressed animals, in which the penetration of zoxazolamine to the brain is identical to that in the controls (Table VI), paralysis developed and remained with levels very much smaller than those necessary to produce paralysis in normal animals (Table VI).

Let us, therefore, look at the level of zoxazolamine in the brain at the end of the latency period. In the normal animal, this level is constant for doses of zoxazolamine (50 and 60 mg/kg) which produce very different durations of paralysis (21 ± 2.6 and 81 ± 7 min. respectively). But in rats which have undergone restraint for 135 mins. and a lowering of their rectal temperature by 13.6 °C, paralysis is engendered even by the 20-mg/kg dose of zoxazolamine, with a very significantly lower cerebral zoxazolamine level (32 ± 3.1 μg/g instead of 76 ± 5.5 and 80 ± 3 μg/g), one which is equal to that for normal animals (37 ± 1.8 μg/g) which have been treated with the same dose (20 mg/kg) but show no sign of paralysis (Table VII).

c. Comparison of effects produced by restraint alone and by restraint accompanied by hypothermia. /51

Restraint accompanied by hypothermia is a stress whose intensity, much greater than that of restraint alone, can be assessed objectively by its consequences to the gastric mucosa, especially by the appearance of so-called restraint ulcers. Although the rats subjected to restraint for 135 mins. with normal body temperatures had practically no lesions of the gastric mucosa, among the hypothermic rats restraint for even 30 mins. led to formation of ulcers in 70% of the animals. Furthermore, as Table VIII shows, gastric attack intensified with length of restraint, which in turn produced deeper and deeper hypothermia.

Thus, it is not surprising that the pharmacological consequences are more severe for hypothermally-restrained rats than for those held /52 at normal body temperature and that in addition they increase with length of immobilization of the hypothermic animals.

Potentialization of the myorelaxant activity of zoxazolamine by restraint alone is small compared to that produced by immobilization accompanied by hypothermia: the ratio of durations of paralysis for restrained rats and free ones goes from 2.2 (normal temperature) to 9.7 (hypothermic).

As for the mechanism of potentialization, it appears that the slowing of zoxazolamine catabolism can be attributed to the hypothermia, since it is unchanged in rats restrained at normal body temperature.

The effect of restraint on the sensitivity of the central nervous system toward zoxazolamine is greater in animals subjected to restraint accompanied by hypothermia. Although a dose of zoxazolamine 20% of the normally effective dose still produces paralysis in the latter, in animals restrained at normal body temperature it is necessary to use a dose four times as large (80% of the limiting effective dose for normal controls). The biochemical results confirm this conclusion: the crebral levels of zoxazolamine which can produce and maintain paralysis can be smaller than those necessary for rats restrained at normal body temperature (Tables III and VI).

Finally, we note that although restraint, whether or not accompanied by hypothermia, does not change cerebral penetration of the myorelaxant (identity of cerebral zoxazolamine levels at the end of the latency period in restrained and in free animals, Tables III and VI), it does increase the permeability of liver tissue for zoxazolamine. At the end of the latency period, and at 10 min. after administration of the zoxazolamine, its concentration in the liver is significantly higher in stressed animals than in control animals (Tables III and VI).

V. CONCLUSIONS

1. We have established the experimental conditions which allow zoxazolamine to exercise its myorelaxant effects on the white rat subjected to forced immobilization, in the absence or in the presence of hypothermia.

2. Restraint of the white rat produces potentialization of the myorelaxant activity of zoxazolamine, much stronger in the presence of hypothermia than in its absence.

3. Determination of cerebral zoxazolamine levels in rats restrained with and without hypothermia provides some data describing the mechanism of the potentialization phenomenon. This is related to the higher reactivity of the central nervous system of the rat to zoxazolamine which is established under the influence of restraint, and is intensified when the restraint is accompanied by hypothermia. In hypothermally restrained animals, a slowing of zoxazolamine catabolism is added to this main cause. /53

TABLE I

Influence of restraint on duration of paralysis produced by zoxazolamine in rats with normal body temperature.

Rats number	treatment ***	Zoxazolamine mg kg	% of animals paralyzed	TL min	DP min	TR °C
10	free	40	0	-	-	-
10	restrained	40	50	6	12 ± 1,6	36,8
40	free	50	72,5	5,5	12 ± 1,5	35,6
40	restrained	50	97,5	3,2	26 ± 1,5 (*)	35,8
10	free	60	100	3,4	53 ± 5,3	34,9
10	restrained	60	100	3,3	89 ± 6,4 (*)	33,6
10(****)	free	60	100	3,3	36 ± 4,5	36,1
10(****)	restrained	60	100	3,3	60 ± 5,5 (**)	36,1

TL: Latency period; DP: duration of paralysis; TR: rectal temperature at end of paralysis.

* P < 0.001

** 0.001 < P < 0.01

*** length of restraint was 135 min

**** rats held at ambient temperature of 27°C instead of 22°C during paralysis.

TABLE II

Duration of potentialization of zoxazolamine action (50 mg/kg) by restraint, rats with normal body temperature

I. Free rats II. Restrained rats(*) Interval between cessation of restraint & administration of zoxazolamine hr	number of rats	Duration of paralysis min.	P, compared to free rats
	20	17 ± 2,2	
0	20	30 ± 6	< 0,001
1	10	26 ± 3,2	< 0,05 > 0,01
2	10	26 ± 3,6	< 0,05 > 0,01
4	10	29 ± 4,6	< 0,05 > 0,01
24	10	25 ± 4	< 0,10
48	10	20 ± 2,5	< 0,50

(*) Length of restraint was 135 min

TABLE III

Influence of 135-min restraint on blood, cerebral and hepatic levels after administration of 50 mg/kg of zoxazolamine, rats with normal body temperature.

Interval between administration of zoxazolamine and sacrifice	treatment of rats	Zoxazolamine levels (*)		
		blood	brain	liver
min		µg/ml	µg/g	µg/g
3,7 (**)	free	37 ± 1,8	61 ± 3,5	116 ± 7,4
3,7 (***)	restrained	41 ± 2,5	65 ± 2,3	139 ± 6,8(.)
10	free	32 ± 1,5	57 ± 3	65 ± 3
10	restrained	33 ± 1,8	57 ± 4,8	83 ± 4,3(..)
30	free	37 ± 1,5	71 ± 6,5	72 ± 4
30	restrained	34 ± 1,5	64 ± 4	72 ± 3
60	free	36 ± 1,3	64 ± 5	74 ± 2,7
60	restrained	34 ± 1,4	61 ± 1,1	61 ± 1,3(...)
90	free	32 ± 2,8	54 ± 5	62 ± 5,5
90	restrained	23 ± 1,7(.)	40 ± 2,5(.)	59 ± 3,6
240	free	17 ± 1,5	36 ± 3,6	39 ± 5
240	restrained	12 ± 1 (.)	24 ± 2,4(.)	29 ± 2,4

(*) averages of 10 determinations
(**) 90% of the rats still not paralyzed
(***) end of latency period, all rats paralyzed

0.05 P 0,01 0,001 P 0,01 P 0,001.

TABLE IV

Influence of restraint on duration of paralysis produced by zoxazolamine in hypothermic rats

No. of rats	length of restraint	Zoxazolamine	% of animals paralyzed	TL	DP	TR 1	TR 2
	min	mg/kg		min	min	°C	°C
Free control rats							
10	0	50	100	4	24 ± 1,4	36,7	35
10	0	45	60	4,5	12 ± 1,3	36,5	35,8
10	0	40	20	5,5	8	36,8	36,2
58	0	20	0	-	-	36,4	-
Restrained rats							
10	30	50	100	4	57 ± 2,7	33,1	35
10	60	50	100	3,9	69 ± 8	30,3	32,2
10	90	50	100	3,7	97 ± 10	29,5	32,3
10	120	50	100	3,3	155 ± 16	27	31,7
10	135	50	100	3,8	233 ± 17(*)	24,2	30,3
10	30	20	0	-	-	30	-
10	60	20	20	10	7	31	29,5
10	90	20	40	10	24 ± 7	27,8	28
10	120	20	100	5,2	42 ± 4	25	26
10	135	20	100	6,5	54 ± 5,5	24,3	27,8
10	120	15	90	5,8	28 ± 4,5	25	26,2
10	120	10	40	7,8	25 ± 6	24,9	24,5

TL : latency period; DP : duration of paralysis
TR 1: rectal temperature before zoxazolamine administration
TR 2: rectal temperature at end of paralysis

TABLE V

Duration of potentialization of zoxazolamine action by restraint, hypothermic rats.

length of restraint	TR 1	TR 2	Interval between cessation of restraint & administration of zoxazolamine	zoxazolamine	% of animals paralyzed	TL	DP
min	°C	°C	hr	mg/kg		min	min
0 (*)	-	36,4	-	20	0	-	-
120	25	25	0	20	100	5,2	42 ± 4
120	25	28	1	20	100	3,6	24 ± 2
120	24,9	32,2	3	20	50	4,6	7 ± 1
120	25,2	34,2	4	20	0	-	-
0 (*)	-	37,5	-	50	100	3,5	18 ± 0,8
90	31	31	0	50	100	3,6	60 ± 5,5 (.)
90	29	38,4	5	50	100	3,9	31 ± 2,5 (.)
90	30,5	37,5	18	50	100	3,4	28 ± 1,6 (.)
0 (*)	-	37,9	-	50	100	3,8	32 ± 6,2
90	29,9	38,8	24	50	100	3,2	52 ± 9
0 (*)	-	37,7	-	50	100	3,6	17 ± 2,9
135	26,1	38,4	48	50	100	3,4	24 ± 2,9

(*) : Free control rats, held at same ambient temp. conditions as restrained rats
TR 1 : Rectal temp. at cessation of restraint
TR 2 : Rectal temp. at moment of zoxazolamine administration
TL : Latency period
DP : Duration of paralysis
(.) : $P < 0.001$ with respect to free controls

TABLE VI

Influence of 120-min restraint on zoxazolamine levels in blood, brain and liver, hypothermic rats.

No. of rats and treatment	Interval between zoxazolamine administration & sacrifice	TR	Zoxazolamine	Zoxazolamine levels		
				blood	brain	liver
	mn	°C	mg/kg	µg/ml	µg/g	µg/g
10 free	4,5	36,5	20	18 ± 1,6	37 ± 1,8	46 ± 2,4
10 restrained	4,5 (*)	26	20	24 ± 1,1 (..)	32 ± 3,1	85 ± 5,7 (.)
30 free	10	36,4	20	16 ± 0,7	26 ± 1,2	34 ± 1,5
30 restrained	10	25,3	20	24 ± 1,2 (.)	36 ± 2,1 (.)	57 ± 2,2 (.)
10 free	35	36,3	20	12 ± 1,2	21 ± 1,6	28 ± 2,2
10 restrained	35	25,1	20	17 ± 0,8 (..)	29 ± 1,2 (..)	39 ± 1,7 (.)
10 free	10	36,8	50	41 ± 1,7	81 ± 6	104 ± 7
10 restrained	10	26	50	50 ± 1,9 (..)	85 ± 3,5	156 ± 14 (..)
10 free	30	36,9	50	33 ± 2	69 ± 7	73 ± 4,4
10 restrained	30	27	50	42 ± 3,8	71 ± 6	82 ± 4
10 free	90	36,7	50	30 ± 1,8	57 ± 2,6	64 ± 4,5
10 restrained	90	26	50	37 ± 1,1 (..)	71 ± 2,8 (..)	71 ± 2,5
10 free	240	36,4	50	18 ± 1,1	30 ± 2,5	36 ± 2,5
10 restrained	240	25,5	50	26 ± 1,6 (..)	45 ± 3,5 (..)	52 ± 3 (.)

TR : Rectal temperature at moment of zoxazolamine administration

(.) P < 0.05, (..) P < 0.01

(*): End of latency period

TABLE VII

	Zoxazolamine mg/kg	latency period min	duration of paralysis min	Cerebral zoxazolamine level at end of latency period µg/g
normal rats	20	-	0	37 ± 1,8 (*)
	50	3,2	21 ± 2,6	76 ± 5,5
	60	2,6	81 ± 7	80 ± 3
stressed rats	20	4,5	42 ± 4	31 ± 3,1

(*) zoxazolamine level determined after a time corresponding to the latency period of stressed animals, 4.5 min.

TABLE VIII

Hypothermic restrained rats		% of animals with ulcers	index
length of restraint min	hyperthermia °C		
30	33,7	70	2,1
60	31	80	2,2
90	27,8	90	2,5
135	24,2	100	4

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