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ADRENOCORTICAL RESPONSE IN RATS SUBJECTED TO A STRESS OF RESTRAINT BY IMMOBILIZATION WHETHER ACCOMPANIED BY HYPOTHERMIA OR NOT.

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ADRENOCORTICAL RESPONSE IN RATS SUBJECTED TO A STRESS OF RESTRAINT BY IMMOBILIZATION WHETHER ACCOMPANIED BY HYPOTHERMIA OR NOT.

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It is well known that different stresses are capable of stim-/1202** ulating the hormonal activity of the adrenal gland. Restraint by immobilization, used with white rats, under conditions that we have described previously [1], constitutes a stress of special type because it engenders, contrary to most of the other stresses, not an increase, but a lowering of the plasma levels of free fatty acids [2].

Investigations already performed by several authors on immobilized or hypothermic rats demonstrate a hyperactivity of the adrenal glands. Simler et al [3], experimenting with rats subjected to a 24 hour restraint according to the procedure of Rossi and Bonfils [4], examine the urinary level of 17-corticosteroids which are directly related to adrenocortical activity: this level does not vary during the restraint, but undergoes a marked elevation on the day after the stress. These results can be compared with those of Knigge et al [5] according to which in immobilized rats the blood level of corticosteroids is clearly increased during the first two hours following restraint and then falls at the third hour. Szot and Murphy [6] indicate an increase of 2 to 3 fold in plasma and adrenal levels of corticosterone in rats after a one hour exposure to an ambient temperature of 6°C or after ligation of a paw. A significant and rapid elevation of these le-

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**Numbers in margins indicate foreign pagination

vels is also observed by Dechezlepretre and Lechat [7] under the influence of a cold stress accomplished by a 5 minute bath in water at 8-10°C. According to Berlin [8], in rats subjected to fluctuations of the exterior temperature, the plasma level of corticosterone is 1.5 to 2 times higher than that of controls. Finally, Beauvallet and Poggioli [9] subjecting rats to a forced swim in 27°C water, create a significant hypothermia and demonstrate, under these conditions, an elevation of adrenal glucocorticoids.

The objective of the present work is to study the adrenocortical reactivity of normo- and hypothermic rats, subjected to more or less severe restraints and to quantitate the kinetics of the disappearance of the eventual variations, by determining the plasma and adrenal levels of corticosterone.

Material and methods. We use female Wistar rats, 8 week old, weighing 180 to 200 gm and deprived of food and water during the restraint. After 3 minutes of ether inhalation, the animals are immobilized in a flexible metallic cylinder [1], for variable times, and maintained in a constant thermal environment either of 28°C (normothermic animals) or of 24° C (slightly hypothermic animals), or of 10°C (clearly hypothermic animals). Unrestrained rats, serving as controls, are placed in the same conditions of ambient temperature, after having undergone all the accessory manipulations to which the restrained rats were subjected. All the animals are sacrificed by decapitation. The measurement of plasma and adrenal corticosterone are performed according to the fluorometric technique of Silber et al [10] and the operating method of Werk et al [11] in those restrained and those unrestrained (controls) at the same moments, either at the end of the stress or after a rest of variable duration at an ambient temperature of 23 $\pm$ 1°C.

The experiments are comparatively performed on groups of 10 rats (free and restrained). The results which appear in the tables

are averages of 10 measurements and the significance of their differences are evaluated by the Student test.

Results. 1. Free rats. 1. In a preliminary study, we determined, in free rats, the variations of plasma and adrenal levels of corticosterone due to the experimental conditions: fasting, ether inhalation and manipulation preceding immobilization Table I).

Table 1.

INFLUENCE OF THE EXPERIMENTAL CONDITIONS ON THE PLASMA AND ADRENAL LEVELS OF CORTICOSTERONE.

State of the animals (free rats)	Corticosterone levels	
	Plasma µg/100 ml	Adrenals µg/gm
Fed	32 ± 4	6.3 ± 1.3
Fasting	40 ±10	6 ± 1.8
15 min. after ether inhalation	83 ± 6	15.5 ± 1.5
30 min. after ether inhalation	60 ± 8	10 ± 1.9
60 min. after ether inhalation	29 ± 8	4 ± 1
15 min. after manipulations preceding immobilization	78 ± 8	17.5 ± 2.3
60 min. after manipulations preceding immobilization.	34 ± 6	6 ± 1.5

Fasting does not change the corticosterone levels although ether inhalations and the manipulations preceding immobilization of the animals do provoke an increase in the plasma and the adrenals [12].* However, since this elevation disappears completely in 60 minutes, we never sacrificed the immobilized animals nor the corresponding controls until at least 60 minutes after the beginning of each experiment.

2. We can conclude from the experiments performed on free /1205 rats exposed to cold that the increase in the plasma and adrenal levels of corticosterone is significant when the animals are maintained for 1 to 2 1/2 hours at 10°C (Table II).

* These results are in accord with those described by Dechez le pretre and Lechat [7].

II. RESTRAINED RATS. Plasma and adrenal corticosterone levels were determined in two distinct series of experiments: 1) at the end of the stress, whose duration varies from 1 to 5 hours, in a thermal environment of 28, 24 or 10°C; 2) at variable time intervals after the end of the restraint.

1. Plasma and adrenal corticosterone levels at the end of restraint (Table II). a. Rats restrained at 28°C (normothermic). After 2 1/2 hours of restraint, the plasma and adrenal corticosterone levels reach respectively 3.3 and 3.1 times the value

TABLE II

PLASMA AND ADRENAL CORTICOSTERONE LEVELS AT THE END OF RESTRAINT

Lots of animals (rats)	Experimental conditions of the rats		Corticosterone levels	
	Duration of the restraint u	Ambient temperature °C	Plasma µg/100ml	Adrenals µg/g
Controls	2 1/2	28	34 ± 3	6.2 ± 1.5
Restrained		28	113 ± 10(1)	19 ± 1.5 (1)
Controls	1	24	28 ± 6	5 ± 1.1
Restrained		24	104 ± 10(1)	18.5 ± 1.5(1)
Controls	2 1/2	24	32 ± 4	6.3 ± 1.3
Restrained		24	106 ± 6(1)	19.6 ± 1.5(1)
Controls	5	24	23 ± 3	3 ± 0.3
Restrained		24	79 ± 7(1)	15 ± 0.6(1)
Controls	1	10	53 ± 6	9.5 ± 1.5
Restrained		10	134 ± 5(1)	21 ± 2.6(1)
Controls	2 1/2	10	79 ± 4	16.7 ± 2
Restrained		10	129 ± 6(1)	22 ± 1.7(2)

of the controls.

b. Rats restrained at 24°C (moderate hypothermia). Lowering of body temperature by about 2°C appears after 40 min. and then is maintained like a plateau for the duration of the restraint [13], which varies from 1 to 5 hours. Whatever the time of immobilization,

the relationship between the plasma corticosterone level of the restrained animals and that of the free controls lies between 3.3 and 3.7. As for the adrenal corticosterone level, it is 3.0 to 3.7 times more elevated after 1 hour and 2 1/2 hours of restraint and 5 times higher after 5 hours of restraint in comparison to the control animals. /1206

c. Rats restrained at 10°C (hypothermic). The depression of body temperature is respectively 8°C and 17°C after 1 hour and 2 1/2 hours of restraint, although it does not exceed 1°C in the free control rats [13]. In restrained rats, the plasma

TABLE III. PLASMA AND ADRENAL CORTICOSTERONE LEVELS AT VARIABLE TIMES AFTER THE END OF RESTRAINT

Lots of animals (rats)	Treatment undergone by the animals: Duration of restraint h	Ambient Temperature °C	Duration of rest after restraint	Corticosterone levels: plasma µg/100 ml	Adrenals µg/g
Controls	1	24	1 1/2	30 ± 7	5 ± 0.9
Restrained		24		63 ± 10(1)	11.5 ± 1.6(2)
Controls	1	24	2 1/2	29 ± 2	6 ± 1
Restrained		24		45 ± 4(2)	7 ± 1.5
Controls	1	24	3 1/2	32 ± 3	6.3 ± 0.8
Restrained		24		35 ± 3	7.7 ± 1
Controls	2 1/2	24	1 1/2	36 ± 5	9 ± 1.4
Restrained		24		93 ± 6(3)	18.5 ± 1.4(3)
Controls	2 1/2	24	2 1/2	45 ± 5	7.5 ± 0.9
Restrained		24		75 ± 10(1)	12.5 ± 2(1)
Controls	2 1/2	24	3 1/2	32 ± 3	6.5 ± 1
Restrained		24		57 ± 6(2)	12 ± 1.9(1)
Controls	2 1/2	24	17	41 ± 6	7.9 ± 0.6
Restrained		24		40 ± 6	6.6 ± 1
Controls	2 1/2	10	3 1/2	29 ± 6	7.5 ± 1.1
Restrained		10		59 ± 7(2)	11.5 ± 1.6(4)
Controls	2 1/2	10	17	31 ± 4	5.6 ± 0.9
Restrained		10		35 ± 5	7 ± 0.8

(1) $0.01 < P < 0.05$; (2) $0.01 < P < 0.001$ (3) $P < 0.001$; (4) $P = 0.05$.

and adrenal corticosterone levels are greater than those of the corresponding controls. Given that, in the free control rats

maintained at 10°C the plasma and adrenal corticosterone levels are increased, the ratio between the levels of the restrained and free animals are less elevated in this thermal environment than in those of 24° C and 28°C; 2.6 for the plasma and 2.2 for the adrenals after 1 hour of restraint; 1.6 for the plasma and 1.3 for the adrenals after 2 hours of restraint. Let us note, however, that the plasma and adrenal corticosterone levels of the rats restrained at 10°C are of the same order of magnitude as those of the restrained rats maintained at 24°C or at 28°C. These levels could constitute a concentration threshold which cannot be surpassed.

2. Plasma and adrenal corticosterone levels at variable times after the end of restraint (Table III). Measurements of corti- /1207 costerone were made 1 1/2 hours, 2 1/2 hours, 3 1/2 hours and 17 hours after the end of the restraint, which was applied 1 hour or 2 1/2 hours, in an environment maintained at 24°C or at 10°C, and at the same times in the free control rats.

a. Rats that had undergone a 1 hour restraint at 24°C. The plasma and adrenal corticosterone levels, which show a large increase at the end of the stress (Table II), are clearly lowered 2 1/2 hours after the end of the restraint; 3 1/2 hours after, they become equal to those of the free controls.

b. Rats that had undergone a 2 1/2 hour restraint at 24°C. The return to normal of the corticosterone levels is slower than in the preceding experiments: 3 1/2 hours after the end of the restraint a significant difference is present again between the previously restrained animals, again normothermic, and the controls; no difference is detectable after 17 hours.

c. Rats that had undergone a 2 1/2 hour restraint at 10°C. In this case, the restraint is accompanied by a significant hypothermia, but this disappears progressively when the animals are

maintained at an ambient temperature of 23°C. After 3 1/2 hours of rest, the control animals manifest no hypothermia and their plasma and adrenal corticosterone concentration, which had sustained an increase under the influence of cold, again becomes normal. In contrast, the animals having been restrained, whose body temperature is not more than 1°C less than that of the controls, again present plasma and adrenal corticosterone levels greater than normal levels. After 17 hours, these levels are identical in the previously restrained animals and the free controls.

Conclusions: 1. Restraint whether accompanied by hypothermia or not, provokes an increase in adrenal activity.

Rats maintained in normothermia or a slight hypothermia sustain an elevation of plasma and adrenal corticosterone concentration that is at least three times the normal concentration, whatever the duration of the restraint (1 to 5 hours) may be.

The rapidity of the return to normal of the plasma and adrenal corticosterone levels is a function of the duration of the restraint.

2. Exposure to cold creates a slight hypothermia in free rats and a significant hypothermia in the restrained rats; this increases the plasma and adrenal corticosteroid levels in the former, but does not potentiate the stimulation of adrenal activity that is provoked by restraint alone in the latter.

Summary. In white rats, restraint by immobilization, whether accompanied by hypothermia or not, provokes an increase in adrenocortical activity, whose return to normal is a function of the duration of the stress.

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