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Effects of Chronic Noise or Daily Water Restriction on the Pituitary-Adrenal Axis in Male Rats

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Pituitary-adrenal function was studied in male rats chronically stressed with noise as well as under water restriction regimen. Chronic noise did not modify either *in vivo* or *in vitro* corticoadrenal function. Daily water restriction decreased body weight and increased relative adrenal weight as well as the serum levels of ACTH and corticosterone. *In vitro* corticoadrenal responsiveness to ACTH was similar in control and water restricted rats. Thus, no evidence for increased adrenal sensitivity to ACTH in the pre-watering period was found in water restricted rats.

Key words: Corticosterone, Adrenal cortex, Noise stress, Water restriction.

It has been reported that daily water restriction increases both basal corticoadrenal activity and response to an acute stress (7, 20). These findings have been explained by a shift in corticoadrenal rhythmicity. Pituitary-adrenal rhythms depend on daily pattern of food intake (15-17, 21), and water restriction likely alters the pituitary-adrenal axis through its effect on the circadian pattern of food intake. Thus, rats which have access to water in the morning show the peak of corticoadrenal activity just before drinking in contrast to control rats which peak at light off (13, 15). As corticoadrenal sensitivity to adrenocorticotropin is higher at light off in control rats (9, 14), it must be expected that pre-watering corticoadrenal response to adrenocorticotropin would be higher in water restricted rats. However, no attempt has been made to demonstrate it.

On the other hand, it has been found that previous chronic noise, alone or combined with flashing light, reduced corticoadrenal response to the same stimuli (4, 5). Since the effect of chronic

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stress on corticoadrenal sensitivity to adrenocorticotropin has provided contradictory reports (11, 18, 19, 22) we have studied here the pituitary-adrenal function not only in water restricted rats but in rats chronically stressed with noise as well.

Materials and Methods

Male Sprague-Dawley rats weighing approximately 350 g were used. They were housed four per cage in a controlled quarter (light on 7.00-19.00, temperature 21° C) and received food and water *ad libitum* unless otherwise stated. Ten days after their arrival the rats were assigned to three experimental groups: (1) control, (2) chronic noise: rats exposed to 4 hours of daily noise (85 dB) between 8.00 and 12.00 for 12 days, and (3) water restriction: rats which had access to water only between 12.00 and 13.00 for 12 days.

Between 8.00 and 9.00 a.m. the rats were killed on the 13th day by decapitation within 30 s after they were taken from their home cages. Trunk blood was collected at 4° C and centrifuged at the same temperature. Serum obtained was frozen at —20° C. Both two adrenals were immediately removed, trimmed of fat and weighed. After being divided into quarters, each adrenal was preincubated in 2 ml of Krebs-Ringer bicarbonate buffer containing 5 mM glucose (KRBG). Preincubation step was done at 37° C under 95 % O_2 : 5 % CO_2 atmosphere. Thirty min later the medium was discarded and stored at —20° C. Then, one adrenal was incubated in the same conditions as previously stated, the other one was incubated with 400 mIU of ACTH (Nuvacthen, Ciba) in 2 ml of KRBG. After 30 min incubation the medium was removed and stored at —20° C. The effect of ACTH on *in vitro* corticosterone release was calculated as A_2-A_1, where A_1 = corticosterone concentration in the second incubation period of the adrenal without ACTH, and A_2 = corticosterone concentration in the second incubation period of the adrenal with ACTH.

Serum ACTH was analyzed using a commercial radioimmunoassay kit (Cea Sorin). Corticosterone was analyzed as previously described (3). All the samples were analyzed in the same assay. Intraassay coefficients of variation were 10 % for ACTH and 8 % for corticosterone.

The results were analyzed with the Student's «t» test or with ANOVA test. The data were log. transformed before their statistical analysis when variances were statistically different. The effect of water restriction on *in vivo* pituitary-adrenal function was analyzed with the one tailed «t» test since the direction of the changes was known by previous works.

Results

The effect of chronic noise or water restriction on body weight, relative adrenal weight and basal levels of ACTH and corticosterone is depicted in table I. Chronic noise did not modify any variable studied. Water restriction decreased body weight ($p < 0.01$) and increased relative adrenal weight ($p < 0.05$), serum ACTH ($p < 0.05$) and serum corticosterone ($p < 0.02$).

Corticosterone release into the medium in the preincubation period was higher in water restricted rats ($p < 0.05$ vs control rats). In the second incubation period without ACTH no significant difference was observed between control and water restricted rats. The analysis of variance revealed a significant effect by ACTH ($p < 0.05$) but not by previous chronic treatments (control,

Table I. *Effects of chronic noise and water restriction on body weight and pituitary-adrenal function in male rats.*

Means ± SEM are represented. Number of animals in parehtheses. * $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$ vs. control group.

Group:	Body weight (g)	Adrenal weight (mg/100 g b.w.)	Serum ACTH (pg/ml)	Serum corticosterone (μg/100 ml)
Control (7)	469 ± 14	10.3 ± 1.4	107 ± 20	1.5 ± 0.6
Chronic noise (6)	434 ± 13	10.7 ± 0.7	128 ± 26	1.8 ± 0.4
Water restriction (6)	416 ± 11***	11.8 ± 0.5*	364 ± 139*	3.8 ± 1.0**

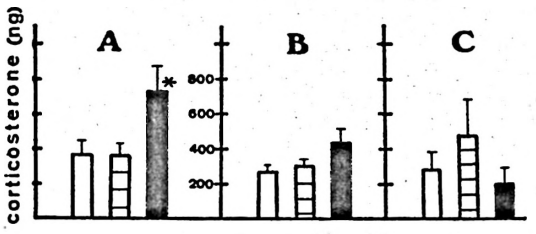

Fig. 1. In vitro *corticoadrenal secretion of male rats.*

Means and SEM of corticosterone released into the medium are represented. In each group n = 5. Open bars indicate control group, shaded bars rats chronically stressed with noise, and closed bars water restricted rats. A) Corticosterone released in the preincubation period; B) Hormone released in the second incubation period without ACTH, and C) Differences between adrenals without ACTH and those with ACTH (400 mIU) in the second incubation period. * $p < 0.05$ vs control group. A significant effect by ACTH ($p < 0.05$) but not by previous chronic treatments on *in vitro* corticosterone secretion was found (C).

noise or water restriction) on corticosterone release into the incubation medium (figure 1).

Discussion

Chronic noise did not modify in vivo pituitary-adrenal activity. Likewise, corcosterone release into the medium during the preincubation period was similar in control and chronically stressed rats. Therefore, there is no evidence for the presence of an inhibitory factor in the adrenals of chronically stressed animals as it has been suggested (18). Sensitivity of the adrenals to ACTH was not altered in rats chronically exposed to noise. It suggests that reduced corticoadrenal response to noise after previous chronic exposure to this stimulus is not due to altered corticoadrenal sensitivity to ACTH. This is corroborated by the finding that rats chronically stressed with noise-light showed a maximal corticosterone response to dog presence similar to that of control rats (2).

Water restricted rats showed decreased body weight and increased relative adrenal weight, serum ACTH and serum corticosterone. These changes induced by the water restriction schedule are in agreement with the results of previous works by us and other authors (1, 6-8, 20). Corticosterone release during the preincubation period was higher in water-restricted than in control rats, likely due to the higher serum ACTH observed in water restricted rats. However, *in vitro* corticoadrenal responsiveness to added ACTH was the same in both groups. Our results do not support the hypothesis that higher absolute corticosterone response to stress of water restricted rats is due to increased corticoadrenal sensitivity to ACTH as a consequence of the shifted circadian

pituitary-adrenal rhythmicity. It has been recently reported that *in vitro* corticoadrenal sensitivity to ACTH in water restricted rats during post-watering period does not explain the rapid drop in serum corticosterone observed *in vivo* just after drinking (10).

Discrepancies between *in vivo* and *in vitro* results are difficult to explain. There is the possibility that some factors which would act *in vivo* were removed or inactivated by *in vitro* incubation. Alternatively, changes either in the distribution volume of corticosterone or in its hepatic metabolism could contribute to high serum corticosterone observed in water restricted rats. In this regard it is known that decreased food intake also decreases hepatic corticosterone metabolism (12), and water restricted rats eat approximately one half the food that control rats (1, 8). Consequently a decreased corticosterone metabolism might be expected.

In vivo and *in vitro* results indicate that chronic noise does not alter pituitary-adrenal function. Water restricted rats show higher pituitary-adrenal activity in the prewatering period, however no sign of an increased corticoadrenal sensitivity to ACTH was found. Although *in vitro* studies cannot be considered as strictly physiological, it appears that factors other than corticoadrenal sensitivity to ACTH should be taken into account in studying the effect of water restriction on prewatering pituitary-adrenal function.

Resumen

Se estudia en ratas macho Sprague-Dawley, el efecto del ruido o la restricción crónica de agua sobre la función corticoadrenal. El ruido crónico no modifica el peso relativo de las adrenales, ni los niveles basales de ACTH o corticosterona. El acceso diario al agua sólo entre las 12 y las 13 horas provoca una reducción del peso corporal, un incremento en el peso relativo de las adrenales y una elevación de los niveles basales de ACTH y corticosterona. Ni el ruido crónico ni la restricción diaria de agua modifican la sensibilidad de la adrenal al ACTH *in vitro*.

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