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Participation of the Nervous System in the Adrenal Cortical Response to Neurogenic Stress *

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The response to immobilization stress has been studied in intact, hypophysectomized and hemidecorticated (bearing removal of the left cortical hemisphere) male rats. Blood and adrenal corticosterone content was measured fluorimetrically in these animals, killed at different intervals after the beginning of the stress.

In intact animals, a monophasic curve, stabilized after 10 minutes, was obtained in blood, and a biphasic response occurred in the adrenal gland, with similar peaks at 5 and 30 minutes.

In hypophysectomized animals a decrease of adrenal corticosterone, parallel to a blood increase, was found during the first 10 minutes. After 15 minutes the elevation was simultaneous in blood and adrenal glands. In hemidecorticated animals no difference was found between the homo and heterolateral glands to the ablation, but the adrenal basal level was higher than that in intact animals.

The gas-liquid chromatographic study has permitted observation of changes in the relative concentrations of the different corticosteroids in the above mentioned groups.

The regulation of the endocrine system is to a great extent automatic, due to feed-back mechanisms and hormonal interactions. The modulatory role of the nervous system on the hypothalamic-hypophyseal axis has also to be considered. Besides, some data suggest a more direct control of the nervous vegetative system on endocrine glands. MELANDER (20) and MARSHALL (19) have brought up the role of the sympathetic nervous system on the thyroid function, through some β_2 adrenergic receptors associated with Adenyl Cyclase activity. In the adrenal gland, the results of previous studies carried out by our research group in animals bearing

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hypophysectomy, adrenal grafts or lesions of the nervous system at different levels support also this hypothesis (8, 10, 28). It has been shown that the vascular response evoked by a neurogenic stress depends on nervous cortical and hypothalamic control, and the parenchymal response seems due to the neurovegetative system, as well as to the hypothalamic-pituitary axis.

In the above mentioned reports the adrenal cortical response to neurogenic stress was studied by means of histological, histochemical and karyometric methods, whose overall evaluation gives a reliable estimation of the functional situation of the gland. Nevertheless, a new approach has been attempted, in order to confirm these results, using a more direct index of the glandular function, such as simultaneous measurement of blood and adrenal corticosterone content. On the other hand, this method allows study of the precocious response to stress.

Materials and Methods

Male Wistar rats, aged between three and four and a half month were used. During the experimental time the animals were held in the same conditions (light, temperature, food, etc.) under which they had been reared. The handling (stress, sacrifice) was carried out in a room adjacent to the stabularium, to minimize uncontrolled stress.

Surgical techniques. The transaural approach was used for hypophysectomies, according with the method of TANAKA (29), modified in this department (8). Twenty four hours were allowed for recovery before the stress was applied. For hemidecortications, once a window was open in the skull, the brain cortex was sucked with a Pasteur pipette connected to a vacuum pump, whose point was bent 90° (fig. 1). The animals were killed 15 days later.

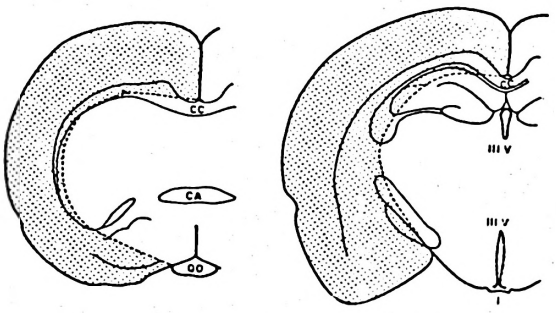

Fig. 1. *Mean extent of the hemidecortications.*
Left: Frontal section at the *Chiasma Opticum* level. Right: Frontal section at the *Commissura Anterior* level. CC: *Corpus Callosum.* CA: *Commissura Anterior.* CQ: *Chiasma Opticum.* III V: Third ventricle. I: *Infundibulum.*

Stress. The neurogenic stress (immobilization) was achieved by having the rats held in a prone position, with their four limbs fixed to a wooden board for five minutes. The stress period commenced from the moment the animals were taken from the cages where they had been living. All rats that survived for more than five minutes waited in individual cages. They were killed by decapitation, to avoid agonic stress.

Quantitative measurement of corticosterone. It was carried out according to the fluorimetric method of DEMOOR (7), except that readings were taken 30 minutes instead of 5 minutes after adding the sulphuric acid-ethanol reagent. This modification was introduced to obtain more reliable data, since the fluorescence intensity increases very quickly at the beginning, reaching a plateau between 25 and 35 minutes. It was verified that, the morphology of the curve intensity-time, as well as fluorescence spectres were similar using standards of samples. Measurement of adrenal protein content was performed following LOWRY (17).

Qualitative determination of corticos-

teroids. Gas-Liquid Chromatography (GLC) was used. Once the dichloromethane extract was purified by thin-layer chromatography (TLC) (2), the procedure of VANDENHEUVEL (31, 32) was followed: reduction of carbonyl groups with sodium borohydride and obtention of trimethylsilyl (TMS) ethers.

Statistical methods. Single (blood samples) or two-way (right and left adrenals) analysis of variance Model I were carried out after logarithmic transformation of the data in order to avoid the heterogeneity of variances (F-max test). Since the overall analysis of variance was found significant in all the cases, and no difference was detected between left and right glands, an SNK *a posteriori* test was applied to study the differences among means. The results are given graphically at the bottom of figures 2, 3 and 4. Statistical methods were taken from SOKAL and ROHLF (27).

Results

Intact animals. The time courses of blood and adrenal corticosterone elevation after stress in intact animals are shown in figure 2. Blood response is monophasic, reaching a plateau after 10 minutes. Adrenal curve is biphasic with a peak at 5 minutes and another of similar size at 30 minutes.

Hypophysectomized animals. The time courses of blood and adrenal corticosterone elevation after stress in hypophysectomized animals are shown in figure 3. For the first 10 minutes, the decrease in the gland parallels the increase in blood, but after 15 minutes a rise occurs simultaneously in blood and in the adrenal gland.

Hemidecorticated animals. The adrenal response to stress has been similar to that shown by intact animals, except that basal levels in the gland were lower in the latter (fig. 4).

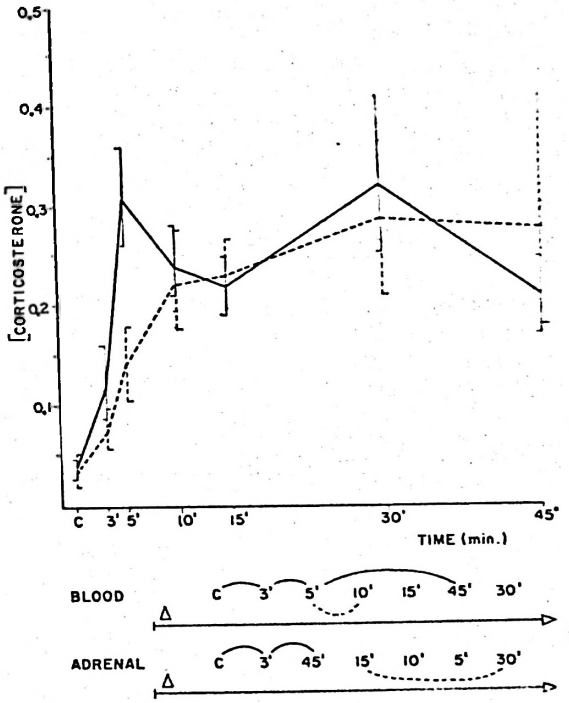

Fig. 2. *Time courses for blood and adrenal corticosterone elevation after immobilization stress in intact rats.*
Mean corticosterone values and confidence limits (n = 9) are plotted against time of survival since the beginning of the stress. ———: adrenal corticosterone (μg/mg Pr); - - - - - : blood corticosterone (μg/ml). Statistical analysis is shown at the bottom: the means are arranged in increasing order as indicated by the arrow. They are significantly different when a line is drawn between: ⌒ = $p < .01$; ⌒ = $P < .05$.

GLC. These are preliminary results, thus, numerical data are not given. The only corticosteroid found in blood was corticosterone. In the adrenal gland, according to increasing retention times, progesterone (P), deoxycorticosterone (DOC) and corticosterone (C) were present in all the groups. In intact animals

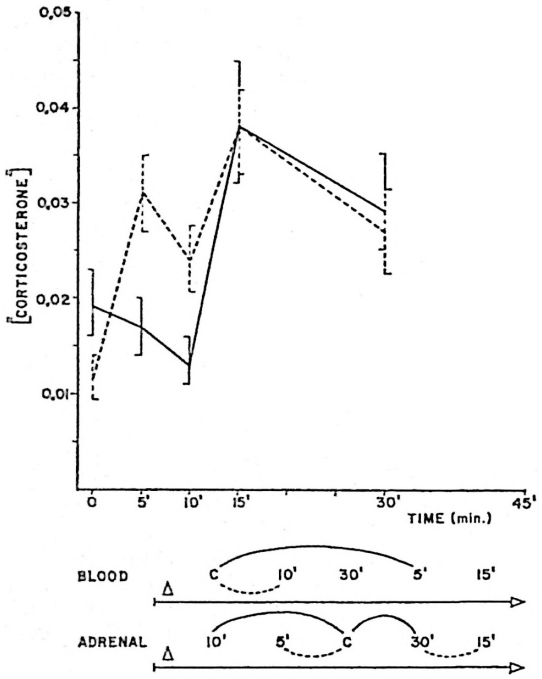

Fig. 3. *Time courses for blood and adrenal corticosterone elevation after immobilization stress in hypophysectomized rats.* See Figure 2 for further details.

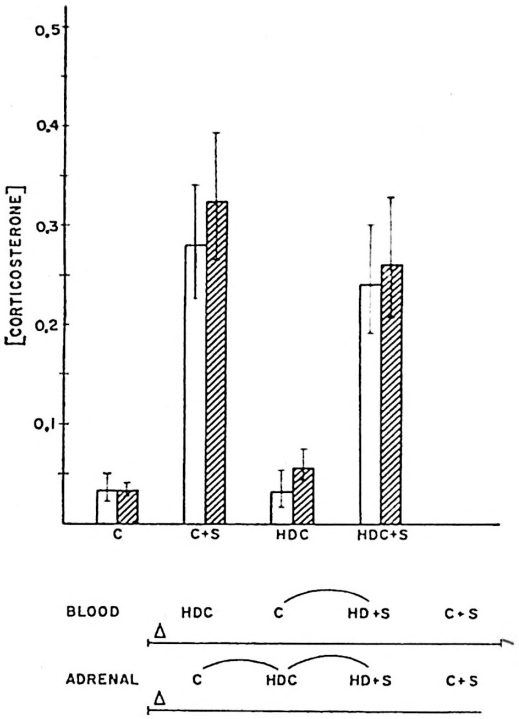

Fig. 4. *Response to immobilization stress in hemidecorticated rats.* Blood (open columns) and adrenal (dashed columns) corticosterone content (n = 12), in basal conditions (C: intact; HDC: hemidecorticated) and 30 min. after the beginning of the stress (C + S: intact; HD + S: hemidecorticated). See Fig. 2 for further details.

with or without stress an unidentified peak appeared, between DOC and C, which was named peak 3. It became very relevant in hypophysectomized and hemidecorticated animals (adrenal homolateral to the ablation) in basal conditions, and disappeared with the stress.

The amount of corticosterone, related to the other steroids, increased in the groups subjected to stress, and the ratio P/DOC was reversed (DOC increased) in hemidecorticated animals, with or without stress, with regard to intact or hypophysectomized animals.

Discussion

Several forms of response to acute stress have been reported, depending on the type of stimulus and the way it has been applied. Ether induces a quick and persistent rise in plasma ACTH and corticosterone. If the animal is previously anaesthetized corticosterone increases slowly, but reaches the maximum more or less at the same time as the former. The handling of the animal for an interval similar to that employed for ether anaesthesia reduces the response to a quick and brief rise of ACTH, as well as corticosterone (5, 6, 15). Thus, two types of response can be described: one is precocious and transient, due to «emotional» stimuli, and the other, slow and persistent, due to «systemic» stimuli. If the emotional component is not eliminated, or the intensity or duration of the stimulus in-

creases (18), a mixed response can be obtained, with a quick and persistent [sometimes in two phases (3)] increase in plasma corticosterone, and a biphasic curve, with a peak between 2 and 3 minutes, and another between 20 and 30 minutes in plasma ACTH (6). A similar behaviour has been described for CRF as well (25).

The curve obtained in plasma under immobilization stress is of the mixed type (fig. 2). The precocious response of the adrenal gland has not been reported yet. The latter rise, around 20 or 30 minutes after the beginning of the treatment, has been studied under ACTH or different types of stress (1, 25). The curve shown in figure 2 is biphasic, and looks strikingly similar to that reported for ACTH, except for a delay of 2 or 3 minutes, which is the latency interval described *in vitro* between the addition of ACTH and the release of corticosterone (4).

The fact that previous attempts have failed to show corticosteroidogenic response to neurogenic stress in hipophysectomized animals can be explained because pentobarbital anaesthetized rats were used (11, 23), and this drug prevents the rise of corticosterone induced by neurogenic stimuli (9). According to the data presented here (fig. 3), it is possible to distinguish two phases in the adrenal response. At the beginning, release of corticosterone seems predominant, since decrease in adrenal content parallels increase in blood. The later increase in adrenal steroid content suggests an active process of corticosterone synthesis. These results are in agreement with those obtained previously with morphological methods, which showed a certain degree of cellular hyperactivity (8).

The existence of an initial period of corticosterone release suggests some form of hormone storage. The lack of organular storage systems (like catecholamine droplets in the adrenal medulla) has been established for cortical adrenal cells. Nevertheless, two pools of corticosterone, dyalizable and non-dyalizable, have been reported, as well as changes in the subcellular distribution of these two forms under different treatments: ACTH, hypophysectomy, etc. (13, 14).

Other authors have been able to show a certain degree of basal corticosterone synthesis in hypophysectomized animals, by studying the transformation of labeled cholesterol into radioactive corticosterone (26). In our study, none of the previous ACTH could be present in the animals, since the half-life of this hormone is about 10 minutes, and they were killed twenty-four hours after the hypophysectomy. Only animals bearing total hypophysectomy were used (fig. 5). A small fragment of *pars tuberalis* of the adenohypophysis, whose function is still unknown (21), remained around the upper pituitary stalk. The infusion of CRF by way of the internal carotid artery has no steroidogenic effect in such animals (22), therefore, the small rate of synthesis observed could not be attributed to the remaining fragment of *pars tuberalis*. On the other hand, the interval between the beginning of the

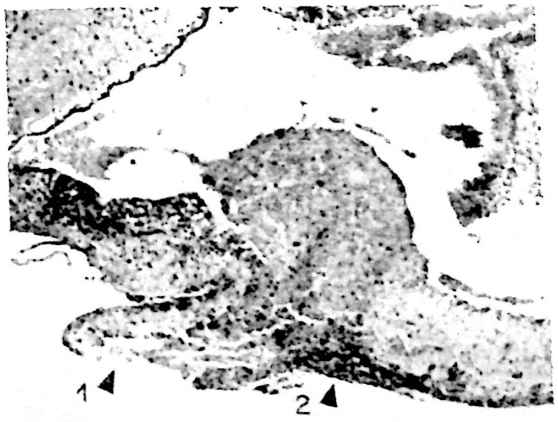

Fig. 5. *Pituitary stalk after transaural hypophysectomy.*
Sagittal section of the diencephalon showing the floor of the third ventricle. 1: Remaining pituitary stalk. 2: Remaining *Pars Tuberalis*.

stress and the increase in adrenal corticosterone (at least 10 minutes) makes unlikely the possibility that the latter is mediated by ACTH, whose effects are apparent after 3 minutes in intact animals.

Once the pituitary factor has been avoided, the steroidogenic response could be attributed to the neurovegetative factor.

The results obtained in animals bearing a removal of the left cortical hemisphere agree with previous results (28) in the slight basal hyperactivity found at the glandular level. Nevertheless, we have been unable to confirm the difference between homo and heterolateral adrenals (24, 28). It could be explained because the vegetative cortico-spinal pathway described by ULLÁN (30) is bilateral, although mainly heterolateral. Thus, the cortical influence has not been completely avoided in the side of the ablation. Moreover, the response obtained in hypophysectomized animals is ten times lower than the normal one, and is probably masked in the presence of ACTH. Furthermore, the process of telencephalization is relatively rudimentary in the rat. 15 days after the operation, the animals showed a practically normal motility, which implies a great degree of substitution by lower centres. Similar events could happen at the neurovegetative system level.

Only two reports dealing with GLC of rat adrenal steroids were found. The absence of some of the steroids mentioned by KITTINGER (16), like aldosterone, dehydrocorticosterone, etc., could be explained because of the different procedures of purification and preparation of derivatives. According to the retention times, peak 3 might correspond to 11-hidroxyandrostenedione, 18-hidroxyprogesterone, 11-ketoprogesterone or some unidentified steroid. HOLZBAUER (12) has studied the effect of the stress on the concentration of pregnenolone, progesterone and corticosterone. The latter shows in all the cases the highest amount. However, its increase, related to those of the other steroids mentioned above, is lower, which does not agree with our results. It should be taken into account that, the kind of stress (cold) as well as the survival period (15 minutes instead of 30 in our study) were different.

The results presented here suggest that some differences exist among the relative concentrations of the various corticosteroids of the rat adrenal gland subjected to different situations, which implies some modifications of the steroid metabolism. Further studies of these modifications can bring to light interesting data in order to understand the function of the adrenal cortex.

Resumen

Se estudia la respuesta al *stress* neurógeno de inmovilización en ratas macho, intactas, hipofisectomizadas y hemidecorticadas, determinando fluorimétricamente el nivel de corticosterona en sangre y en ambas suprarrenales, en animales sacrificados a distintos intervalos desde el comienzo del *stress*.

En ratas intactas se ha obtenido, en sangre, una curva monofásica, con estabilización a partir de los 10 minutos, y en suprarrenal, una respuesta bifásica, con un pico a los 5 minutos y otro de similar magnitud a los 30.

En animales hipofisectomizados se ha encontrado, en los primeros 10 minutos, un descenso de corticosterona en suprarrenal paralelo al aumento en sangre. A partir de los 15 minutos la elevación ha sido simultánea en sangre y suprarrenal.

En animales hemidecorticados no se han hallado diferencias entre las glándulas homo y heterolaterales a la ablación, si bien el nivel basal en suprarrenal era superior al que presentaban los animales intactos.

En el estudio por cromatografía en fase gaseosa se observan diferencias en las concentraciones relativas de los distintos corticosteroides en los grupos citados.

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