Endocrine status of immature female rats treated with Wy-18,185

Group	No. of rats	Body wt (g)	Thyroid wt (mg)	Adrenal wt (mg)	Uterine wt (mg)	Ovarian wt (mg)	Anterior pituitary wt (mg)	Day vagina opened
Oil control	20	128.6±2.6*	8.9±0.50	36.3±2.0	154.7±16	33.4±1.9	4.88±0.35	36.0±0.52
Wy-18,185	10	125.6±1.9	8.5±0.30	34.3±0.9	133.0±17	30.5±2.2	3.96±0.43	36.3±0.68

* Mean ± SE.

mature and adult female rats in relation to the complex interplay of peptide sensitivity and the steroid milieu[6], especially since Wy-18,185 has been shown to dually inhibit, by independent mechanisms, the release of hypothalamic LH-RH[7] and the release of pituitary LH[8]. Furthermore, Wy-18,185 and structurally related antagonists effectively block the pre-ovulatory gonadotropin surge and subsequent ovulation in the cyclic laboratory rodent, a rhythmic event that has yet to occur in the immature animal.

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The effects of microinjection of carbachol or hemicholinium into the amygdala on the levels of plasma and adrenal corticosterone in rats

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Summary. Microinjection of 0.4 µg of carbachol into the amygdala caused a rise of corticosterone (CS) in the morning, when the prestimulating level of CS was lower. But the same procedure with a larger dose had no effect in the afternoon, when the prestimulating level of CS was higher.

It is widely recognized that the regulation of the pituitary-adrenocortical (PA) functions requires the presence of elements in both the hypothalamic and the extrahypothalamic central nervous systems. Some conflicting evidence[1-6], however, regarding the role of the amygdala in the regulation of PA activities has been reported. This work deals with the effects of microinjection of carbachol (Carb) or hemicholinium (HC-3) into the amygdala on the levels of plasma and adrenal corticosterone (CS) in rats.

Materials and methods. Male Wistar rats weighing 300–380 g were used. Permanent cannuli, for microinjection of chemicals into the bilateral amygdala, were implanted stereotaxically. Microinjection was carried out 14 days after surgery. The doses were 0.1, 0.4 or 0.8 µg for Carb and 0.5, 1.0 or 5.0 µg for HC-3. The chemicals were dissolved in 0.5 µl of 0.9% saline and injected into the amygdala in a volume of 0.5 µl for each site. The same quantity of the vehicle was given to the control animals. Then experiments were carried out at 08.00 and 15.00 h. The animals were killed 40 min after microinjection of the chemicals. Trunk blood was collected and centrifuged. The right adrenal gland was removed, weighed, homogenized in saline containing 20% ethanol. The samples were kept frozen until assayed fluorometrically for their CS content, according to

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	n	Plasma corticosterone (µg/100 ml)	Adrenal corticosterone (µg/100 mg)
Morning			
Control	7	5.99± 1.57	0.12±0.12
Carb (0.4 µg)	7	19.94±15.26*	1.41±2.18
Control	7	5.31± 1.78	0.03±0.03
HC-3 (1.0 µg)	8	5.99± 2.53	0.02±0.04
Afternoon			
Control	8	21.71± 9.98	1.15±0.74
Carb (µg)			
0.1	8	20.91±10.13	1.01±0.43
0.4	8	21.66±11.30	0.80±1.04
0.8	8	23.30±15.35	1.54±1.37
Control	8	16.50± 8.46	0.92±1.20
HC-3 (µg)			
0.5	7	21.11±11.11	1.79±1.70
1.0	8	14.27± 5.60	0.86±1.31
5.0	7	22.11±10.02	1.90±1.40

Control animals were injected with saline. Values are expressed as mean ± SD. n, refers to the number of animals used; *: $p < 0.05$ as compared with controls; Student's t-test.

the method of Guillemin et al.[7]. The brains were histologically examined for verification of the injected sites.

Results. The results are summarized in the table. The prestimulating levels of plasma and adrenal CS observed in the morning experiments were significantly lower than those observed in the afternoon experiments.

Microinjection of Carb in doses of 0.1, 0.4 or 0.8 µg showed no effect on the levels of plasma and adrenal CS in the afternoon, when the prestimulating level was higher. But microinjection of 0.4 µg of Carb caused a significant rise of plasma CS in the morning, when the prestimulating level of the hormone was lower.

Microinjection of HC-3 in doses of 0.5, 1.0 and 5.0 µg in the afternoon or in a dose of 0.4 µg in the morning had no effect on the levels of plasma and adrenal CS. No significant behavioral response was detected in the animals whose amygdala had been injected with Carb or HC-3.

Discussion. It is evident that the microinjection procedure itself has no stress-effect, because either microinjection of Carb in the afternoon, or of HC-3 in the morning as well as in the afternoon, has no effect on the levels of plasma and adrenal CS. Thus it is reasonable to believe that the rise of plasma CS resulting from microinjection of 0.4 µg of Carb into the amygdala in the morning is not the result of non-specific stimulation, but that of the specific stimulation of acetylcholine receptor in the amygdala.

It is also apparent that the rise of CS does not result from behavioral excitements, since the animals showed no significant behavioral responses. Some authors[8,9] reported that adrenocortical responses following electrical stimulation or lesion of the hippocampus were dependent on the circadian rhythm of adrenocortical activities. The present study shows that adrenocortical response to a specific stimulation of acetylcholine receptor in the amygdala is also dependent on the circadian rhythm of PA acitivity. An alternate explanation for this result is that the effects of this stimulation are dependent on the prestimulating levels of CS; in other words, one might except a higher response with a lower prestimulating level.

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Effects of chronic treatment with ACTH on the intracellular levels of cyclic-AMP and cyclic-GMP in the rat adrenal cortex[1]

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Summary. The effects of chronic ACTH treatment on the increase in the intracellular concentration of cyclic-AMP and cyclic-GMP acutely elicited by ACTH in the rat adrenal cortex were investigated. The results are consistent with the hypothesis that chronic ACTH treatment stimulates a) the de novo synthesis of adenylate- and guanylate-cyclase or b) the synthesis of new specific membrane receptors for ACTH.

It is generally accepted that ACTH increases acutely the intracellular concentration of cyclic-AMP (cAMP) and cyclic-GMP (cGMP) in the adrenal cortex, by activating adenylate- and guanylate-cyclase, respectively[2]. As there was some evidence that cAMP and cGMP, besides mediating the rapid action of ACTH, function as intracellular mediators of the trophic action of ACTH on rat adrenocortical cells[3-5], it seemed worth while to investigate whether chronic treatment with ACTH affects the adrenal production of cAMP and cGMP elicited acutely by ACTH.

Materials and methods. 114 adult male albino Wistar rats weighing about 200 g were divided into 19 groups, with 6 animals in each group. In the 1st experiment 6 groups received i.p. 2,4,6,8,10 or 12 IU/kg of ACTH (Acthar, Armour-Erba, Milan, Italy) 15 min before sacrifice. A 7th group served as a control. In the 2nd experiment 5 groups received 10 IU/kg of ACTH i.p. 5,15,30,60 or 180 min before sacrifice. A further group was the control. A 3rd experiment analogous to the 2nd one was performed using 6 groups of animals which were treated for 6 consecutive days with daily doses of ACTH (10 IU/kg).

Each rat's right adrenal, having had the capsular fat and its zona medullaris removed, was used for the measurements of cAMP and cGMP concentrations. The cyclic nucleotides were extracted according to Sharma et al.[2] and separated from each other by the method of Murad et al.[6]. The determination of cAMP and cGMP was then accomplished by the methods respectively of Gilman[7] and Murad and Gilman[8], using commercial kits from the Radiochemical Centre, Amersham (England). Each assay was made in duplicate using a pool of 2 adrenal glands. The left adrenal of each rat was halved. One half was processed for optical microscopy and the other for electron microscopy in order to evaluate by morphometric methods[9-11] the number of parenchymal cells in each adrenal cortex. By knowing the weight of the adrenal gland, the average number of cells per mg of adrenocortical tissue was calculated and the concentration of cyclic nucleotides was expressed as pM per 10^6 parenchymal cells. This was made in order to overcome the difficulty arising from the fact that the increase in the adrenal weight would obscure, in the chronically ACTH-treated groups, the ACTH-elicited changes in the intracellular concentration of cyclic nucleotides, if this parameter is expressed as pM/mg of adrenal tissue (table).

Results and discussion. According to previous investigations[11-12], morphometry indicates that chronic ACTH treatment induced a significant increase in the volume of the adrenal gland and in the number of its parenchymal cells, which was almost exclusively due to the changes in the zona fasciculata (table). Therefore, our biochemical find-