EXPERIMENTAL BIOLOGY

Interrelationship between the Membrane Potential of Adrenocorticocytes and Functional Activity of the Adrenal Glands in Adult and Old Rats

E. N. Gorban'

UDC 612.452.014.1:612.67

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 117, № 3, pp. 302-303, March, 1994
Original article submitted August 12, 1993

Experiments on adult (5-7 months old) and old (28-30 months old) male rats reveal that ACTH induces a hyperpolarization of the plasma membrane of adrenocorticocytes from the fasciculate zone in both age groups of animals 24 hours after a single administration of the hormone. Actinomycin D, an inhibitor of protein synthesis, prevents the development of hyperpolarization.

Key Words: *adrenal glands; aging; membrane potential; protein synthesis*

Recent studies have demonstrated a relationship between the membrane potential (MP) of adrenocorticocytes (ACC) in the fasciculate zone of the adrenal cortex and the secretory response of the cortical substance to ACTH [6,7]. Two types of effects are involved in the mechanism of the effect of ACTH on crinoparenchyma of the adrenal cortex: first, immediate effects consisting in a sharp activation of the secretion of corticosteroids due to activated synthesis of pregnenolone from precursors and, second, delayed effects related to an activation by the tropic hormone of the ACC genome and protein synthesis in ACC [5]. Until now little has been known about the relationships between the electrical properties of the plasma membrane (PM) of ACC and the functional state of the adrenal cortex in the manifestation of the delayed effects of ACTH on the adrenal cortex and the age-related peculiarities of these relationships. Yet these relationships are important for two reasons. First, a relation between the rate of protein synthesis in the cell and its MP has been found in various types of cells, except in secretory cells of endocrine tissues. Namely, the activation of protein synthesis in the cell is accompanied by hyperpolarization of the PM, which in turn has a substantial effect on its specific functional activity [3]. Second, age-related changes in the functional activity of the adrenal glands (AG) largely determine the nature of the adaptive responses of the organism in senility.

Thus, the objective of the present study was to investigate the relationship between the MP of ACC of the fasciculate zone of the adrenal cortex in adult and old rats at late stages after a single injection of ACTH (24 hours), when an increase in the incorporation of radiolabeled amino acids into proteins of the adrenal tissue is observed [5].

MATERIALS AND METHODS

Adult (5-7 months old) and old (28-30 months old) male Wistar rats were used in the experiments. The membrane potential of ACC of the

Laboratory of Radiobiology, Ukrainian Research Institute of Gerontology, Academy of Sciences of Ukraine, Kiev. (Presented by D. F. Chebotarev, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0003-0305$12.50

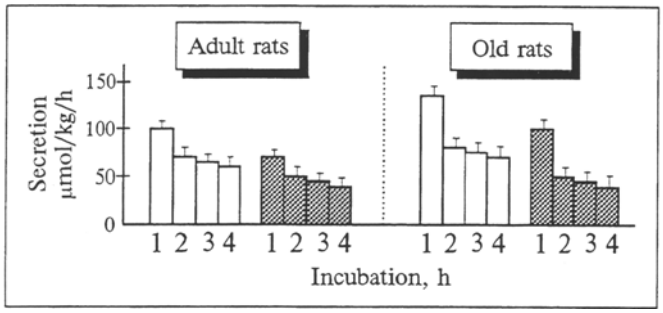

Fig. 1. Basal secretion of 11-OCS by isolated "hyperpolarized" adrenal glands from adult and old rats. Here and in Fig. 2. open bars denote control, shaded bars denote "hyperpolarized" adrenal glands, and an asterisk denotes reliable differences in comparison with the control.

fasciculate zone of isolated AG (IAG) as well as the basal and ACTH-stimulated secretion of 11-oxycorticosteroids (11-OCS) in IAG were studied 24 hours after a single injection of ACTH (100 ED/kg body weight, intramuscularly). Control animals received a single injection of physiological saline. The effect of a single injection of ACTH on the above parameters of the functional state of the adrenal cortex was also studied against the background of chronic (24 hours) administration of the inhibitor of protein synthesis actinomycin-D (Act-D, in a dose of 100 μg/kg body weight, four times per day). ACTH was injected 2 hours after the first administration of blocker.

Each AG obtained from one animal was cut into two parts. Both halves of one IAG were fixed on plastic substrate and placed in a chamber containing 5 ml Krebs-Henseleit solution [8]. Each of the two AG obtained from one animal was incubated separately at 37°C for 4 hours. The incubation medium was aerated with a 5% CO_2 and 95% O_2 mixture and replaced every 10 minutes. In the removed portions the content of 11-OCS was measured fluorometrically [2]. Each member of the pair of IAG from one animal was used for the study of the basal and ACTH-stimulated secretion of 11-OCS, respectively. To this end the first IAG

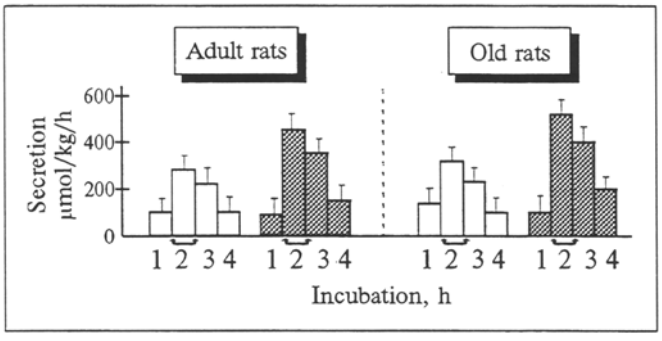

Fig. 2. ACTH-stimulated secretion of 11-OCS by isolated "hyperpolarized" adrenal glands from adult and old rats. Arrows indicate incubation in the presence of ACTH (10 U/liter).

was incubated in standard Krebs-Henseleit solution, while the second was incubated in a medium where ACTH (10 U/liter) was added 2 hours after the start of the experiment. The membrane potential of ACC of the fasciculate zone was measured after a 20-30-min incubation [1].

RESULTS

Twenty-four hours after the injection of ACTH a hyperpolarization of PM of ACC was observed in rats of both age groups. In adult control animals (n=10) MP ACC was -55.7±0.4 mV and 24 hour after injection of ACTH (n=12) -62.6±0.8 mV p<0.001. In old rats MP ACC was -53.9±0.8 mV in the control (n=6) and -60.9±0.8 mV 24 hours after injection of ACTH (n=6), p<0.001. In animals of both age groups the injection of Act-D prevented the hyperpolarization of PM in ACC 24 hours after a single injection of the tropic hormone. Twenty-four hours after a single injection of ACTH against the background of Act-D the MP of ACC in IAG of the adult animals (n=6) was -57.1±1.7 mV and in old animals (n=6) it was -52.5±0.4 mV, i.e., it did not differ reliably from the control. Injection of ACTH alone during 24 hour induced no reliable differences in MP of ACC in the fasciculate zone of the AG in animals of both groups.

The basal and ACTH-stimulated secretion of 11-OCS by the "hyperpolarized" IAG was also studied. In both age groups the basal secretion by the "hyperpolarized" IAG was lower in comparison with the control. Figure 1 demonstrates an hour-by-hour diagram of the basal secretion of 11-OCS in IAG of adult and old rats 24 hours after a single injection of ACTH or isotonic saline (control).

The reactivity of "hyperpolarized" IAG to ACTH was considerably higher compared to the control in both age groups. Figure 2 presents the hour-by-hour dynamics of ACTH-stimulated secretion of 11-OCS in IAG of adult and old rats 24 hours after a single injection of ACTH or isotonic saline (control).

The obtained results suggest the existence of a relationship between the level of polarization of ACC PM and the functional activity of the adrenal cortex at late stages after ACTH injection. The absence of age-related differences in the nature of the reaction of "hyperpolarized" IAG to ACTH allow us to conclude that the membrane-genomic mechanism of the regulation of glucocorticoid function of AG remains unchanged also during the late ontogeny.

Since the secretory products of the adrenal cortex are nonprotein substances, the hyperpolarization of PM of ACC long after the injection of ACTH due to activation of protein synthesis in ACC may be thought to be related to an activated synthesis of the enzymes of steroidogenesis. This may be what provides for the enhanced secretion of corticosteroids by "hyperpolarized" AG during *in vitro* stimulation of steroidogenesis in the adrenal cortex by the tropic hormone.

At the same time, other mechanisms underlying the enhanced reactivity of the "hyperpolarized" AG to ACTH cannot be ruled out. In particular, it has been shown that the development of hyperpolarization of cell PM resulting from the activated protein synthesis in tissues is accompanied by activation of Na,K-ATPase of the PM. In addition, the appearance of peptide compounds, so-called invertors, has been demonstrated in hyperpolarized tissues, which are capable of activating Na,K-ATPase of cell PM in intact tissues and thereby affect their functional activity [4]. From this point of view, the hyperpolarization of PM of ACC which develops against the background of activated protein synthesis may serve as an invertor-mediated interface in the chains of direct and feedback regulatory influences, thus modulating the reactivity of ACC to the tropic hormone.

REFERENCES

1. P. G. Kostyuk, *Microelectrode Technique* [in Russian], Kiev (1960).
2. A. G. Reznikov, *Methods of Hormone Assay* [in Russian], Kiev (1980).
3. V. V. Frol'kis, *Aging. Neurohumoral Mechanisms* [in Russian], Kiev (1981).
4. V. V. Frol'kis, *Dokl. Akad. Nauk SSSR*, **324**, 229 (1992).
5. N. A. Yudaev, C. A. Afinogenova, A. A. Bulatov, *et al.*, *Biochemistry of Hormones and Hormonal Regulation* [in Russian], Moscow (1976).
6. J. R. Lymangrover, E. K. Matthews, and M. Saffran, *Endocrinology*, **110**, 462 (1982).
7. J. R. Lymangrover, N. K. Valego, and R. D. Shilling, *Mech. Age. Dev.*, **43**, 269 (1988).
8. E. K. Matthews and M. Saffran, *J. Physiol. (Lond.)*, **189**, 149 (1967).

Age-Specific Effects of Insulin on the Secretion of Somatotropic Hormone

V. P. Fedotov, T. V. Mamaeva, and V. I. Gudoshnikov

UDC 612.433.65.06:612.349.7.018.2].06:612.66

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 3, pp. 304-306, March, 1994
Original article submitted September 12, 1993

It is shown that insulin is able to alter the secretion of somatotropic hormone directly at the level of the pituitary. The direction of the regulatory effect of insulin depends on the age of the animals donating the pituitary cells, while the intensity of the effect of insulin is largely modulated by glucocorticoid and thyroid hormone.

Key Words: *somatotropic hormone; cell culture; pituitary; insulin; postnatal development*

It is now undisputed doubt that there are direct and feedback relationships between the somatotropic hormone (STH) of the pituitary and insulin. As is well known, STH directly stimulates insulin secretion and biosynthesis, as well as the proliferation of β-cells of the pancreatic islets (islets of Langerhans) [7,14]. On the other hand, insulin has been found to directly inhibit the secretion of STH in cultures of normal and tumor cells of the

Laboratory of Biological Research of Hormonal Compounds, Institute of Experimental Endocrinology, Scientific Center of Endocrinology, Russian Academy of Medical Sciences, Moscow. (Presented by Yu. A. Pankov, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0003-0307$12.50