MEMBRANE MECHANISMS OF ACTION OF TESTOSTERONE

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Steroid hormones exert an early action on target cells from the membrane [3, 10, 11]. It was shown previously that hyperpolarization of cell plasma membranes develops under the influence of certain steroid hormones [5, 6]. It has been suggested that a specific hyperpolarizing factor is synthesized under the influence of these hormones [6].

The aim of this investigation was to discover the mechanisms of the effect of testosterone on the membrane potential (MP) of hepatocytes and adrenocorticocytes of rats.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 6-8 months. MP of cells in the liver and zona fasciculata of the adrenal cortex was measured by means of "Pyrex" glass microelectrodes with a tip under 1 μ in diameter, and filled with 2.5 M KCl by the method in [2]. MP of the adrenocorticocytes was measured under the influence of testosterone in vivo and in vitro. The adrenals were incubated in Krebs–Henseleit medium [8] at 37°C by the method described in [1]. The plasma membrane fraction was isolated from the hepatocytes and purified as described in [12]. The degree of purification was determined by measuring enrichment of the fraction with the plasma membrane marker enzyme 5'-nucleotidase [9]. The protein concentration in the samples was determined by Lowry's method [7]. Activity of Na,K-ATPase was measured as in [4]. The plasma membrane fraction isolated from hepatocytes was incubated in vitro with rat cytosol and blood serum on an ice bath for 40 min with constant shaking. The adrenals were incubated in vitro in Krebs–Henseleit solution for 1 h at 37°C. The testosterone concentration in the incubation medium was 20 μg/ml. The results were subjected to statistical analysis by one-factor analysis of variance.

EXPERIMENTAL RESULTS

Significant hyperpolarization of the hepatocyte and adrenocorticocyte membranes was observed 1 h after intraperitoneal injection of testosterone (1 mg/kg). Preliminary (30 min before testosterone) injection of 2-aminopyridine (10 mg/kg), a K channel blocker, into the animals did not affect the development of hyperpolarization, whereas actinomycin D (50 μg/kg) and cycloheximide (250 μg/kg), inhibitors of protein biosynthesis, prevented the hyperpolarizing action of the hormone on both hepatocytes and adrenocorticocytes. Ouabain (73 μg/kg), a specific Na,K-ATPase inhibitor, also prevented hyperpolarization of the hepatocyte and adrenocorticocyte plasma membranes by testosterone. The effect of testosterone on the amplitude of MP was linked with its direct action on the target cells. This is shown by experiments in which isolated adrenals were incubated with testosterone in vitro. As Fig. 1 shows, addition of testosterone to the incubation medium of the adrenal caused significant hyperpolarization of the adrenocorticocyte membranes.

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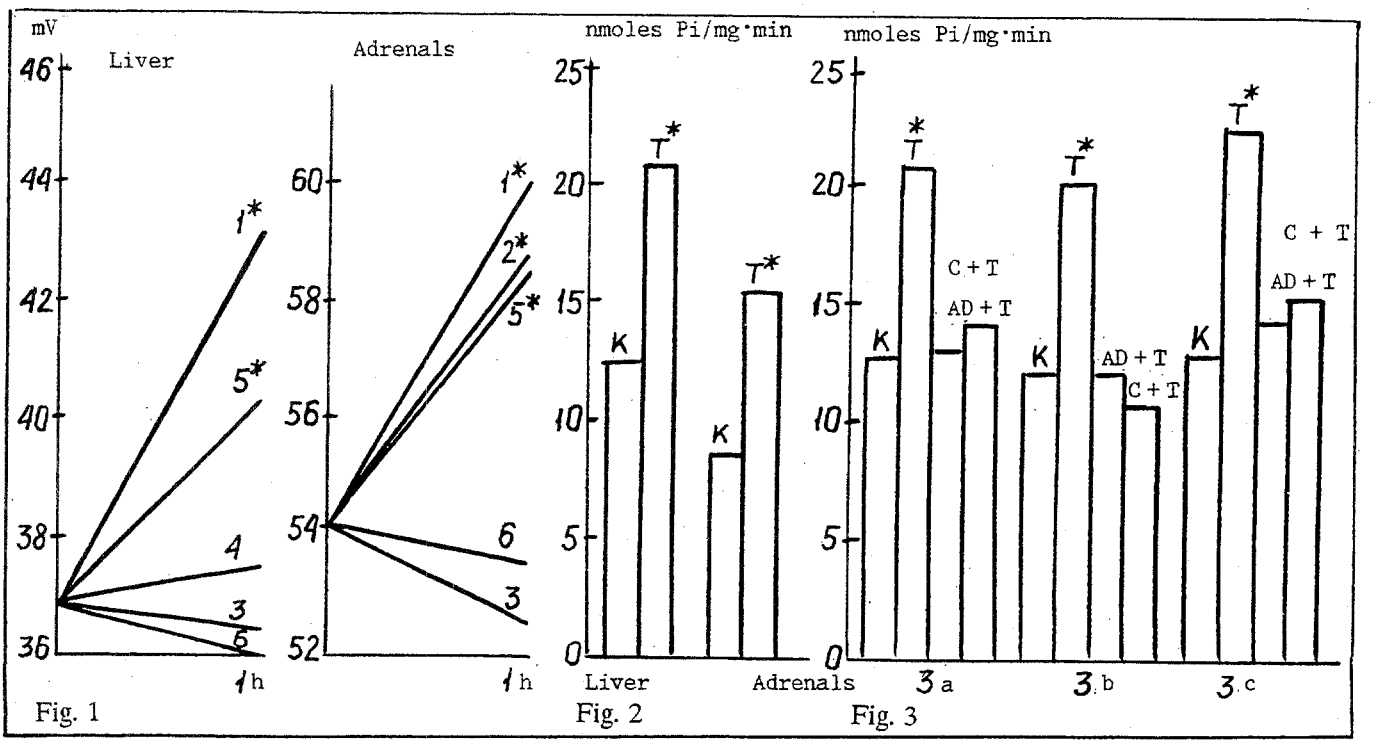

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Effect of testosterone on membrane potential of rat hepatocytes and adrenocorticocytes: 1) membrane potential 1 h after injection of testosterone into animals; 2) membrane potential after incubation with testosterone in vitro; 3) membrane potential of hepatocytes and adrenocorticocytes of rats receiving actinomycin D; 4) cycloheximide; 5) 2-aminopyridine, and 6) ouabain 30 min before testosterone; *$p < 0.05$.

Fig. 2. Effect of testosterone on Na,K-ATPase activity of rat hepatocytes and adrenocorticocytes. K) Na,K-ATPase activity in control; T) after injection of testosterone into animals; *$p < 0.05$.

Fig. 3. Na,K-ATPase activity in isolated plasma membrane fraction of rat liver under influence of testosterone, hepatocyte cytosol, and blood serum: a) Na,K-ATPase activity in isolated plasma membrane fraction; b) Na,K-ATPase activity in fraction of intact isolated plasma membranes after incubation in vitro with hepatocyte cytosol; c) Na,K-ATPase activity in fraction of intact isolated plasma membranes after incubation in vitro with blood serum; K) control animals; T) animals receiving testosterone; AD + T) animals receiving actinomycin D 30 min before testosterone; C + T) animals receiving cycloheximide 30 min before testosterone; *$p < 0.05$.

It can thus be postulated that the mechanisms of the hyperpolarizing action of testosterone on liver and adrenal cells are similar and are due, not to its influence on membrane K^+-channels, but indirectly through activation of the membrane enzyme Na,K-ATPase. Prevention of testosterone-induced hyperpolarization by inhibitors of protein biosynthesis supports the view that under the influence of this hormone, and as a result of stimulation of protein biosynthesis in the cell, a hyperpolarizing factor, which may perhaps possess an activating action on Na,K-ATPase, appears in the cell.

The next series of experiments was carried out to study the effect of testosterone on the Na,K-ATPase activity of rat hepatocytes and adrenocorticocytes. Intraperitoneal injection of testosterone caused significant activation of Na,K-ATPase (1 h after injection) of both hepatocytes (by 49%) and adrenocorticocytes (by 71%) (Fig. 2). Na,K-ATPase activity in plasma membrane fractions isolated from the liver of rats which had received one of the inhibitors of protein biosynthesis (actinomycin D or cycloheximide) 30 min before testosterone, was unchanged (Fig. 3a). Separate series of experiments showed that testosterone, actinomycin D, and cycloheximide alone, in vitro, did not affect the Na,K-ATPase activity of plasma membranes isolated from rat hepatocytes.

The results suggest that as a result of activation of protein biosynthesis a factor activating membrane Na,K-ATPase appears in the cytosol of the cells. This conclusion is confirmed by experiments in which fractions of isolated hepatocyte plasma membranes of intact rats were incubated in vitro with cytosol of hepatocytes from animals receiving testosterone (Fig. 3b). The cytosol of these animals caused significant activation of the enzyme, whereas the cytosol of intact animals did not affect basal Na,K-ATPase activity. Cytosols of the hepatocytes of rats receiving an inhibitor of protein biosynthesis (actinomycin D or cycloheximide) 30 min before testosterone, likewise did not activate the Na,K-ATPase of intact plasma membranes (Fig. 3b).

The factor activating Na,K-ATPase enters the blood stream. Incubation of the fraction of isolated plasma membranes with blood serum from rats receiving testosterone caused activation of the enzyme compared with incubation in serum from intact animals (Fig. 3c). Actinomycin D and cycloheximide, injected into the animals 30 min before testosterone, prevented the appearance of this Na,K-ATPase-activating factor in the blood serum of the rats also (Fig. 3c).

The experiments thus showed that testosterone induces hyperpolarization of the plasma membranes of rat hepatocytes and adrenocorticocytes. This hyperpolarization is due, in all probability, to activation of the membrane enzyme Na,K-ATPase. It can be tentatively suggested that under the influence of testosterone a factor with a stimulating action on cellular Na,K-ATPase appears in the cytosol and blood serum of rats. Actinomycin D and cycloheximide, inhibitors of protein synthesis, prevent the appearance of this factor both in the cell cytosol and in the blood serum of rats. It can be postulated that this mechanism is transmitted through the cell genome and plays a definite role in the effect of testosterone on plasma membranes.

LITERATURE CITED

1. E. N. Gorban', Fiziol. Zh. (Kiev), **24**, No. 6, 395 (1979).
2. P. G. Kostyuk, Microelectrode Techniques [in Russian], Kiev (1960).
3. T. M. Morozova, V. E. Volchkov, T. I. Merkulova, and I. N. Nagibneva, Dokl. Akad. Nauk SSSR, **272**, No. 6, 748 (1983).
4. R. I. Potapenko, Neirokhimiya, **5**, No. 1, 57 (1986).
5. V. V. Frol'kis, Fiziol. Zh. (Kiev), **26**, 558 (1980).
6. V. V. Frol'kis, Aging. Neurohumoral Mechanisms [in Russian], Kiev (1981).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. I. Randall, J. Biol. Chem., **193**, No. 1, 265 (1951).
8. E. K. Matthews and M. S. Safran, J. Physiol. (London), **189**, 149 (1967).
9. R. H. Michell and J. N. Hamthorn, Biochem. Biophys. Res. Commun., **21**, No. 4, 333 (1965).
10. R. Pietras and C. Szego, Biochem. Actions Horm., **8**, No. 1, 307 (1981).
11. M. Savart and Y. Cabilie, Biochim. Biophys. Acta, **813**, No. 2, 87 (1985).
12. K. Yamamoto, S. Omata, T. Onishi, and H. Terayama, Cancer Res., **33**, 567 (1973).