PHYSIOLOGY

A Putative Inverter Mechanism of Hormonal Na,K-ATPase Regulation

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Insulin, testosterone, and thyroxine activated the Na,K-ATPase of hepatocyte plasma membranes in adult rats. Incubation of intact plasma membranes from rat hepatocytes with hepatocyte cytosol or blood serum from adult rats injected with one of the three indicated hormones led to elevated Na,K-ATPase activity in the incubated plasma membranes. It is suggested that hormones induce the appearance in liver cells and blood serum of a factor, called inverter, that is ultimately responsible for the observed Na,K-ATPase activation.

Key Words: *Na,K-ATPase; insulin; thyroxine; testosterone; hepatocytes*

We showed previously that activation of protein synthesis in cells results in hyperpolarization of the plasma membrane [3,5]. The hyperpolarization thus induced may be due to alterations in the active and/or passive ion transport. On the other hand, plasma membrane hyperpolarization that occurs as a consequence of partial hepatectomy, blood loss, or hormonal action (e.g., by insulin, testosterone, or estradiol propionate) can be prevented by the protein biosynthesis inhibitors actinomycin D and cycloheximide and by the specific Na,K-ATPase inhibitor ouabain [4,6].

In this study we examined responses of hepatocyte plasma membranes to insulin, testosterone, and thyroxine. These hormones were selected because they all activate protein synthesis in the cells, although they do so by different mechanisms.

MATERIALS AND METHODS

Wistar rats aged 6-8 months were used. The plasma membrane fraction was isolated from their livers and purified as described by Yamamoto *et al.* [11], the degree of purification being evaluated by its enrichment with 5'-nucleotidase, a marker enzyme for plasma membranes [8]. Protein concentration in the samples was determined by Lowry's method [7], Na,K-ATPase activity was assessed by the difference between total and magnesium-stimulated ATPase activities [1,10]. Inorganic phosphorus was measured according to Rathburn and Betlach [9].

The isolated plasma membranes (100 μg protein per sample) were incubated with one of the hormones (insulin, testosterone, or thyroxine), rat hepatocyte cytosol, or rat serum (150 μl per sample) for 20 min (thyroxine) or 40 min (insulin, testosterone) in a medium for determining total ATPase activity (1×10^{-1} M NaCl, 2×10^{-2} M KCl, 3×10^{-3} M $MgCl_2$, and 5×10^{-2} M Tris-HCl, pH 7.5) or in one for determining magnesium-stimulated ATPase activity (same medium as above but supplemented with 1×10^{-4} M ouabain); all incubations were carried out in an ice bath with constant shaking.

The data were statistically treated by one-way analysis of variance (ANOVA).

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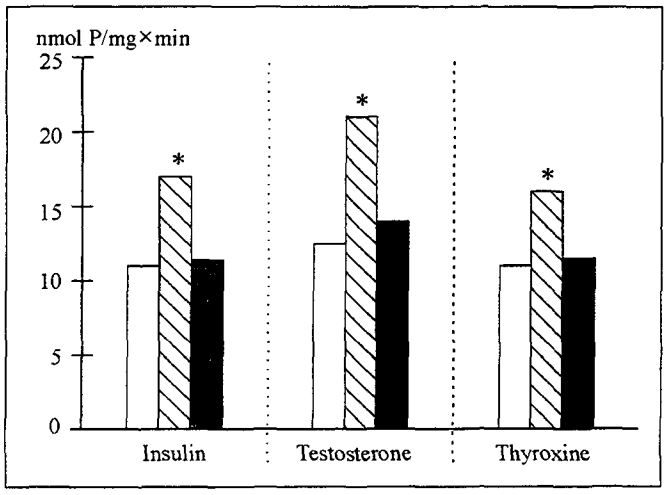

Fig. 1. Na,K-ATPase activity of plasma membranes of rat hepatocytes. Here and in Figs. 2 and 3: white bars, intact rats; hatched bars, hormone-treated rats; black bars, rats treated with actinomycin D+hormone. *$p<0.05$ in comparison with intact rats.

RESULTS

Insulin (1.6 U/kg body weight) and testosterone (1 mg/kg) significantly increased Na,K-ATPase activity of isolated hepatocyte plasma membranes 40 min and 60 min postinjection, respectively, while thyroxine (33 μg/kg) increased this activity by 32% 20 min postinjection (Fig. 1). None of the hormones altered Na,K-ATPase activity on *in vitro* incubation with hepatocyte plasma membranes from intact rats. Consequently, these hormones acted on the enzyme via the hepatocyte cytoplasm rather than directly. Na,K-ATPase activation was prevented by actinomycin D, a blocker of protein biosynthesis at the transcription level, injected at 50 μg/kg 30 min before insulin or testosterone and 50 min before thyroxine (Fig. 1). The elevation of Na,K-ATPase activity was therefore mediated by hormone-dependent stimulation of protein biosynthesis in hepatocytes.

In order to check the validity of our hypothesis that Na,K-ATPase may be activated by factors appearing in the cell during the activation of protein synthesis, tests with cell hybridomas were undertaken, in which isolated hepatocyte plasma membranes from intact rats were incubated with the cytosol of hepatocytes from hormone-injected rats. (The incubation times for these tests was chosen taking into account the times at which Na,K-ATPase activation was maximal in the tests described above.) It was found that while the cytosol of hepatocytes from intact rats had no effect on the Na,K-ATPase activity of isolated intact plasma membranes, the cytosols of hepatocytes from rats injected with insulin, testosterone, or thyroxine activated the enzyme by 52%, 66.1%, and 40.3%, respectively (Fig. 2). No Na,K-ATPase activation was observed with the cytosol of hypatocytes from rats administered actinomycin D (Fig. 2) or cycloheximide before the hormone.

The findings presented above indicate that the hormones caused the emergence of a Na,K-ATPase activating factor in the rat hepatocyte cytosol. The presence of such an activator in cytosol was associated with the hormone-dependent intensification of protein biosynthesis in the cells.

The activating factor can probably enter the circulation, as was indicated by our tests where isolated hepatocyte plasma membranes were incubated with sera from intact and hormone-treated rats. In these tests, sera from intact rats had little or no effect on Na,K-ATPase activity, whereas those from

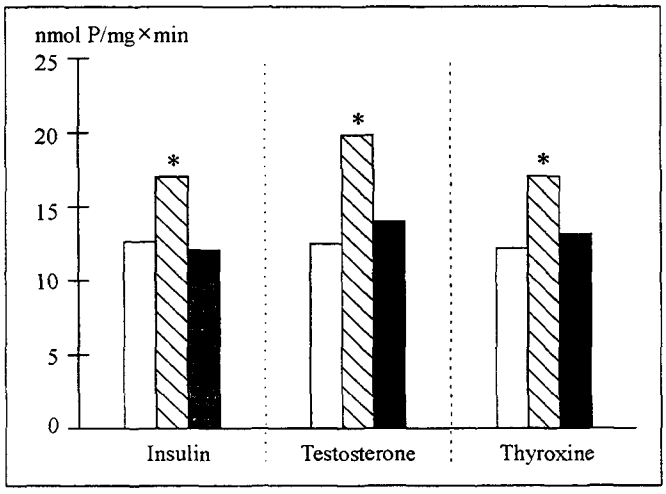

Fig. 2. Na,K-ATPase activity of rat hepatocyte plasma membranes after incubation of the intact plasma membrane fraction with rat hepatocyte cytosol.

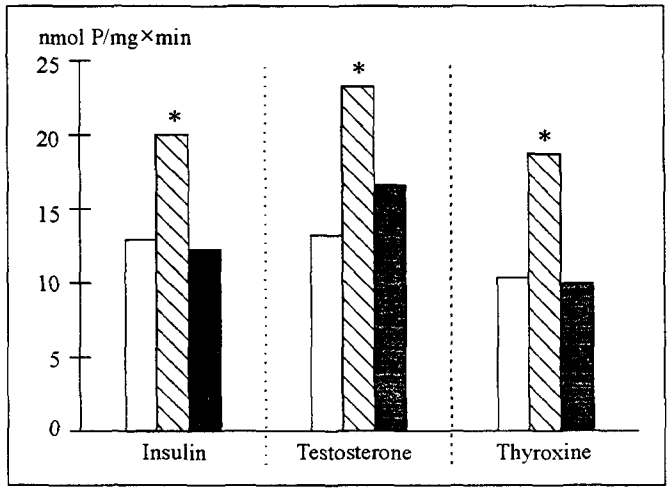

Fig. 3. Na,K-ATPase activity of rat hepatocyte plasma membranes after incubation of the intact plasma membrane fraction with rat serum.

animals that had received insulin, testosterone, or thyroxine activated the enzyme by 43.6%, 69.9%, and 82.9%, respectively (Fig. 3). The sera from rats administered actinomycin D before the hormone also failed to activate the enzyme (Fig. 3).

Hormonal regulation of Na,K-ATPase activity may be presumed to occur via a mechanism whereby a given hormone stimulates biosynthetic processes in liver cells and thus causes the appearance in the cytosol and serum of a factor, which we previously called inverter [2], that activates the Na,K-ATPase. Such inverters, synthesized under genomic control, may be responsible for membrane-genome linkages, in particular those existing when cells are acted upon by certain hormones.

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