RAPID STIMULATION OF Na^+,K^+-ATPase BY GLUCAGON, EPINEPHRINE, VASOPRESSIN AND cAMP IN PERFUSED RAT LIVER

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1. Introduction

Hormones which promote glucose production by the liver have a pronounced effect on ion distribution in this organ. The redistribution of ions is prompt and involves several ions. Glucagon [1,2], epinephrine [1,2], norepinephrine [1], phenyl epinephrine [3,4] and vasopressin [5] affect K^+ movement across the plasma membrane. A similar response is evoked by cAMP [1,2,6]. The effect of hormonal stimuli on K^+ flux is biphasic, consisting of an initial uptake of K^+, followed by release when the liver plasma membrane hyperpolarizes [7–9]. The efflux of K^+ and the associated hyperpolarization have been attributed to an increase in the K^+ permeability of the liver cell membrane [7,8]. The uptake phase has been less studied and conflicting results on hormonal effects on Na^+ fluxes render the mechanism responsible for it less clear. Gluconeogenic hormones have been reported to cause Na^+ release [10], uptake followed by release [1], uptake [7] or to have no effect on Na^+ movements.

Since K^+ uptake occurs against a concentration gradient, we undertook to determine whether gluconeogenic hormones promote K^+ uptake by activating the membrane-bound Na^+,K^+-ATPase. Here, gluconeogenic hormones are shown to stimulate the enzyme. Insulin, which antagonizes the effect of the gluconeogenic hormones [6], had no similar effect, but prevented the stimulation by glucagon.

2. Experimental

Experiments were performed with male Sprague-Dawley rats (85–135 g body wt). The following materials were purchased from Sigma (MO): ATP disodium salt (from equine muscle); ouabain octahydrate (Strophantin G); EGTA (ethyleneglycol-bis-(β-aminoethyl ether) *N,N'*-tetraacetic acid); sodium azide; DDT (D,L-dithiothreitol); 1-O-*n*-Octyl-β-D-glucopyranoside; Hepes (*N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid); albumin, Cohn fraction V; vasopressin (grade VI); and cAMP, sodium salt. Glucagon (for injection USP) and insulin (1000 U/ml) were from Eli Lilly (IN). Epinephrine was obtained from Parke and Davis (MA).

2.1. *Liver perfusion*

The liver perfusion technique was detailed in [11]: rats were anesthetized with an i.p. injection of Nembutal, and livers perfused in situ via the portal vein with Krebs–Ringer bicarbonate buffer (pH 7.4) containing 4% bovine serum albumin, in a recirculating system for 45 min. If not indicated otherwise, the appropriate hormone or cAMP was present during the last 5 min of perfusion. It takes 2 min for any agent added to the reservoir to reach the liver. Following perfusion, the livers were removed and cut into fragments of ~1 g. These pieces were either homogenized immediately or frozen in liquid N_2 and stored at −80°C. No difference was noted between these two treatments.

2.2. *Preparation of liver homogenate for Na^+,K^+-ATPase activity determination*

A 10% dispersion of liver in medium (0.25 M sucrose, 2 mM EGTA, 10 mM Tris–HCl (pH 7.4) was made with a Polytron homogenizer (20 s at low speed). The homogenate was then filtered through nylon mesh (190 mesh).

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2.3. *Rat liver plasma membrane isolation*

This was as in [12].

2.4. *Assay of Na^+,K^+-ATPase activity*

Protein (2 mg/ml) was preincubated for 30 min at 0°C with occasional shaking in 50 mM Tris–HCl (pH 7.4); 1 mM EGTA; 2 mM octyl-glucoside. This solution (25 μl) was transferred into a reaction mixture containing 50 mM Tris–HCl (pH 7.4); 5 mM $MgCl_2$; 5 mM sodium azide; 150 mM NaCl; 15 mM KCl; ±1 mM ouabain in 1.0 ml total vol. The incubation was started with 3 mM ATP and continued for 15 min at 37°C in a shaking water bath. The reaction was terminated and phosphate determined by adding 1.75 ml 1.43% ammonium molybdate, 1.43 N H_2SO_4, 7% $FeSO_4 \cdot 7\ H_2O$ and measuring the absorbance at 740 nm after 15 min at room temperature (modified from [13]). A sample with protein added after terminating the reaction was used as the blank. The measurements were obtained in a Beckman UV 5260 spectrophotometer. All determinations were carried out in triplicate. The Na^+,K^+-ATPase was calculated from the difference ± ouabain.

2.6. *Protein determination*

This was as in [14], using bovine albumin as standard.

3. Results and discussion

The administration of a group of gluconeogenic hormones (glucagon, epinephrine, or vasopressin) or of cAMP to the perfused liver caused stimulation of the reaction rate of Na^+,K^+-dependent ATPase, as measured in a subsequently obtained tissue homogenate. The degree of stimulation varied, but was significant in all cases (table 1). Enzymic activity was measured subsequent to the hormonal stimulus, after homogenization of the liver. Therefore, the effect persisted after dilution of the hormone. However, the response to the stimulus was rapid; in the case of glucagon, maximal stimulation was obtained 3 min after the hormone reached the liver, and diminished afterwards (fig.1). This is in contrast to the induction by glucagon of the de novo synthesis of Na^+,K^+-ATPase in isolated hepatocytes, a process requiring several hours [15]. Since the synthesis and insertion into membrane of new enzyme molecules is unlikely to take place within 3 min, we interpret our data as indicating a modification of existing enzyme. The nature of this modification is being studied. The fact that enzyme activation is still expressed after homogenization of the tissue and purification of plasma membranes on a density gradient (now shown) could indicate that it is not caused by a dissociable regulator. A covalent modification appears more likely.

Epinephrine and vasopressin caused a similar stimulation of Na^+,K^+-ATPase. The effectiveness of cAMP suggests a possible involvement of this effector. Insulin, which by itself had no effect (table 1), blocked the stimulation caused by glucagon when the two hormones were administered simultaneously. This behavior is compatible with the general physiological roles of these hormones.

The significance of the hormonal stimulation of Na^+,K^+-ATPase cannot be fully explained yet. Since

Table 1
Na^+,K^+-ATPase activity in homogenates of perfused livers

Addition to perfusate	Na^+,K^+-ATPase (mU/mg)	% Stimulation	Significance[a]
None	15.5 ± 3.8 (n = 26)	–	–
Glucagon (2.8×10^{-8} M)	27.6 ± 3.9 (n = 6)	83%	$p = 0.02$
Epinephrine (5.5×10^{-8} M)	18.5 ± 3.4 (n = 10)	19%	$p = 0.02$
Vasopressin (3×10^{-8} M)	20.6 ± 3.7 (n = 8)	33%	$p = 0.05$
cAMP (10^{-4} M)	19.2 ± 3.2 (n = 9)	23%	$p = 0.02$
Insulin (2.6×10^{-7} M)	12.4 ± 2.3 (n = 7)	–20%	n.s.
Insulin (2.6×10^{-7} M) + glucagon (2.8×10^{-8} M)	16.9 ± 2.9 (n = 3)	8%	n.s.

[a] Wilcoxon paired signed ranks test; n.s., not significant

The additions to the perfusate are listed. Details of perfusion and subsequent measurements of enzyme activity in liver homogenates are in section 2. Results are expressed as mean ± SD with (□) the no. determinations

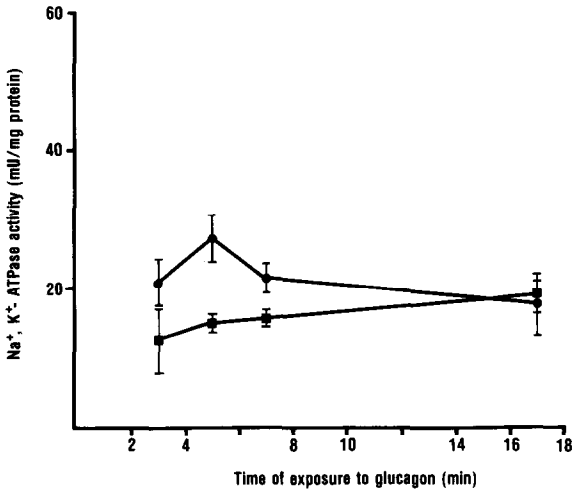

Fig.1. Dependence of the stimulation of Na^+,K^+-ATPase by glucagon on the time of exposure to the hormone. Livers were perfused for 40 min (section 2) after which glucagon (2.8×10^{-8} M) was added to the perfusate. It takes 2 min for any substance added to reach the liver. At the times indicated (taking addition of hormone as time 0) livers were removed from perfusion and activity of Na^+,K^+-ATPase was determined in tissue homogenates. Each point represents the mean of 4 determinations: (⊢⊣) standard deviation; (■) control; (●) glucagon.

glucagon exerts its effect on the perfused liver at physiological concentrations (2.8×10^{-8} M; the stimulation is 3-times lower at 2.8×10^{-9} M), it can be inferred that the Na^+,K^+-ATPase is affected by hormones in the intact organism. We consider the observed magnitude of stimulation to be a minimal estimate. Not knowing the regulatory mechanism operating on the Na^+,K^+-ATPase, we are not able to adjust experimental conditions to avoid taking an already partially activated enzyme as the control. Moreover, we did not control the hormonal status of the animal before perfusion. Therefore, in vivo stimulation of activity by glucagon could be considerable. We have no direct evidence that the observed changes in enzyme activity are accompanied by increased Na^+ and K^+ fluxes, but indirect information to support this notion is available. Hormonally evoked ion redistribution has been mentioned. This redistribution is accompanied by a hyperpolarization of the plasma membrane [9], which is sensitive to ouabain [16]. The data suggest the following sequence of events: a hormonal stimulus (e.g., glucagon) causes plasma membrane hyperpolarization by both an initial K^+ uptake by the electrogenic Na^+,K^+-ATPase and a subsequent K^+ efflux via a different K^+ permeability. Cyclic AMP is likely to be involved in mediating these responses. The ensuing membrane potential (or the ion redistribution itself) might influence other cellular processes. Therefore, we think that the modulation of Na^+,K^+-ATPase activity may serve as a second messenger in regulation of cellular metabolism. The effects of disturbing the activity of this enzyme on several metabolic parameters will be described in [17].

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