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Hormonal control *in vitro* of plasma membrane-bound (Na^+–K^+)-ATPase of rat liver

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SUMMARY

Epinephrine, glucagon and cyclic AMP reduced *in vitro* the activity of (Na^+–K^+)-ATPase of plasma membranes isolated from rat liver. Insulin did not significantly modify the activity, but partially counteracted the effect of both hormones. Propranolol, a β-blocking agent, prevented the action of epinephrine. These data suggest that epinephrine, glucagon and insulin may modulate the activity of plasma membrane (Na^+–K^+)-ATPase, by an action probably mediated by cyclic AMP.

The hormonal control *in vitro* of liver plasma membrane adenylate cyclase has been investigated by several workers[1–6] for the physiological implications of cyclic AMP as a second messenger (for review, see ref. 7). Conversely, other plasma membrane-bound enzymes have received little attention.

The importance of (Na^+–K^+)-activated ATPase in the active transport of cations[8,9] prompted us to investigate whether or not the activity of this enzyme might be controlled *in vitro* by hormones.

The data to be described here suggest that glucagon and epinephrine inhibit the enzyme activity, while insulin counteracts this effect.

Liver plasma membranes were isolated from fed male rats (180–200 g body weight), according to the procedure of Ray[10], as previously reported[5,6]. The mean recovery was about 1 mg membrane protein per g fresh tissue. The purity of the preparations was checked by electron microscopy and by an assay of marker enzymes, 5'-nucleotidase and glucose-6-phosphatase.

(Na^+–K^+)-dependent ATPase activity was measured from the amount of P_i released,

essentially according to the procedure of Bonting[11]. The assay medium (2.2 ml final volume) contained: membrane suspension (80–130 μg protein), 92 mM Tris buffer (pH 7.5), 5 mM $MgSO_4$, 60 mM NaCl, 5 mM KCl and 0.1 mM EDTA. After 10 min equilibration at 37 °C, in a shaking incubator, reaction was started by addition of ATP (disodium or Tris salt) at a final concentration of 4 mM, and of the hormones under investigation. At the end of the incubation period, reaction was stopped by addition of 2 vol. of ice-cold trichloroacetic acid (10%, w/v). All experiments were run in triplicate or duplicate.

The rate of ATP hydrolysis catalysed by the $(Na^+–K^+)$-ATPase was routinely derived by subtrating the rate of ATP hydrolysis obtained in the absence of K^+ from that obtained in the medium described above. Alternatively, in numerous experiments the activity of $(Na^+–K^+)$-ATPase was derived by subtracting the average rate of hydrolysis measured: (a) in the absence of Na^+; (b) in the absence of K^+; and (c) in the presence of K^+, Na^+ and ouabain ($1 \cdot 10^{-4}$ M). When Na^+ or K^+ were omitted they were replaced by K^+ or Na^+, respectively; in the case of Na^+ absence, ATP (Tris salt) was employed. However, it was found that the value was essentially similar to the one obtained in the absence of K^+, as shown by Bakkeren and Bonting[12] in total aqueous homogenates of liver. In Table I the results of preliminary experiments concerning ATPase and 5'-nucleotidase activities of plasma membrane, as compared with the activities of aqueous homogenates, are reported. The baseline activity of $(Na^+–K^+)$-ATPase of our membrane preparations is quite similar to that reported by Emmelot and Bos[13]

TABLE I

ATPase AND 5'-NUCLEOTIDASE ACTIVITY OF PLASMA MEMBRANE AND HOMOGENATE OF RAT LIVER

Enzymatic activity is reported as μmoles P_i ± S.E./mg protein per 5 min; in parentheses, the number of experiments. For experimental details see text.

	ATPase	
	Total activity	*$(Na^+–K^+)$-ATPase*
Plasma membrane *		
Complete system	5.45 ± 0.14 (9)	–
Idem, $-K^+$	3.86 ± 0.13 (8)	1.59
Idem, $-Na^+$	4.05 ± 0.32 (3)	1.40
Idem, + ouabain ($1 \cdot 10^{-4}$ M)	4.35 ± 0.11 (8)	1.10
Homogenate **		
Complete system	0.085 ± 0.025 (3)	–
Idem, $-K^+$	0.059 ± 0.011 (3)	0.026
Idem, + ouabain ($1 \cdot 10^{-4}$ M)	0.068 ± 0.017 (3)	0.017
	5'-Nucleotidase	
Plasma membrane	2.41 ± 0.07 (3)	
Homogenate	0.235 ± 0.037 (3)	

* Membrane protein recovery was 1.27 ± 0.25 mg/g wet liver.
** Protein content of the homogenate was 190 ± 22 mg/g wet liver.

for liver plasma membranes isolated by a similar procedure.

5'-Nucleotidase was assayed in a medium containing 65 mM KCl, 5 mM $MgSO_4$, 92 mM Tris buffer (pH 7.5), 0.1 mM EDTA and 4 mM 5'-AMP, final volume 2.2 ml.

Protein was estimated according to Lowry *et al.*[14] using bovine serum albumin as a standard.

ATP (disodium or Tris salt); L-epinephrine bitartrate; crystalline glucagon; bovine serum albumin; DL-propranolol were from Sigma. Insulin, glucagon-free, was a gift of Dr R. Chance, Eli Lilly Co., Indianapolis, Ind. Cyclic AMP (adenosine 3':5'-monophosphoric acid), dibutyryl cyclic AMP (N^6-2'-*O*-dibutyryl adenosine 3':5'-monophosphoric acid, sodium salt), 5'-AMP were products from Boehringer-Mannheim, Germany.

In preliminary experiments it was found that the (Na^+–K^+)-ATPase reaction was linear for about 8–10 min. However, an experimental time of 5 min was routinely employed in order to avoid possible alterations of the membranes and thus of the response to the hormones under investigation, due to the presence of proteolytic enzymes[15,16]. On the other hand such a period of time appeared reasonably sufficient to measure modifications related to the hormonal treatment.

Epinephrine ($4 \cdot 10^{-5}$ M) and glucagon ($1 \cdot 10^{-7}$ M) significantly reduced (Na^+–K^+)-ATPase activity (Table II), the latter being more effective than the former (–64% *versus* –47% inhibition).

TABLE II

EFFECT OF EPINEPHRINE, INSULIN, GLUCAGON, CYCLIC AMP AND PROPRANOLOL ON LIVER PLASMA MEMBRANE (Na^+–K^+)-ATPase

Enzyme activity is expressed as μmoles P_i ± S.E./mg protein per 5 min; in parentheses, the number of experiments. *P* has been calculated by Student's *t* test. For experimental conditions, see text.

Treatment	*ATPase activity*	*P value*
None	1.60 ± 0.20 (7)	–
Epinephrine ($4 \cdot 10^{-5}$ M)	0.85 ± 0.18 (4)	<0.05 *
Epinephrine ($4 \cdot 10^{-5}$ M) + insulin ($1 \cdot 10^{-9}$ M)	1.86 ± 0.15 (3)	–
Insulin ($1 \cdot 10^{-9}$ M)	1.40 ± 0.17 (8)	–
Glucagon ($1 \cdot 10^{-7}$ M)	0.58 ± 0.025 (5)	<0.01 *
Glucagon ($1 \cdot 10^{-7}$ M) + insulin ($1 \cdot 10^{-9}$ M)	0.91 ± 0.044 (5)	<0.05 * <0.01 **
None	1.21 ± 0.22 (7)	–
Cyclic AMP ($5 \cdot 10^{-5}$ M)	0.50 ± 0.11 (7)	<0.05 *
Dibutyryl cyclic AMP ($5 \cdot 10^{-5}$ M)	0.10 ± 0.007 (4)	<0.01 *
None	1.44 ± 0.15 (8)	–
Epinephrine ($4 \cdot 10^{-5}$ M)	0.84 ± 0.17 (8)	<0.05 *
Epinephrine ($4 \cdot 10^{-5}$ M) + propranolol ($5 \cdot 10^{-5}$ M)	1.20 ± 0.21 (8)	–
Propranolol ($5 \cdot 10^{-5}$ M)	1,20 ± 0.19 (4)	–

* With respect to controls.
** With respect to glucagon.

A dose–response study indicated that glucagon started to inhibit the enzyme at a concentration of $1 \cdot 10^{-9}$ M; at $1 \cdot 10^{-6}$ M it completely blocked the activity. On the other hand, the lowest inhibitory concentration of epinephrine was $1.5 \cdot 10^{-5}$ M (Fig. 1).

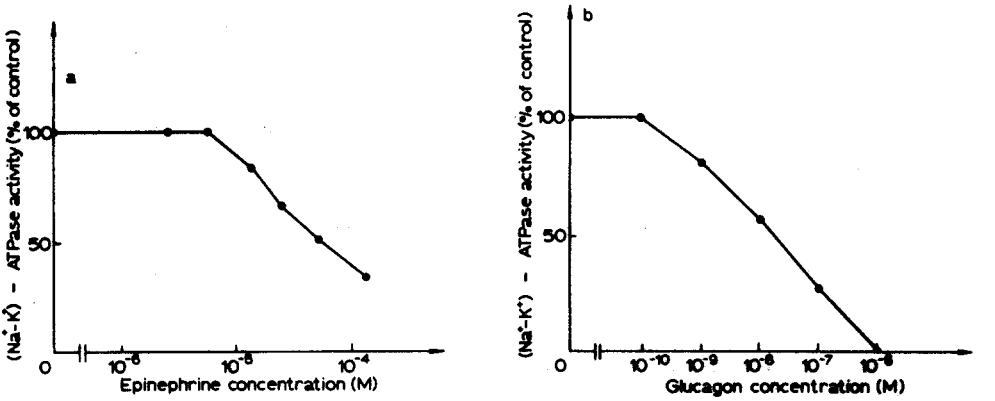

Fig. 1. (a) Effects of L-epinephrine concentration on $(Na^+–K^+)$-ATPase activity. Each point is the average of at least three experiments. Activity of the control was 1.72 ± 0.14 μmoles P_i/mg protein per 5 min. For experimental conditions, see text. (b) Effects of glucagon concentration on $(Na^+–K^+)$-ATPase activity. Each point is the average of at least three experiments. Activity of the control was 1.48 ± 0.19 μmoles P_i/mg protein per 5 min.

In several experiments the effect of epinephrine was measured over longer incubation periods (10 and 20 min). The hormonal effect was unchanged until 10 min, while it was reduced to only −20% after 20 min of incubation (not shown).

Insulin did not significantly modify the baseline activity, while it partially counteracted the effect of both epinephrine and glucagon (Table II). In these experiments insulin was employed at a concentration of $1 \cdot 10^{-9}$ M. A 100-fold higher dose ($1 \cdot 10^{-7}$ M) did not modify the response (not shown).

Epinephrine and glucagon are known to enhance the activity of adenylate cyclase of isolated liver plasma membranes[1–6]. Insulin, on the other hand, probably enhances the activity of cyclic AMP phosphodiesterase[17] and reduces that of adenylate cyclase[2,18]; in addition, it partially counteracts the stimulating effect of glucagon on the latter enzyme[19]. Therefore, the possibility was considered that the hormonal effects described above could be mediated by cyclic AMP. It was found that both cyclic AMP and its dibutyryl derivative strongly inhibited ATPase (−60% and 92% inhibition, respectively). No interpretation can be given at present for the higher activity of the dibutyryl derivative of cyclic AMP.

Propranolol, a β-blocking agent, was effective in preventing the inhibitory action of epinephrine, although used alone it did not significantly alter the enzyme activity.

These data, in our opinion, may be interpreted to mean that the activity of plasma membrane (Na^+–K^+)-ATPase is modulated *in vitro* by epinephrine, glucagon and insulin, by an action probably mediated by cyclic AMP.

Recently, a direct stimulation by insulin of an ATPase activity of lymphocyte plasma membrane has been shown by Hadden *et al.*[20], while an inhibition by cyclic AMP of Ca^{2+}-activated ATPase of heart sarcolemma has been registered by Dietze and Hepp[21].

In the past years, attention has been paid to the effect of epinephrine and glucagon on the release of K^+ from the hepatic tissue, a process usually (although not necessarily) related to glycogenolysis (see refs 22–24 and 25 for review). A release of K^+ may be due either to an enhanced cation permeability and therefore to a passive loss, or to a reduced uptake of K^+ by the Na^+/K^+ pump. Even if it is difficult to compare *in vivo* with *in vitro* effects, the data of this paper may suggest that an inhibition of the (Na^+–K^+)-ATPase may be involved in the loss of K^+ from liver.

In addition, the partial prevention by insulin of the glucagon and epinephrine effects on the membrane ATPase has its counterpart in the findings of Williams *et al.*[24], indicating that insulin prevents to a large extent the release of K^+ produced by cyclic AMP in the isolated rat liver perfused with a recirculating medium. The data of the present paper are also in agreement with the insulin-caused recovery of K^+ previously lost from perfused rat liver as an effect of glucagon infusion[26].

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