ACTION OF LULIBERIN ON Na,K-ATPase AND Ca-ATPase ACTIVITY IN THE RAT HEART SARCOLEMMA

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The effect of luliberin, one of the hypothalamic releasing factors, on ATPase activity in the rat heart sarcolemma was investigated. Luliberin was found to depress Na,K-ATPase activity and to increase Ca-ATPase activity. It was also shown that cyclic AMP and noradrenalin inhibit Na,-K-ATPase activity. It is suggested that the effect of luliberin on Na,K-ATPase activity of the rat heart sarcolemma is attributable to direct action on activity of the enzyme and indirect action through cyclic AMP.

KEY WORDS: luliberin; cyclic AMP; noradrenalin; ATPase activity; sarcolemma.

Some peptide hormones of the hypothalamic–hypophyseal system of the brain (somatostatin, vasopressin) can affect the activity of the cardiovascular system [1, 4]. The activity of several enzyme systems of the heart is modified in this way [2], including ATPase activity [3].

With the discovery in recent years of a whole group of new peptide hormones in the hypothalamus, the so-called releasing factors, the study of the role and place of these hormones in the regulation of activity of the visceral organs, including the heart, has assumed special importance.

The object of this investigation was to study the effect of one of the hypothalamic releasing factors, the decapeptide luliberin (pro-glu-his-try-ser-tyr-gly-leu-arg-pro-gly-NH_2) on Na,K-ATPase and Ca-ATPase activity of the sarcolemma of the rat heart.

EXPERIMENTAL METHOD

The sarcolemma was removed from the ventricles of noninbred male albino rats weighing 180-220 g by the method in [5]. Experiments to study the action of luliberin on sarcolemma ATPase were carried out during the first 3-4 h after isolation of the hormone, corresponding to the time of maximal activity of Na,K-ATPase and Ca-ATPase.

ATPase activity in the sarcolemma was determined as acidification of the medium [7] by means of a PHM-64 pH-meter (from Radiometer, Denmark). Measurements were made in 2 ml medium with a protein concentration of 0.25 mg/ml and at 37°C. The protein concentration was determined by Lowry's method [11]. The incubation medium for measurement of Na,K-ATPase activity contained (in mM): $MgCl_2$ 5, NaCl 120, KCl 30, Tris-HCl 5, ATP-Na_2 3, EGTA 2, ouabain 0.5 (pH 7.4). Na,K-ATPase activity was determined as the difference between activity of total ATPase and of its ouabain-sensitive component. The incubation medium for measurement of activity of transport Ca-ATPase contained (in mM): $MgCl_2$ 5, NaCl 140, Tris-HCl 5, ATP-Na_2 3. Activity of Ca-ATPase was determined as the increase in ATPase activity after addition of various quantities of $CaCl_2$ (from 0.6 to 3 mM) to the incubation medium against the background of 2 mM EGTA. The ratio of total ATPase activity to the activity of its ouabain-sensitive components was 1.7-1.9, and the ratio to the activity of its EGTA-dependent component was 1.3-1.4.

The following reagents were used: EDTA, EGTA, and cyclic 3',5'-AMP were from Sigma, USA, the ATP-Na_2 and ouabain from Calbiochem, USA, and the noradrenalin bitartrate was from Serva, FRG.

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TABLE 1. Effect of Cyclic AMP, Luliberin, and Noradrenalin on Na,K-ATPase Activity in Rat Heart Sarcolemma

Experimental conditions	Na,K-ATPase activity, neq $H^+ \cdot min^{-1} \cdot mg^{-1}$ protein	P
Control	15,7±2,0	—
Cyclic AMP ($2 \cdot 10^{-6}$ M)	10,8±2,1	<0,05
Cyclic AMP (10^{-4} M)	9,8±1,6	<0,01
Noradrenalin ($5 \cdot 10^{-5}$ M)	8,5±2,1	<0,05
Luliberin ($5 \cdot 10^{-6}$ M)	7,8±1,3	<0,001
Noradrenalin ($5 \cdot 10^{-5}$ M) + luliberin ($5 \cdot 10^{-6}$ M)	5,7±1,1	<0,001

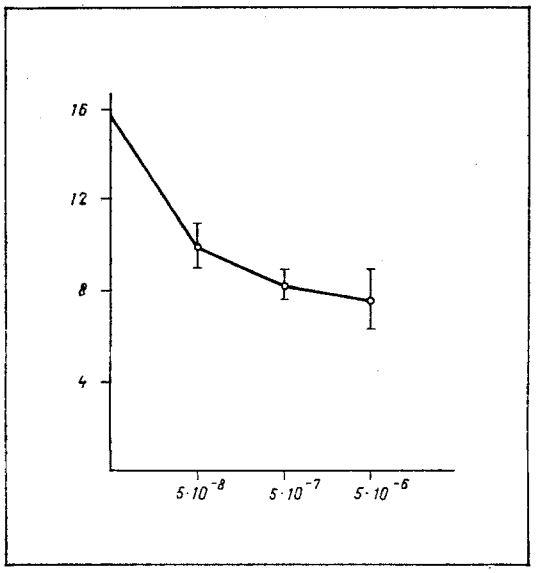

Fig. 1. Effect of luliberin on Na,K-ATPase activity in the rat heart sarcolemma. Abscissa, concentration of luliberin (in M); ordinate, Na,K-ATPase activity (in neq $H^+ \cdot min^{-1} \cdot mg^{-1}$ protein).

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that luliberin inhibits activity of transport Na,K-ATPase in the sarcolemma; the peptide was most effective in a concentration of $5 \cdot 10^{-6}$ M, when it reduced Na,K-ATPase activity by 50%. Two possible ways of reducing activity of Na,K-ATPase can be postulated: by direct action of the hormone on the combining sites of the enzyme or indirectly, through cyclic AMP. The decrease in Na,K-ATPase activity in the sarcolemma of the rat heart in the present experiments under the influence of cyclic AMP is evidence in support of the latter hypothesis (Table 1).

Inhibition of Na,K-ATPase activity in the rat heart sarcolemma and Na,K-ATPase activity in the plasma membranes of other organs of the rat by cyclic AMP have been found in other investigations [7, 10]. However, as Table 1 shows, cyclic AMP in a concentration (10^{-4} M) several times greater than is required to exhibit its hormonal effect, did not completely reproduce the effect of luliberin, a result which may perhaps indicate that the hormone also has a direct action on enzyme activity.

Catecholamines and, in particular, noradrenalin, are known to increase the adenylate cyclase activity of the plasma membranes of the heart [8]. It was important to discover if the action of luliberin on Na,K-ATPase

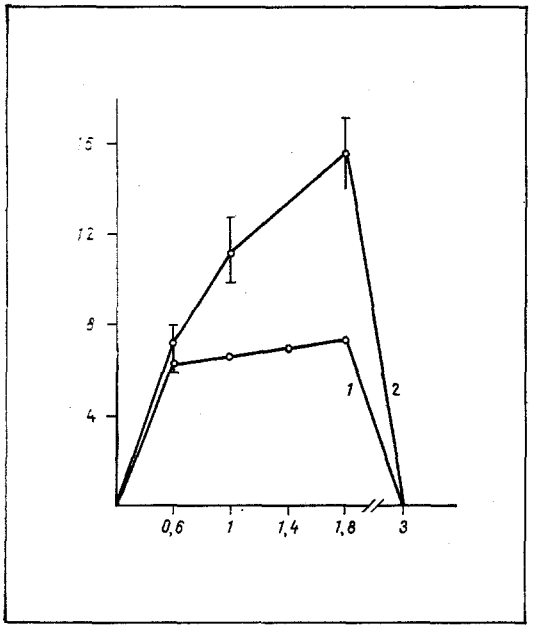

Fig. 2. Effect of luliberin on Ca-ATPase activity in rat heart sarcolemma. Abscissa, Ca^{++} concentration (in mM); ordinate, Ca-ATPase activity (in neq $H^+ \cdot min^{-1} \cdot mg^{-1}$ protein). 1) Control, 2) in presence of $5 \cdot 10^{-6}$ M luliberin.

activity in the sarcolemma would still be exhibited in the presence of noradrenalin. As Table 1 shows, during the combined action of noradrenalin and the peptide, Na,K-ATPase activity was inhibited much more strongly (a decrease of 64%) than by their action separately (decreases of 50 and 46% respectively). This fact could be further proof that, besides inhibiting Na,K-ATPase in the sarcolemma of the rat heart by its action through cyclic AMP, the hypothalamic hormone luliberin has a direct action on the activity of this enzyme.

Besides the decrease in Na,K-ATPase activity under the influence of luliberin in these experiments, activity of Ca^{++}-activated Mg^{++}-dependent ATPase also was changed. The presence of Ca-ATPase in the sarcolemma of the heart in rats and other animals has been demonstrated in several investigations [5, 7].

Dose–effect curves for stimulation of the Ca^{++}-activated component of Mg^{++}-dependent ATPase in the sarcolemma by Ca ions is illustrated in Fig. 2. The level of hydrolysis of the substrate was maximal when the Ca^{++} concentration in the medium was 2 mM. Addition of luliberin ($5 \cdot 10^{-6}$ M) to the incubation medium caused an increase in Ca-ATPase activity by 2.2 times compared with the control.

Luliberin thus not only participates in the regulation of liberation of the pituitary trophic hormones but can also modify the Na,K-ATPase and Ca-ATPase activity of the sarcolemma of the rat heart, and the changes produced are in different directions.

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