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STIMULATION OF (Na^+—K^+)-ATPase OF RAT LIVER PLASMA MEMBRANE BY AMINO ACIDS

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Summary

The effect of twelve L-amino acids on the activity of liver plasma membrane (Na^+—K^+)-ATPase has been tested. Histidine and arginine significantly enhanced the activity. The activation by histidine showed saturation kinetics with an apparent K_a of about 8 mM, and was evident over a wide range of Na^+ concentrations. The same amino acid did not significantly affect the Mg^{2+}-dependent ATPase activity.

Amino acid stimulation of (Na^+—K^+)-dependent ATPase has been reported on a few occasions [1—4] in more or less purified enzymatic preparations from different sources, and a possible role of (Na^+—K^+)-ATPase in amino acid transport has been critically evaluated in two recent reviews [5,6].

The observations to be reported here concern the effect of some L-amino acids on plasma membrane-bound (Na^+—K^+)-dependent ATPase of rat liver. Stimulation was observed for two amino acids only while a stimulating effect of EDTA could not be shown.

Liver plasma membranes were isolated from fed male rats (150—180 g body weight) following the procedure of Ray [7]. After removal of the plasma membrane fraction from the sucrose gradient, the membranes were washed twice, first with the homogenizing medium (1 mM $NaHCO_3$, 0.5 mM $CaCl_2$; pH 7.5), then with 0.015 M EDTA (sodium salt, pH 7.5); and finally resuspended in bidistilled water. The purity of the preparation was checked by electron microscopy and by the assay of marker enzymes as reported previously [8].

(Na^+—K^+)-dependent ATPase activity was measured as previously reported [8]. The assay medium (2.2 ml final volume) contained: membrane

suspension (80—120 μg proteins), 92 mM Tris buffer (pH 7.5), 5 mM $MgSO_4$, 5 mM KCl, 60 mM NaCl and 0.1 mM EDTA (unless otherwise stated). The assay medium also contained the amino acids to be tested at the concentrations reported in the text. After 10 min equilibration at 37° C in a shaking incubator, the reaction was started by addition of ATP (disodium or tris salt) at a final concentration of 4 mM. After 5 min the reaction was stopped by adding two volumes of ice-cold trichloroacetic acid (10%, w/v). All experiments were run in triplicate.

ATP hydrolysis was measured from the amount of P_i released, and the method employed was not affected by the presence of histidine and other amino acids [9]. (Na^+—K^+)-ATPase activity was routinely obtained, as previously reported [8], by subtracting the rate of ATP hydrolysis in the absence of K^+ from the complete medium reported above.

Protein was estimated by the method of Lowry et al. [10] using bovine serum albumin as a standard.

Of the twelve amino acids tested, only histidine and arginine exerted a significant stimulation (Table I). This stimulation took place in the presence of 0.1 mM EDTA. Concentrations of this chelating agent above 0.1 mM were

TABLE I

EFFECT OF DIFFERENT AMINO ACIDS ON LIVER PLASMA MEMBRANE (Na^+—K^+)-ATPase.

Enzyme activity is reported as μmoles P_i ± S.E./mg protein per 5 min; in parentheses, the number of experiments. Final concentration of amino acids was 10 mM. *P* has been evaluated by Student's *t* test. For experimental details, see text. n.s., not significant.

Treatment	ATPase activity	*P*
—	1.02 ± 0.12 (8)	—
Glycine	0.93 ± 0.05 (5)	n.s.
—	0.92 ± 0.05 (6)	—
L-Alanine	0.88 ± 0.14 (6)	n.s.
—	1.08 ± 0.13 (4)	—
L-Methionine	1.09 ± 0.14 (4)	n.s.
—	1.04 ± 0.23 (3)	—
L-Phenylalanine	0.90 ± 0.20 (3)	n.s.
—	1.15 ± 0.14 (7)	—
DL-Phenylalanine	1.05 ± 0.10 (7)	n.s.
—	1.03 ± 0.07 (6)	—
L-Tryptophan	1.00 ± 0.05 (5)	n.s.
L-Leucine	1.00 ± 0.03 (5)	n.s.
—	1.04 ± 0.06 (13)	—
L-Histidine	1.40 ± 0.06 (13)	<0.001
—	1.23 ± 0.28 (4)	—
L-Aspartic acid	1.27 ± 0.18 (4)	n.s.
—	1.09 ± 0.09 (4)	—
L-Glutamic acid	0.92 ± 0.16 (4)	n.s.
—	1.13 ± 0.06 (11)	—
L-Arginine	1.38 ± 0.10 (11)	<0.05
L-Lysine	1.26 ± 0.06 (10)	n.s.

TABLE II

EFFECT OF EDTA CONCENTRATION ON RAT LIVER PLASMA MEMBRANE (Na^+—K^+)-ATPase

Enzyme activity is reported 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 details, see text. n.s., not significant. *P* was evaluated with respect to 0.1 mM EDTA; or 0.5 mM EDTA (*); 1.0 mM EDTA (†).

EDTA Concentration (mM)	ATPase activity	*P*
0	0.95 ± 0.007 (4)	n.s.
0.1	1.18 ± 0.19 (5)	—
0.5	0.99 ± 0.24 (4)	n.s.
1.0	0.59 ± 0.06 (4)	<0.05
		<0.05 (*)
10	0.10 ± 0.11 (3)	<0.01
		<0.05 (†)

inhibitory to (Na^+—K^+)-ATPase activity; its complete removal resulted in a slight, and statistically insignificant diminution of the activity (Table II). These results are at variance with those previously reported concerning EDTA activation of (Na^+—K^+)-ATPase preparations from kidney outer medulla [3] or Ehrlich cell plasma membrane [4].

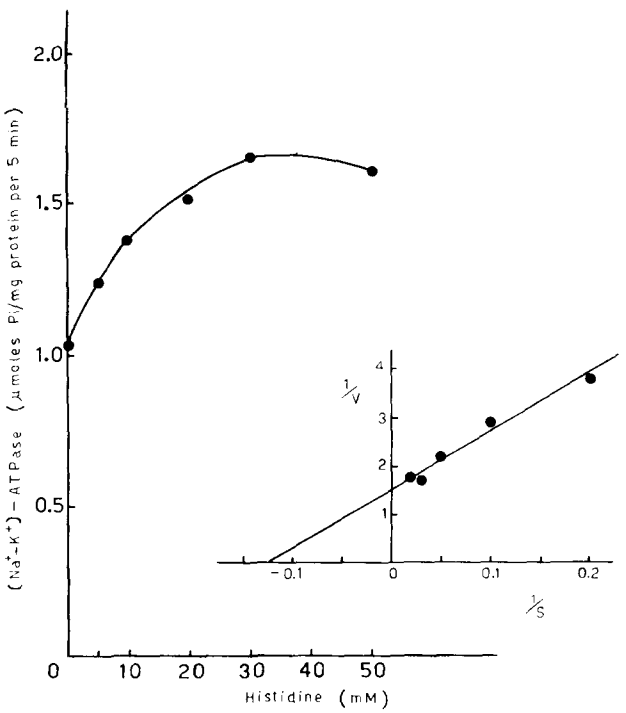

Fig.1. Concentration effect of L-histidine in stimulating (Na^+—K^+)-ATPase of rat liver plasma membranes. The points are means of at least three experiments carried out on different membrane preparations, as reported in the text. The insert represents a Lineweaver—Burk plot of the histidine stimulation curve when the reciprocal of (Na^+—K^+)-ATPase activity, from which the basal (Na^+—K^+)-ATPase activity in the absence of histidine has been subtracted, is plotted against the reciprocal of histidine concentration. The straight line has been drawn by the least squares method.

At the histidine concentration reported in Table I we observed a stimulation of Mg^{2+}-dependent ATPase measured, as previously reported [8], in the absence of K^+ and in the presence of 60 mM Na^+. The average value of 22 experiments was 5.58 ± 0.42 versus 6.54 ± 0.41 (μmoles P_i ± S.E./mg protein per 5 min), but the difference was not statistically significant. On the basis of these data we can state that ATPase activity stimulation requires, in the experimental conditions we employed, the presence of Na^+ and K^+; even if a (Na^+—K^+)-independent ATPase stimulation, as found by Ronquist and Christensen [11] in Ehrlich cell membrane preparations, cannot be ruled out completely.

Histidine (10 mM) failed to exert its stimulatory effect when $1 \cdot 10^{-4}$ M ouabain was added to the reaction medium (not shown). Under these conditions, the (Na^+—K^+)-ATPase activity was inhibited by 70% as in our previous experiments [8].

(Na^+—K^+)-dependent ATPase stimulation by increasing concentrations of histidine is reported in Fig. 1. The activation of ATPase shows saturation kinetics with an apparent K_a of about 8 mM.

The Na^+ activation of ATPase activity in the presence of Mg^{2+} and K^+, both at 5 mM, gives an apparent K_a of about 19 mM which is in good agreement (Fig. 2) with previous reports [9,12]. Histidine stimulation of ATPase activity, under the same conditions as reported above, proved to be effective over the same range of Na^+ concentration investigated, giving an apparent K_a

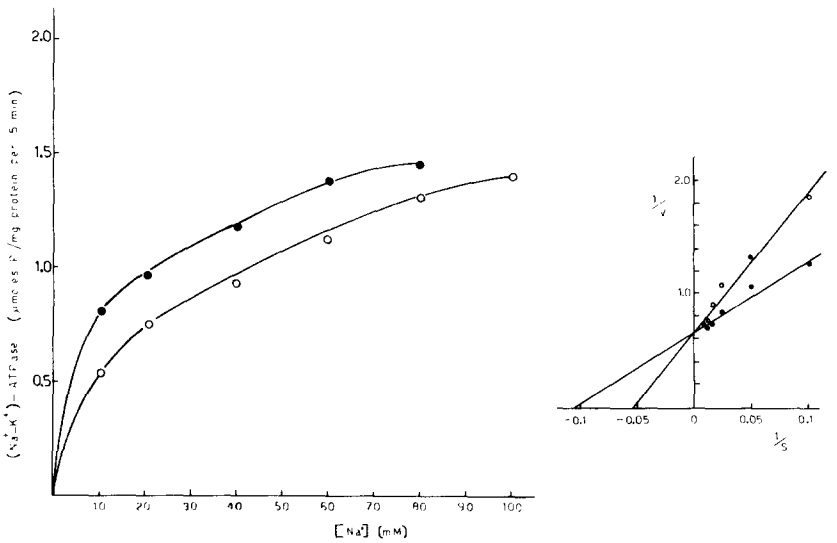

Fig. 2. Na^+ concentration effect on (Na^+—K^+)-dependent ATPase activity in the presence (●—●) and absence (○—○) of 10 mM L-histidine; Mg^{2+} and K^+ concentration was kept constant at 5 mM. The points are means of at least three experiments carried out on different membrane preparations. The insert represents a Lineweaver—Burk plot of the Na^+ dependence curve of (Na^+—K^+)-ATPase, under the conditions reported above, when the reciprocal of (Na^+—K^+)-ATPase activity is plotted against the reciprocal of Na^+ concentration. The straight line has been drawn by the least squares method.

of about 9 mM. Na^+ omitted was not replaced in any way, following Bonting's suggestion [9] that the osmolarity of the medium is not a critical point as far as ATPase activity is concerned.

The data presented so far may be interpreted as excluding, for rat liver plasma membrane preparations, any possible stimulation of (Na^+—K^+)-dependent ATPase due to the presence of a chelating agent; conversely, it is not easy to explain amino acid stimulation in terms of a simple chelation. On the other hand, it seems difficult not to link the observed stimulation of ATPase activity to a possible cation-dependent transport system present at the plasma membrane level, even if very recent observations argue against a direct coupling between ATP hydrolysis and amino acid transport [13].

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