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STIMULATION OF Na^+-DEPENDENT AMINO ACID UPTAKE BY ACTIVATION OF THE Ca^{2+}-DEPENDENT K^+ CHANNEL IN THE EHRLICH ASCITES TUMOR CELL

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The activation of Ca^{2+}-dependent K^+ channel by propranolol or by ascorbate-phenazine methosulphate stimulates Na^+-dependent transport of α-aminoisobutyric acid. This stimulation arises from a membrane hyperpolarization due to the specific increase of membrane K^+ conductance. The same treatment does not modify the Na^+-independent uptake of the norbornane amino acid.

A large variety of animal cells possess in their membrane K^+ channels whose activity is controlled by the cytoplasmic Ca^{2+} concentration. The involvement of these channels in the control of membrane potential at rest as well as in the genesis of post-spike hyperpolarizations has been shown in several nerve and muscle cells [1]. A role for this transport system in the response of different epithelial and secretory cells to humoral modulators has also been suggested [2–4]. We have reported recently the presence of Ca^{2+}-dependent K^+ channels in the Ehrlich cell membrane [5]. Here we report the effects of the activation of such channels on amino acid transport.

Amino acid transport was measured as described previously [5–7]. Membrane potential was assessed from the distribution of triphenylmethylphosphonium (TPMP) in the presence of tetraphenylboron [8]. The incubation medium had the following composition (mM): NaCl, 120; $CaCl_2$, 0.5; KCl, 1; choline chloride, 29; Hepes-NaOH buffer, 20 (pH 7.4). In several experiments KCl was increased to 5 or 30 mM, replacing an equimolar amount of choline chloride. Propagation and handling of the Ehrlich cell were as described previously [7].

The addition of 0.5 mM propranolol or 20 mM sodium ascorbate (replacing an equimolar amount of NaCl) + 0.1 mM phenazine methosulphate (asc-PMS) to the incubation medium activates the Ca^{2+}-depenent K^+ channel of the Ehrlich cell [5]. Fig. 1 shows that this activation is accompanied by an stimulation of the uptake of α-aminoisobutyric acid, a model substrate for the Na^+-dependent transport system A [9]. This effect was prevented by quinine, a known inhibitor of the Ca^{2+}-dependent K^+ channel in the Ehrlich cell [5] as well as in other cell systems [10,11].

The effects of the addition of asc-PMS or propranolol did not appear when the K^+ concentration of the medium was increased to 30 mM (Table I). Under these conditions there is no net movement of K^+ (Table I), as could be expected from the values of the transmembrane potential estimated for these cells [12]. Propranolol in Na^+-free medium did not modify the uptake of 0.1 mM 2-aminonorbonyl-2-carboxylic acid, a model substrate for the Na^+-independent system L [9] (not shown). The above results strongly suggest that the

Abbreviations: TPMP, Triphenylmethylphosphonium; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; asc-PMS: 20 mM sodium ascorbate + 0.1 mM phenazine methosulphate.

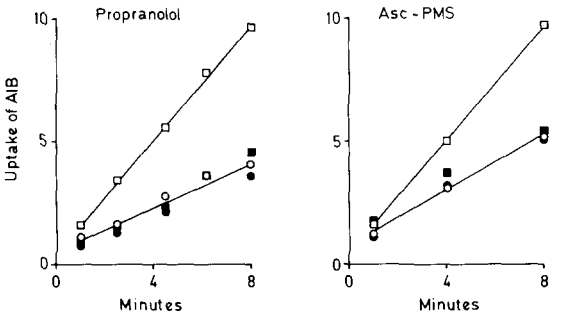

Fig. 1. Stimulation of the uptake of α-aminoisobutyric acid (AIB) by propranolol or asc-PMS. The uptake of 1 mM AIB was studied at 37°C and under N_2 from medium containing 1 mM K^+, and is expressed as the distribution ratio (cpm per l of cell water/cpm per l of medium) reached. Additions: ○——○, none; ●——●, 1 mM quinine; □——□ 0.5 mM propranolol (left) or 20 mM sodium ascorbate+0.1 mM phenazine methosulphate (right); ■——■, same+ 1 mM quinine.

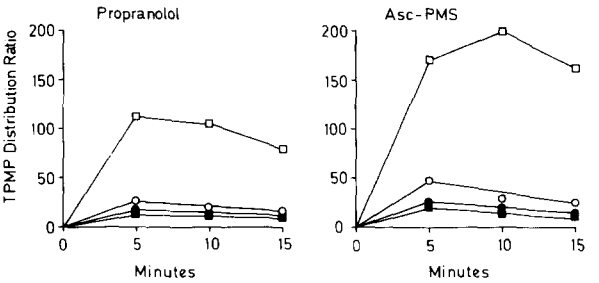

Fig. 2. Effects of propranolol or asc-PMS on the distribution ratio of TPMP. The initial concentrations of TPMP and tetraphenylboron in the incubation medium were 4.4 and 3.3 μM, respectively. Other conditions and symbols as in Fig. 1. Similar results were obtained in Na^+-free (substituted by choline) medium. The experiments with propranolol and with asc-PMS were done with two different cell batches.

increased uptake of α-aminoisobutyric acid observed under treatment with asc-PMS or propranolol is due to an increase of the membrane permeability to K^+ that generates a membrane hyperpolarization. Since Na^+-dependent amino acid transport is electrogenic [13–15], the increase of the electrochemical gradient for Na^+ resulting from the membrane hyperpolarizacion is expected to stimulate amino acid uptake through the Na^+-dependent system A but not through the Na^+-independent system L, as observed. The occurrence of membrane hyperpolarization was demonstrated by the fact that asc-PMS or propranolol increased the distribution ratio reached by TPMP. This effect was also prevented by quinine (Fig. 2) or by increasing the K^+ concentration of the medium to 30 mM (not shown).

These results extend previous observations on the effects of propranolol on amino acid transport in the Ehrlich cell [16], and suggest that the degree of activation of the Ca^{2+}-dependent K^+ channels of the plasma membrane could modulate Na^+-dependent transport through changes in membrane potential. Several responses to hormones and other humoral modulators are known to include changes of amino acid transport [17]. In some cases, Ca^{2+}-dependence [18–20] and/or

TABLE I

EFFECTS OF MEDIUM K^+ CONCENTRATION ON THE STIMULATION OF THE UPTAKE OF 1 mM α-AMINOISOBUTYRIC ACID (AIB) INDUCED BY PROPRANOLOL OR asc-PMS

Each value is mean±S.D. of four experiments.

Condition	5 mM K^+		30 mM K^+	
	Uptake [a] of AIB	Cell K^+ [b]	Uptake [a] of AIB	Cell K^+ [b]
Control [c]	12.2±2.9	94±1	10.0±0.9	115±2
asc-PMS [c]	20.4±1.8	79±2	10.2±0.1	111±5
Control	26.6±1.3	142±3	14.4±0.5	158±4
Propranolol	32.5±1.4	119±8	13.6±0.8	168±6

[a] 8 min distribution ratio.
[b] mequiv./l cell water.
[c] These two sets of experiments were performed in anaerobiosis.

simultaneous changes of K^+ permeability [20–22] and membrane potential [20,23,24] have been reported. On these basis, the possible involvement of the Ca^{2+}-dependent K^+ channel in such responses deserves to be investigated.

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