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INHIBITION OF ADENOSINE MEDIATED RESPONSES IN ISOLATED HEPATOCYTES BY DEPOLARIZING CONCENTRATIONS OF K^+

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Summary: Isolated rat hepatocytes, incubated in a high K^+ medium which depolarizes their plasma membrane, were used to investigate the response to adenosine. High K^+ concentration blocked both the adenosine mediated increase of calcium influx and the increase in the rate of urea synthesis. It is concluded that a) adenosine stimulates calcium influx in hepatocytes probably through receptor-operated Ca^{2+} channels which are closed by depolarization of the plasma membrane, b) the higher cytosolic calcium concentration triggers a regulatory step that fully stimulates the rate in urea synthesis.

Incubation of isolated rat hepatocytes with micromolar concentrations of adenosine stimulates glycogenolysis (1) ureagenesis (2) and gluconeogenesis (3). Since an increase in the cytosolic free calcium concentration (Ca^{2+} cyt) has been observed in hepatic cells after addition of adenosine and since the absence of calcium in the incubation mixture suppressed the adenosine-stimulated ureagenesis, calcium has been proposed as the transducing signal for adenosine in the hepatic cell (4). The elevation of Ca^{2+} cyt produced by adenosine seems to be predominantly due to a stimulation of the influx of Ca^{2+} across the plasma membrane (4).

The Ca^{2+} inflow systems involved in the hormonal stimulation of Ca^{2+} uptake in the liver are not well characterized, but it has been shown that they are distinct from the voltage - gated channels responsible for Ca^{2+} influx in excitable cells (5, 6). In rat liver, hormone dependent Ca^{2+} influx has been suggested to occur through receptor operated Ca^{2+} channels (7-8). Incubation of isolated hepatocytes with high external K^+, enough to produce depolarization of their liver plasma membranes (7, 9), abolished the stimulated Ca^{2+} influx induced by co-

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administration of glucagon (or cAMP) and vasopressin (6), or vasopressin alone (7). Even more, incubation of hepatocytes in high K^+ concentration impaired the stimulation of metabolic step(s) promoted by hormones or their agonist, i. e.: phosphorylase by phenylephrine (10), gluconeogenesis by glucagon (9) and phosphorylase a by vasopressin (7). A likely explanation for the activation of phosphorylase includes a rise in the concentration of cytosolic Ca^{2+} (7, 10) which could not be reached due to the action of external K^+ impairing, by membrane depolarization, the hormone-stimulated Ca^{2+} influx (7).

This report includes the effect of high external K^+ medium in isolated hepatocytes to explore the characteristics of the adenosine-mediated stimulation in Ca^{2+} influx and in the rate of urea synthesis.

MATERIALS AND METHODS

Hepatocyte isolation. Male Wistar rats weighing 150 to 200 g and fed Purina Rat Chow ad-libitum were anesthetized with ether. Hepatocytes were isolated using slight modifications (2) to the method of Berry and Friend (11) and suspended in Krebs-Ringer bicarbonate (12): 120 mM NaCl, 5.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 12 mM $NaHCO_3$, 1.2 mM $CaCl_2$, adjusted to pH 7.4 at 37°C and equilibrated with O_2/CO_2 (95/5%).

Quin-2 loading of hepatocytes. The procedure was done according to Charest et al. (13). Briefly, isolated hepatocytes were diluted in Krebs-Ringer bicarbonate, to approximately 50 mg ww/ml and incubated at 37°C under an O_2/CO_2 (95/5%) atmosphere for 10 min. The hepatocytes were incubated for an additional 20 min in the presence of 100 μM quin-2/AM added from a 20 mM stock solution in DMSO. After incubation with quin-2/AM, the cells were washed twice by centrifugation at 500 rpm/3 min in a clinical centrifuge. Liver cells were distributed in 200 μl aliquots in Eppendorf microfuge tubes and immersed in ice until used (4).

Measurement of urea. Urea was measured according to Gutman and Bergmeyer (14).

RESULTS

The effect of adenosine on the Ca^{2+} cyt of isolated hepatocytes was different depending on the K^+ concentration in the incubation solution. In low KCl medium, i.e., in the original Krebs-Ringer bicarbonate solution containing 5.7 mM KCl, Ca^{2+} cyt increased from 70 nM to above 100 nM at all the adenosine concentrations tested (10^{-9}-10^{-6}M) (Fig. 1). The higher KCl concentration in the Krebs-Ringer solution (from 5.7 mM to 114 mM KCl), had no effect on the basal level of Ca^{2+} cyt but abolished the ability of adenosine to increase Ca^{2+} cyt (Fig. 1). The effect of 10^{-6} M adenosine on the uptake of external Ca^{2+} into the Ca^{2+} cyt was

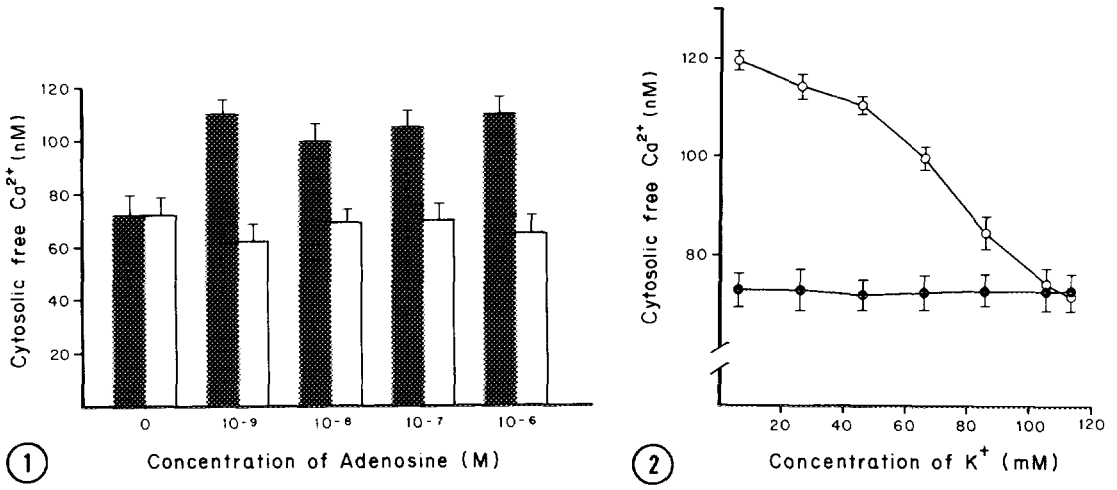

Fig. 1. Effect of different concentrations of adenosine on the Ca^{2+} cyt of isolated rat hepatocytes at two different KCl concentrations. Quin-2 loaded liver cells (25 to 30 mg wet weight) were incubated in 2.5 ml of Krebs - Ringer bicarbonate containing normal K^+ concentration (■ 5.7 mM KCl and 120 mM NaCl, reference 12) or high K^+ concentration (□ 114 mM KCl and 37 nM NaCl). Each value represents the average ± standard error, n=3.

Fig. 2. Effect of varying the external K^+ concentrations on the adenosine - mediated increase in Ca^{2+} cyt. The original concentrations of NaCl and KCl in Krebs-Ringer solution (12) were modified in order to reach the KCl concentration indicated in the figure, at the expense of decreasing NaCl concentration; in the absence of adenosine (•), and with 10^{-6} M adenosine (o). Other indications as in Fig. 1.

progressively inhibited by increasing the KCl concentration, at the expense of NaCl, in the incubation mixture of isolated liver cells (Fig. 2). In contrast, the basal levels of Ca^{2+} cyt were not modified by K^+ changes in the incubation mixture. There is an excellent correlation between the progressive diminution in plasma membrane potential (7) and the diminution in the adenosine - mediated increase in Ca^{2+} cyt (Fig. 2). Both actions were equally impaired as a function of the K^+ concentration in the Krebs-Ringer medium in which liver cells were incubated.

The increase in the rate of urea synthesis was affected in a complex way by the depolarization of the hepatocyte plasma membrane produced by incubating cells in increasing K^+ concentrations. A dose-dependent stimulation in the rate of urea synthesis was produced by adenosine using unmodified Krebs-Ringer solution (Fig. 3). The increase has statistical significance ($p < .001$) beginning at a nucleoside concentration of 10^{-8} M. Additionally, the incubation of

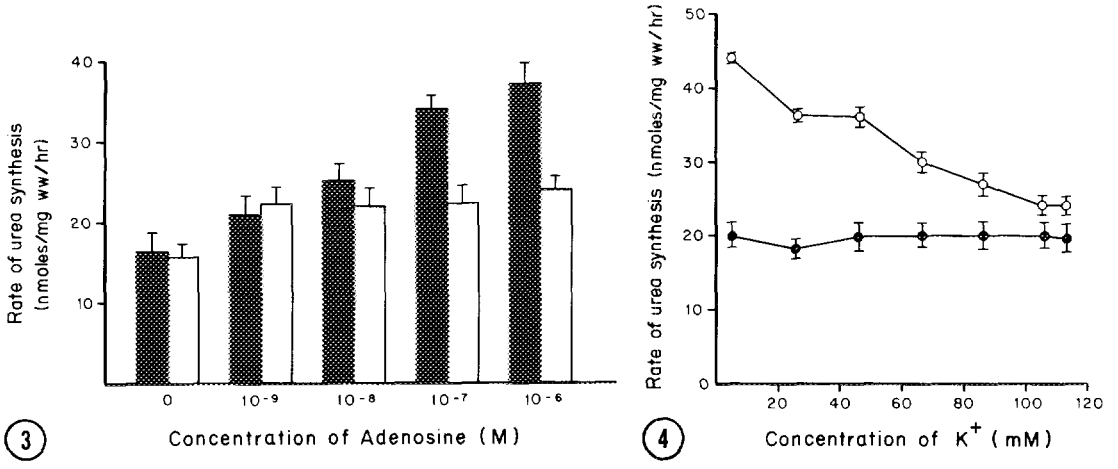

Fig. 3. Effect of different concentrations of adenosine on the rate of urea synthesis in isolated hepatocytes (25 to 30 mg wet weight) incubated in 1 ml Krebs-Ringer bicarbonate,pH 7.4, with normal or high K^+ concentration (see Fig. 1). The incubation medium was supplemented with 3 mM ornithine, 10 mM glucose and 5 mM $(NH_4)_2 CO_3$. Each value represents the average ± standard error, n=4.

Fig. 4. Effect of varying the external K^+ concentration on the adenosine-mediated stimulation in the rate of urea synthesis in isolated hepatocytes. The concentrations of NaCl and KCl were modified in the original Krebs-Ringer medium as indicated in Fig. 2. In the absence of adenosine (●), and with 10^{-6} M adenosine (o). Other conditions as in Fig. 3.

hepatic cells at 114 mM KCl resulted in a smaller increase in urea synthesis rate due to the presence of the nucleoside, which is statistically significant ($p < .05$) but is independent of the employed doses of adenosine. (Fig. 3).

Basal urea synthesis rate was unaffected by progressive substitution of NaCl by KCl in the Krebs-Ringer solution where the isolated hepatocytes were incubated; however, the stimulation in the rate of urea formation by 10^{-6} M adenosine was inhibited as the concentration of KCl in the incubation medium increased (Fig. 4). This inhibition in the rate of urea formation with KCl is analogous to the one impairing the rise in Ca^{2+} cyt, and interestingly, both effects of KCl were observed by using the same concentration of adenosine (Figs. 2 and 4).

DISCUSSION

There are 3 described routes in which Ca^{2+} can move through the plasma membrane of animal cells from the extracellular space into their cytosol: voltage-operated channels, receptor-operated

channels and a process of facilitated diffusion (5). Voltage-operated channels are only present in excitable cells (15,16), and, specifically, no voltage-dependent inward Ca^{2+} currents were identified in avian hepatocytes (17). Ca^{2+} facilitated diffusion is not modified by depolarization of the membrane or by the binding of an agonist to its receptor (18). The data obtained here with isolated hepatocytes: adenosine mediated Ca^{2+} influx and its blockage at elevated K^+ external concentrations (Figs. 1 and 2), which produced depolarization of liver plasma membrane (7, 9), suggest the presence of receptor-operated Ca^{2+} channels opened by adenosine, and are thus responsible for the nucleoside stimulated Ca^{2+} influx. A direct action of high K^+ incubation medium on the Ca^{2+} channel can be discarded since the basal uptake of Ca^{2+} was not modified (Figs. 1 and 2).

It is interesting to compare the opposite responses of adenosine-receptors upon agonist stimulation, either in the nervous system and in the hepatocyte; in the former adenosine closes Ca^{2+} channels and opens K^+ channels (19), in the latter the nucleoside opens Ca^{2+} channels (Figs. 1 and 2). In addition, the depolarization of plasma cell membranes from nerve or hepatic cells is accompanied by opposite responses in their respective Ca^{2+} channels: in nerve cells they are opened (19) but in hepatic cells they are closed (7,9).

A small increase in the rate of urea synthesis was present even in the absence of a net Ca^{2+} influx from the extracellular medium, but a full increase in the rate of urea synthesis required a net Ca^{2+} influx and was adenosine dose-dependent (Figs. 3 and 4). Consequently, an adenosine-mediated rise in the concentration of cytosolic calcium likely impinges a regulatory step in the extramitochondrial route of urea biosynthesis. Increases in the uptake of ornithine and in the synthesis of citrulline due to the presence of Ca^{2+} ions have been documented (20, 21). Therefore, it is proposed that the complete stimulation in the rate of urea synthesis by adenosine depends on the uptake of external calcium through a receptor-operated channel.

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