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α_1-Adrenergic stimulation causes Mg^{2+} release from perfused rat liver

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The possibility that Mg^{2+} mobilization is stimulated in perfused liver by α_1-adrenergic agonists was studied by measuring Mg^{2+} release in response to 0.5 and 20 μM phenylephrine. During preperfusion exogenous Mg^{2+} was added to the medium to give 1.2 mM. 5 min before starting the addition of phenylephrine the infusion of exogenous Mg^{2+} was stopped. Mg^{2+} in the perfusate leaving the liver was measured by atomic absorption spectroscopy. Analysis of the Mg^{2+} decay curves with two exponential models indicated that phenylephrine caused dose-dependent Mg^{2+} release from perfused rat livers.

Mg^{2+}; Perfusion; Adrenergic agonist, α_1-; (Rat liver)

1. INTRODUCTION

Mg^{2+} is an important cofactor for several types of enzymes and binds to coenzymes, metabolic intermediates and proteins or phospholipids of cellular membranes. Theoretically, changes in subcellular Mg^{2+} concentrations could influence cellular regulation in various ways but experimental evidence for a regulatory role of Mg^{2+} has remained difficult or impossible to obtain. In hepatocytes, numerous low-affinity binding sites for Mg^{2+} and no significant gradient of free Mg^{2+} between the cytosolic and mitochondrial compartments have been found, supporting the conclusion that short-term metabolic regulation by Mg^{2+} is unlikely [1]. More recently, however, increased mitochondrial Mg^{2+} content has been observed in rats in vivo after intravenous injection of vasopressin or vasopressin + glucagon [2]. This indicates that subcellular Mg^{2+} may be mobilized and taken up by mitochondria if cytosolic free Ca^{2+} is increased in response to hormones.

In perfused rat liver, Ca^{2+} mobilization by α_1-adrenergic stimulation is inhibited when the extracellular Mg^{2+} concentration is above 10 mM [3]. In addition, effects of exogenous Ca^{2+} mimicking those of phenylephrine on glycogenolysis and K^+ uptake by perfused livers are reduced by high perfusate Mg^{2+} concentrations [4], suggesting competition or antagonism between the two ions at regulatory sites. The possibility that Mg^{2+} is displaced from cellular sites following α_1-adrenergic stimulation has been studied by measuring Mg^{2+} release from perfused rat livers in response to phenylephrine. The results have been presented in preliminary form (18th FEBS Meeting, Ljubljana, 1987, Abstract).

2. EXPERIMENTAL

Livers from male fed rats (200-220 g) were perfused with medium containing 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 10 μM $MgSO_4$ and 10 μM $CaCl_2$. No metabolic substrates were added and the medium was not recirculated [3]. During the first 35 min of perfusion $MgCl_2$ was con-

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Table 1

Comparison of models

Model	[Phenylephrine] (μM)	k_1 (10^{-5} M)	k_2 (min^{-1})	k_3 (10^{-5} M)	k_4 (min^{-1})	Sums of squares	$P<$
A							
$Y = k_1\exp(-k_2X')$	0.5	1.1578	0.0634	-	-	0.0533	-
	20	1.4890	0.0731	-	-	0.1673	-
B							
$Y = k_1\exp(-k_2X') +$	0.5	1.0525	0.1552	0.5038	0.3206	0.0111	0.001
$k_3(1-\exp(-k_4X'))$	20	1.2940	0.2000	0.7248	0.4274	0.0094	0.0001

Parameters and minimal residual sums of squares obtained by fitting the data points of fig.1B to the models are indicated. All data points shown in the figure were used to calculate k_1, k_2 and the sums of squares of model A for each dose of phenylephrine. In the case of model B, background parameters (k_1, k_2) were first calculated by using only the decay part of the model and the data points before phenylephrine infusion; k_1 and k_2 were kept constant for the subsequent calculation of k_3 and k_4 from the remaining data points. For model B, the sums of squares shown were calculated by adding the sum obtained from the two separate parts of the fitting procedure. Y, Mg^{2+} concentration (10^{-5} M); X, time of perfusion (min). Note that $X' = X - 40$. P values indicate the significance of the improvement of the sums of squares by using model B instead of model A. Model comparison by F test [9]

tinuously infused into the medium entering the liver to give a final concentration of 1.2 mM. The perfusate was saturated with O_2/CO_2 (95%/5%) in a disc oxygenator and pumped into the liver via portal vein at a constant rate of 20 ml/min. Phenylephrine infusion was started at the times indicated in the figures. Aliquots of the perfusate leaving the liver were collected at 30-s intervals and analyzed for glucose by optical test (glucose oxidase, peroxidase; Boehringer) and for Mg^{2+} by atomic absorption spectroscopy.

Curves were fitted to the models shown in table 1 with a weighted iterative least-squares procedure based on the method of steepest descent with an anti-oscillator control. Reciprocals of standard deviations were used as weights. The estimated variance of fitted curves was obtained by dividing the minimal residual sum of squares by the difference of the numbers of experimental points minus the number of estimated parameters. The square root of the variance was used as standard error of the estimate.

3. RESULTS

In order to be able to measure the possible release of small amounts of Mg^{2+} from liver, infusion of Mg^{2+} was stopped after 35 min of preperfusion, i.e. 5 min before starting the addition of phenylephrine. This α_1-adrenergic agonist was

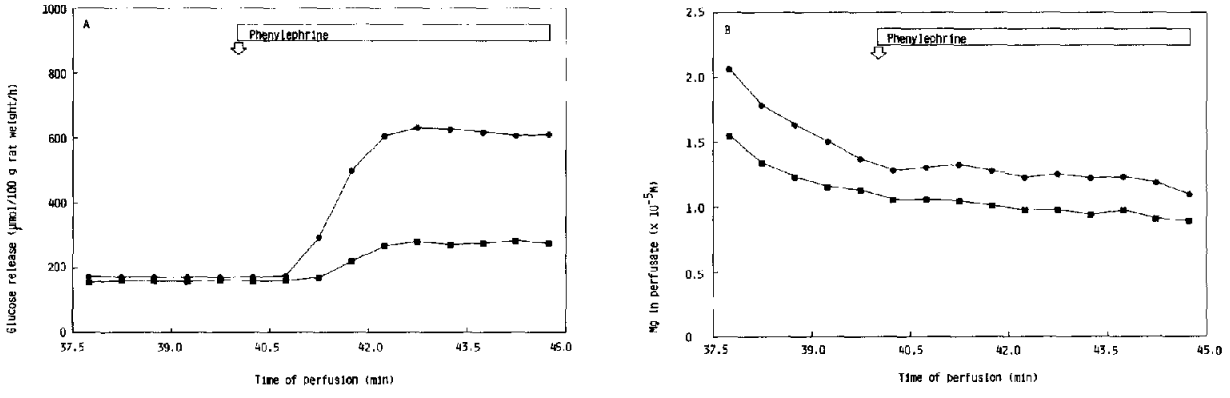

Fig.1. Effects of two concentrations of phenylephrine on rat livers perfused without metabolic substrates. Infusion of exogenous Mg^{2+} into the medium entering the liver was stopped at 35 min of perfusion. Phenylephrine was infused from 40 min to the end of the experiments to give final concentrations of 0.5 μM (■) and 20 μM (●). (A) Glucose production. In 3 of the 4 experiments with livers perfused with 20 μM phenylephrine, glucose production was measured and mean values are shown. At 0.5 μM phenylephrine, glucose was measured only in one of the 6 experiments of this group. (B) Mg^{2+} concentrations in the effluent. Mean values of 6 and 4 experiments with 0.5 and 20 μM phenylephrine, respectively, are shown. SE values were below 1.5 μM within each group.

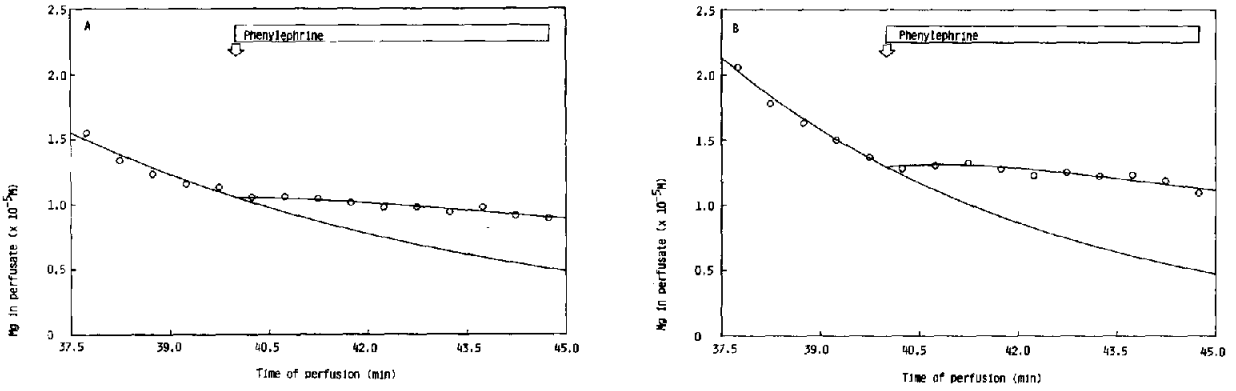

Fig.2. Regression lines obtained by fitting the values from fig.1B to a two-component exponential model. A monoexponential decrease (background Mg^{2+} release; control without phenylephrine) was fitted to the time points before 40 min of perfusion and was kept constant during calculation of the regression in the presence of phenylephrine (model B, table 1). Final concentrations of phenylephrine were 0.5 μM (A) and 20 μM (B).

previously shown to enhance glucose and lactate production from endogenous glycogen if 1.3 mM or 10 μM Ca^{2+} and 1.2 mM Mg^{2+} were present in the medium [3,5]. Glucose production, reflecting glycogen breakdown in response to 0.5 and 20 μM phenylephrine, is shown in fig.1A, demonstrating that metabolic responses were not impaired by switching to low Mg^{2+} perfusion. Mg^{2+} concentrations in the effluent are shown in fig.1B. A monoexponential decay of Mg^{2+} concentration was initiated by stopping the infusion of Mg^{2+} at 35 min of perfusion. 20 μM phenylephrine appeared to slow the decay but no unequivocal short-term release of Mg^{2+}, comparable to the release of Ca^{2+} [3], was observed. Therefore the curves of Mg^{2+} efflux were analyzed in more detail. If no phenylephrine was infused, Mg^{2+} decay was adequately described by a monoexponential curve (not shown). Since a satisfactory fit of each of the two curves shown in fig.1B to a monoexponential decay model would indicate that phenylephrine had no effect on Mg^{2+} release, this possibility (model A in table 1) was compared with a model composed of a monoexponential decay as background and a monoexponential increase starting with the infusion of the agonist (model B, table 1). As shown in table 1, the sums of squares were significantly lower with model B than model A, indicating that phenylephrine had a dose-dependent effect on Mg^{2+} release. The curves defined by the parameters of model B are shown in fig.2. Phenylephrine-induced Mg^{2+} release (fig.3) was obtained from parameters k_3 and k_4 while k_1 and k_2 (background parameters) were set to zero. By numerical integration of curves in fig.3, (interval 0–5 min) total amounts of Mg^{2+} released of 1.3 (range 0.9–1.6) μmol and 2.1 (range 1.8–2.5) μmol were obtained at 0.5 and 20 μM phenylephrine, respectively.

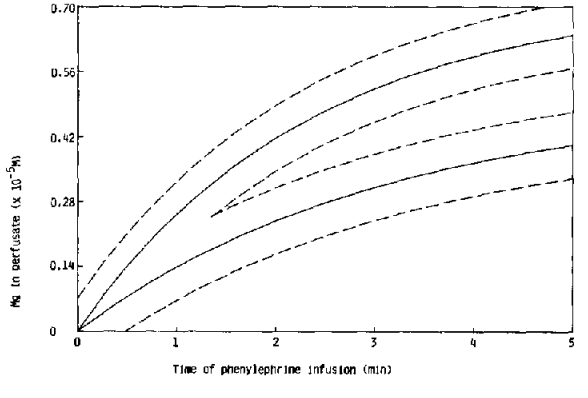

Fig.3. Phenylephrine-induced Mg^{2+} release (——; upper curve 20 μM; lower curve, 0.5 μM phenylephrine) is the difference between the curves obtained in the presence of phenylephrine and the monoexponential decay in the absence of the agonist. Standard errors of estimates of each pair of curves were added and the highest value was used for drawing the error lines shown (– – –).

4. DISCUSSION

α_1-Adrenergic agonists mobilize cellular Ca^{2+} from rat livers perfused with low Ca^{2+} concentrations. A transient release of Ca^{2+} into the perfusate

has been measured by a Ca^{2+}-selective electrode during the first 5 min of phenylephrine infusion into the medium entering the livers [3,6]. The present results indicate that Mg^{2+} is also mobilized and released from perfused livers in response to phenylephrine. Mg^{2+} release is dose-dependent and occurs more slowly than the release of Ca^{2+}, reaching its maximum only after 5 min of agonist infusion.

Subcellular mobilization of hepatic Ca^{2+} and Mg^{2+} has been observed in rats in vivo after intravenous injection of vasopressin and glucagon. In this case, a decrease in Ca^{2+} content of the endoplasmic reticulum and an increase in mitochondrial Mg^{2+} content have been measured. Mitochondrial matrix free Mg^{2+} concentrations have been calculated to increase from 590 to 800 μM, suggesting an activating effect of Mg^{2+} on pyruvate dehydrogenase [2].

Generally, the mobilization of extramitochondrial Mg^{2+} may be followed by mitochondrial Mg^{2+} uptake and release of Mg^{2+} from hepatocytes, resulting in a decrease in total cellular Mg^{2+} content. In the rat, total hepatic Mg^{2+} contents are 23–43 nmol/mg dry wt [1,2]. By assuming a factor of approx. 0.3 for wet wt to dry wt conversion for perfused livers, a maximum Mg^{2+} loss of 0.7 nmol/mg dry wt, corresponding to 2–3% of total hepatic Mg^{2+}, can be calculated from the present results. This is only a minor fraction of the total Mg^{2+} but cytosolic concentrations of free Mg^{2+} appear to be sensitive to small changes in total Mg^{2+} because of the presence of numerous low-affinity binding sites in liver cells [1]. Whether this amplification mechanism is efficient enough to influence metabolic regulation in our experiments remains questionable. In addition, net release of Mg^{2+} has been measurable only at low extracellular Mg^{2+} concentrations. At present it remains unknown as to whether Mg^{2+} release in response to hormones also occurs at physiological extracellular Mg^{2+} and Ca^{2+} levels and in vivo.

Alternatively, Mg^{2+} mobilization from the plasma membrane may induce changes of the phospholipid order of the membrane and thereby contribute to transmembrane signal transduction in the presence of hormones. Mg^{2+}-induced changes in lipid order and conformation of renal (Na^+,K^+)-ATPase have been described [7]. α_1-Adrenergic stimulation of ouabain-sensitive K^+ uptake by perfused rat livers has been reported earlier [8], leaving open the possibility that release of membrane-associated Mg^{2+} affects the regulation of (Na^+,K^+)-ATPase.

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