Norepinephrine evokes a marked Mg^{2+} efflux from liver cells

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The addition of norepinephrine to perfused rat livers and to collagenase isolated hepatocytes induced a marked and dose-dependent magnesium efflux. The addition of β-adrenergic receptor antagonists, but not α-antagonists, completely blocked the Mg^{2+} efflux. The Mg^{2+} efflux could also be induced by forskolin and by permeable cAMP analogues. By contrast, the addition of carbachol or vasopressin induced a Mg^{2+} influx into isolated hepatocytes. These results indicate that a significant Mg^{2+} efflux from liver cells can be induced through the β-adrenergic receptors and that it is mediated through the cytosolic cAMP levels.

Mg^{2+}; Hepatocyte; β-Adrenergic receptor, cAMP; Rat liver

1. INTRODUCTION

Magnesium is one of the more abundant cations inside cells [1,2] where it is involved as a cofactor in a variety of intracellular enzymatic processes [1,3–8] and as a stabilizer of membrane integrity [9]. Heretofore, a large body of information on Mg^{2+} transport has been obtained from bacteria [9–11] and giant cells [9,12]. Yet little or inconsistent data are available in the literature about Mg^{2+} intracellular compartmentation [4,7,13] or Mg^{2+} mobilization in response to external stimuli [14–16] in mammalian cells. Changes in Mg^{2+} concentration have been observed in mitochondria in normal [7] or in stimulated conditions [8] and very recently attention has been directed to a possible Mg^{2+} movement in perfused livers [17] or hearts [18].

In the present study we report the presence of an Mg^{2+} flux across the plasma membrane of liver cells, both in perfused rat liver as well as in isolated hepatocytes. We also demonstrate that this movement is regulated via modulation of the cytosolic cAMP level.

2. MATERIALS AND METHODS

Male Sprague-Dawley rats (200–250 g) fed on stock diet were used as liver donors after anaesthetic treatment with intraperitoneal injection of phentobarbital. Livers were removed and perfused with a medium containing (mM): NaCl 120, KCl 3, Hepes 10, KH_2PO_4 1.2, glucose 10, $NaHCO_3$ 12, $CaCl_2$ 1.25, $MgCl_2$ 1.2 (pH 7.2 with O_2/CO_2 95:5 by vol., 37°C). After 10 min the perfusion medium was switched to a similar perfusion medium containing 0 mM Mg^{2+}. The flow was 20 ml/min without recirculation. Different doses of NE, agonists and antagonists were directly added in the perfusing medium as indicated. The effluent perfusate was collected every 15 s and the Mg^{2+} content of the perfusate measured by atomic absorbance spectrophotometry (Varian AA-575).

Hepatocytes isolated by collagenase digestion were prepared according to Seglen [19] and utilized up to 3–4 h after isolation. During this time the viability (85 ± 3%, $n = 9$) did not change significantly. The hepatocytes were washed four times and incubated at a concentration of 100–150 μg protein/ml in the same medium in the absence of Mg^{2+} at 37°C. After the addition of the cells the Mg^{2+} present as contaminant in the incubation buffer, measured by atomic absorbance spectrophotometry, ranged between 10 and 20 μM. After 5 min of equilibration, in absence or presence of antagonists, adrenergic agonists were added. At the time indicated in the figures samples were withdrawn and centrifuged in microfuge tubes. The Mg^{2+} content of the supernatant was measured by atomic absorbance spectrophotometry.

The protein was determined according to Bradford [20].

2.1. *Chemicals*

Collagenase type CLS-1 was from Worthington Biochemical Corp. (Freehold, NJ, USA). All other chemical reagents and drugs were from Sigma (St. Louis, MO, USA), except Sotalol that was obtained from Bristol Myers Co. (Evansville, IN, USA).

3. RESULTS

Fig. 1 shows the result of an experiment where Mg^{2+} content of the perfusate was measured in the absence (Fig. 1A) and in the presence (Fig. 1B) of norepinephrine (NE) stimulation. In order to increase the sensitivity of the Mg^{2+} measurements and to magnify Mg^{2+} efflux, 5 min before the measurements were carried out, the perfusion medium was changed to one containing 0 Mg^{2+}. The collected perfusate was assayed as described in section 2. In the control liver (Fig. 1A) the Mg^{2+} efflux as measured as Mg^{2+} content of the perfusate was about 2 μM and continued

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Abbreviations: Hepes, 4-(2-hydroxymethyl)-1- piperazine ethane sulphonic acid; 8-Cl-cAMP, 8-(4-chlorophenylthio)-adenosine 3′:5′-cyclic monophosphate; 8-Br-cAMP, 8-bromoadenosine 3′:5′-cyclic monophosphate; dBucAMP, N^6,2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate

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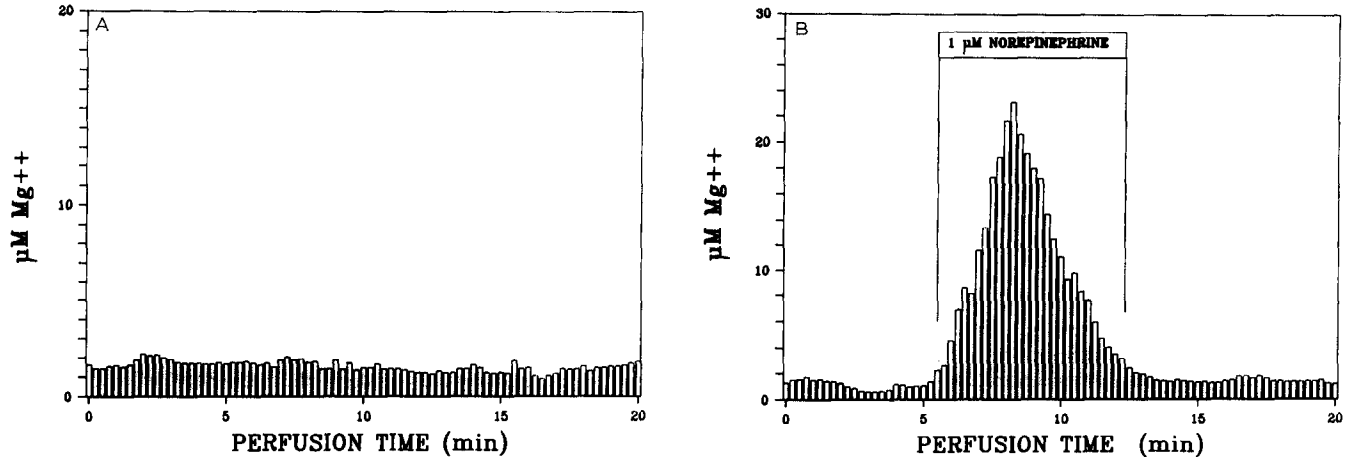

Fig. 1. Mg^{2+} efflux in control perfused rat liver (A) and after addition of norepinephrine (B). One experiment typical of three both for the control and norepinephrine (NE) stimulated livers is shown.

throughout the perfusion period. By contrast, in the stimulated liver (Fig. 1B) the addition of 1 μM norepinephrine (NE) evoked a large and reproducible Mg^{2+} efflux. Under the same experimental conditions, no detectable release of LDH was observed (not shown), indicating that the Mg^{2+} efflux was not secondary to plasma membrane damage and increase in permeability.

To avoid possible interference from cell heterogeneity, perfusion rate and other metabolic conditions, the following experiments were carried out using collagenase dispersed hepatocytes. As shown in Fig. 2, the addition of NE at concentrations of 1 nM or 1 μM to a suspension of hepatocytes evoked an efflux of Mg^{2+} in the extracellular medium corresponding to a release of 6 and 10 nmol Mg^{2+}/million cells, within 6 min, respectively. Qualitatively similar results were induced by the addition of the β-adrenergic agonist isoproterenol (not shown).

Because the targets of adrenergic stimulation are the α and β receptor located in the plasma membrane of liver cells [21,22] we used both α and β blockers [23–25] to identify the type of receptor involved in the regulation of Mg^{2+} efflux after adrenergic stimuli. The addition of 2 μM propranolol (a non-selective β-antagonist) blocked the Mg^{2+} efflux induced by NE (Fig. 3). The inhibition lasted for at least 12 min after the addition of NE (not shown). In contrast, the addition of α-blocker as regitine was far less effective (Fig. 3). Qualitatively similar responses were also obtained by using the β-blocker, sotalol, or the α-blockers, prazosin and yohimbine (not shown).

Table I shows that the above Mg^{2+} efflux is dependent on an increase in cytosolic cAMP level. The addition of 1 μM NE stimulates an Mg^{2+} efflux in the extracellular medium corresponding to approximately 10 nmol Mg^{2+}/million cells in 5 min. The addition of forskolin at a concentration directly activating adenylate cyclase [26], induced an Mg^{2+} efflux at an extent similar to that obtained with NE. Virtually iden-

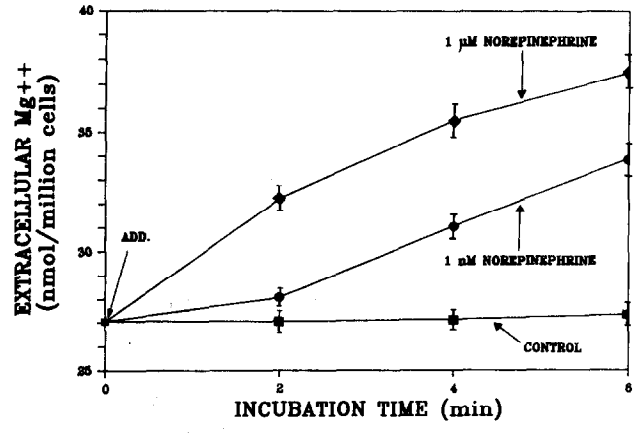

Fig. 2. Mg^{2+} efflux induced by different doses of norepinephrine on isolated hepatocytes. Where indicated 1 nM or 1 μM norepinephrine was added to a suspension of isolated hepatocytes. The data are means ± SE of 4 different preparations.

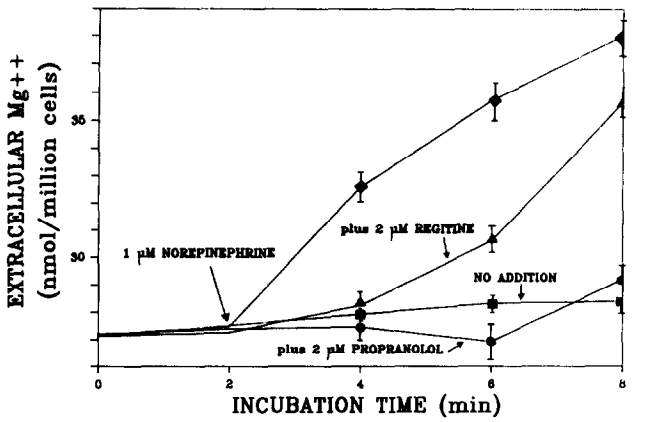

Fig. 3. Inhibition by α- and β-adrenergic receptor blocking agents on the Mg^{2+} efflux induced by norepinephrine in isolated hepatocytes. Where indicated, 1 μM norepinephrine (NE) was added in the absence or presence of 2 μM regitine or 2 μM propranolol. The data are the means ± SE of 4 different preparations.

Table I

Effect of norepinephrine, forskolin and permeant cAMP analogues on Mg^{2+} efflux in isolated hepatocytes

Agent	Extracellular Mg^{2+} content (nmol/million cells)		
	Incubation time (min after addition)		
	1	3	5
Control	27.15 ± 0.53	27.40 ± 0.55	27.20 ± 0.65
1 μM NE	32.78 ± 0.70	35.27 ± 0.67	37.50 ± 0.55
50 μm forskolin	31.25 ± 0.75	33.75 ± 1.10	38.05 ± 1.80
100 μM dBucAMP	30.78 ± 0.23	35.08 ± 1.40	38.73 ± 0.53
100 μM 8-Cl-cAMP	33.53 ± 0.80	36.93 ± 0.65	39.10 ± 0.85
250 μM 8-Br-cAMP	32.98 ± 0.48	36.70 ± 0.75	39.40 ± 0.35

Data are means ± SE of 10 experiments for control and norepinephrine (NE) stimulated cells and of 3 experiments for forskolin and cyclic-AMP analogues stimulated cells, respectively

tical Mg^{2+} movements were also observed after the addition of three different permeable cAMP analogues. By contrast Fig. 4 shows that the additions of 100 μM carbachol or 10 nM vasopressin, substances able to inhibit the adenylate cyclase activity via activation of G_i [27,28], stimulated a sizable cellular uptake of Mg^{2+}.

4. DISCUSSION

The present data, obtained from perfused rat liver and isolated hepatocytes, indicate the presence of a new mechanism involved in Mg^{2+} transport across the plasma membrane of liver cells. Recently, Jakob et al. [17] have shown an Mg^{2+} release in perfused liver induced by phenylephrine, via stimulation of the α_1-adrenergic receptor. Our data show that the Mg^{2+} efflux from perfused liver and liver cells is occurring through the adrenergic stimulation of β-receptors and that the net Mg^{2+} efflux is clearly related to the cAMP levels of the liver and hepatocytes. The decreased Mg^{2+} efflux we observed in presence of regitine could be accounted for by either a mobilization of Mg^{2+} after stimulation of α receptors [17] or, more likely, to an absence of complete specificity of regitine towards the α-receptors.

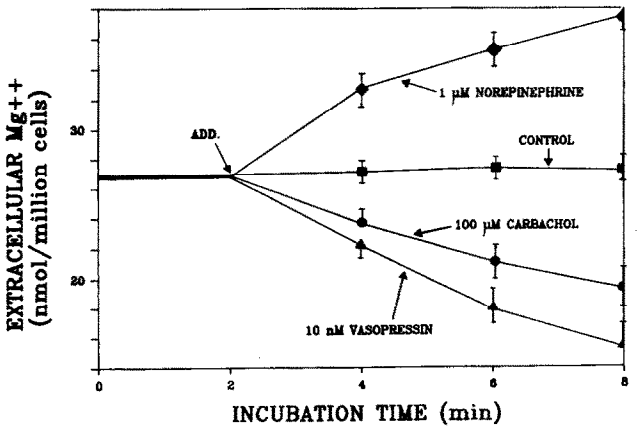

Fig. 4. Fluxes of Mg^{2+} across isolated hepatocytes: stimulation of efflux by norepinephrine and of uptake by carbachol and vasopressin. Where indicated carrier (as control), 1 μM norepinephrine (NE), 100 μM carbachol or 10 nM vasopressin were added. The data are the means ± SE of 3 different preparations.

The increase in cellular cAMP concentration via β-adrenergic stimulation as well as the direct additions of permeable cAMP analogues or forskolin induce a consistent Mg^{2+} efflux through the plasma membrane of liver cells. Since our experiments were carried out at very low (μM) concentrations of external Mg^{2+}, the presence of a concentration gradient between the inside and the outside of the cells may have resulted in an amplification of Mg^{2+} efflux. It is noteworthy that under those conditions, both vasopressin and carbachol were able to stimulate Mg^{2+} uptake against a concentration gradient. The action of vasopressin and carbachol in liver cells is complex but a major effect is the decrease of cytosolic AMP levels [27,28]. Bond et al. [8] observed that the addition of vasopressin to rat liver in vivo resulted in an increase in the mitochondrial Mg^{2+} content. Therefore, it is possible that in our experimental conditions a major intracellular redistribution of Mg^{2+} is occurring in the cells. More specifically this could be consistent with an increase of Mg^{2+} transport across the mitochondrial inner membrane which should have a major effect on the mitochondrial choice of substrate utilization.

In conclusion, our data show that there is an apparent correlation between cAMP levels in liver cells and net Mg^{2+} efflux. The observed Mg^{2+} efflux could be the result of regulation by cyclic AMP of one or several Mg^{2+} transport pathways in liver cells. A working hypothesis, consistent with but not proven by the present data, is that there are at least two pathways operating Mg^{2+} fluxes in hepatocytes: one for Mg^{2+} efflux and one for Mg^{2+} influx, the latter being inhibited by cAMP.

Many other experiments need to be carried out to define in more detail the mechanism(s) involved in Mg^{2+} transport and to examine a possible redistribution of intracellular Mg^{2+} occurring after adrenergic and/or hormonal stimulations.

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REFERENCES

[1] Grubbs, R.D. and Maguire, M.E. (1987) Magnesium 6, 113–117.
[2] Walker, G.M. (1986) Magnesium 5, 9–23.
[3] Askari, A., Huang, W.H. and McCormick, P.W. (1983) J. Biol. Chem. 258, 3453–3460.

[4] Gunther, T. (1986) Magnesium 5, 53–59.
[5] Maguire, M.E. (1984) Trends Pharmacol. Sci. 5, 73–77.
[6] Garlid, K.D. (1980) J. Biol. Chem. 255, 11273–11279.
[7] Corkey, B.E., Duszynski, J., Rich, T.L., Matschinsky, B. and Williamson, J.R. (1986) J. Biol. Chem. 261, 2567–2574.
[8] Bond, M., Vadasz, G., Somlyo, A.V. and Somlyo, A.P. (1987) J. Biol. Chem. 262, 15630–15636.
[9] Flatman, P.W. (1984) J. Membr. Biol. 80, 1–14.
[10] Lusk, J.E., Williamson, J.P.R. and Kennedy, E.P. (1968) J. Biol. Chem. 243, 2618–2624.
[11] Jasper, P. and Silver, S.J. (1978) J. Bacteriol. 133, 1323–1328.
[12] Scarpa, A. and Brinley, F.J. (1981) Fed. Proc. 40, 2646–2652.
[13] Grubbs, R.D., Collins, S.D. and Maguire, M.E. (1984) J. Biol. Chem. 259, 12181–12192.
[14] Maguire, M.E. (1982) Mol. Pharmacol. 22, 274–280.
[15] Erdos, J.J. and Maguire, M.E. (1983) J. Physiol. 337, 351–371.
[16] Maguire, M.E. (1984) Trend Pharmacol. Sci. 5, 73–77.
[17] Jakob, A., Becker, J., Schotti, G. and Fritzsch, G. (1989) FEBS Lett. 246, 127–130.
[18] Romani, A. and Scarpa, A. (1990) FASEB J. 4, A294, abstr. 166.
[19] Seglen, P.O. (1976) Methods Cell Biol. 13, 29–83.
[20] Bradford, M.M. (1976) Biochemistry 72, 248–254.
[21] Exton, J.H. (1982) Trends Pharmacol. Sci. 3, 111–115.
[22] Osnes, J.-B., Refsum, H., Skomedal, T. and Oye, I. (1978) Acta Pharmacol. Toxicol. 42, 235–247.
[23] Molinoff, P.B. (1984) Drug 28 (Suppl. 2), 1–15.
[24] Bylund, D.B. (1988) Trends Pharmacol. Sci. 9, 356–361.
[25] Weiner, N. (1985) in: Goodman and Gilman's The Pharmacological Basis of Therapeutics (Goodman Gilman, A., Goodman, S.L., Rall, T.W. and Murad, F. eds) pp. 181–214, Macmillan, New York.
[26] Zheng, J.S., De Young, M.B., Wiener, E., Levy, M.N. and Scarpa, A. (1990) Ann. NY Acad. Sci. (in press).
[27] Jakobs, K.H., Aktories, K. and Schultz, G. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 310, 113–119.
[28] Birnbaumer, L., Yatani, A., Codina, J., VanDongen, A., Graf, R., Mattera, R., Sanford, J. and Brown, A.M. (1989) in: Molecular Mechanism of Hormone Action, pp. 147–177, Springer-Verlag, Berlin.