

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

A. Romani and A. Scarpa

*Department of Physiology and Biophysics, Case Western Reserve University, School of Medicine, Cleveland, OH 44106, USA*

Received 18 May 1990, revised version received 15 June 1990

The addition of norepinephrine to perfused rat livers and to collagenase isolated hepatocytes induced a marked and dose-dependent magnesium efflux. The addition of  $\beta$ -adrenergic receptor antagonists, but not  $\alpha$ -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  $\beta$ -adrenergic receptors and that it is mediated through the cytosolic cAMP levels.

$Mg^{2+}$ ; Hepatocyte;  $\beta$ -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 ( $85 \pm 3\%$ ,  $n = 9$ ) did not change significantly. The hepatocytes were washed four times and incubated at a concentration of 100–150  $\mu$ g 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  $\mu$ M. 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  $\mu$ M and continued

*Correspondence address:* A. Romani, Department of Physiology and Biophysics, Case Western Reserve University, School of Medicine, Cleveland, OH 44106, USA

*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-dibutyladenosine 3':5'-cyclic monophosphate

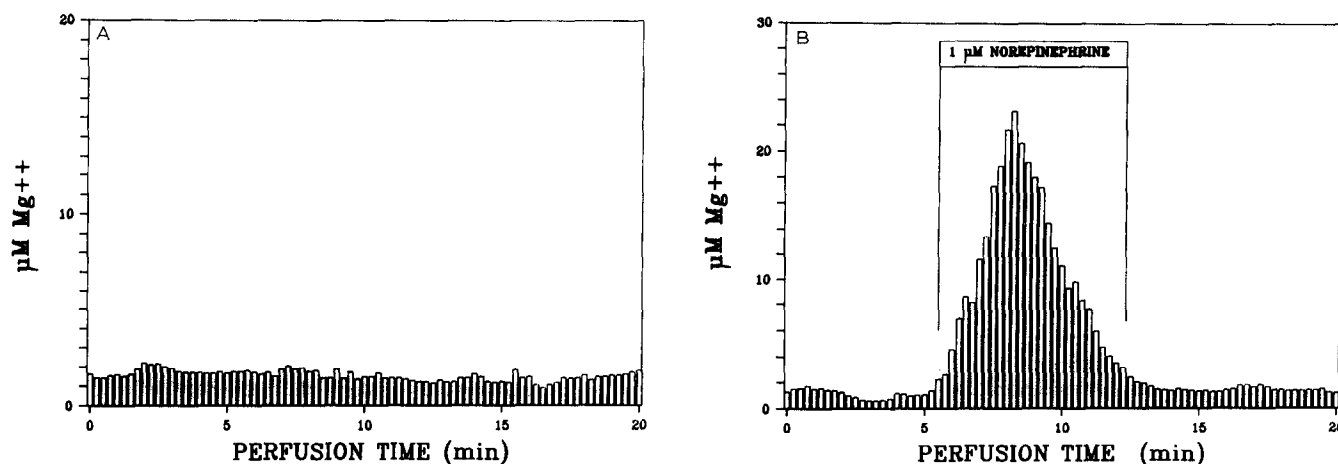


Fig. 1.  $\text{Mg}^{2+}$  efflux in control perfused rat liver (A) and after addition of norepinephrine (B). One experiment typical of three both for the control and norepinephrine (NE) stimulated livers is shown.

throughout the perfusion period. By contrast, in the stimulated liver (Fig. 1B) the addition of 1  $\mu\text{M}$  norepinephrine (NE) evoked a large and reproducible  $\text{Mg}^{2+}$  efflux. Under the same experimental conditions, no detectable release of LDH was observed (not shown), indicating that the  $\text{Mg}^{2+}$  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  $\mu\text{M}$  to a suspension of hepatocytes evoked an efflux of  $\text{Mg}^{2+}$  in the extracellular medium corresponding to a release of 6 and 10 nmol  $\text{Mg}^{2+}$ /million cells, within 6 min, respectively. Qualitatively similar results were induced by the addition of the  $\beta$ -adrenergic agonist isoproterenol (not shown).

Because the targets of adrenergic stimulation are the

$\alpha$  and  $\beta$  receptor located in the plasma membrane of liver cells [21,22] we used both  $\alpha$  and  $\beta$  blockers [23–25] to identify the type of receptor involved in the regulation of  $\text{Mg}^{2+}$  efflux after adrenergic stimuli. The addition of 2  $\mu\text{M}$  propranolol (a non-selective  $\beta$ -antagonist) blocked the  $\text{Mg}^{2+}$  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  $\alpha$ -blocker as regitine was far less effective (Fig. 3). Qualitatively similar responses were also obtained by using the  $\beta$ -blocker, sotalol, or the  $\alpha$ -blockers, prazosin and yohimbine (not shown).

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

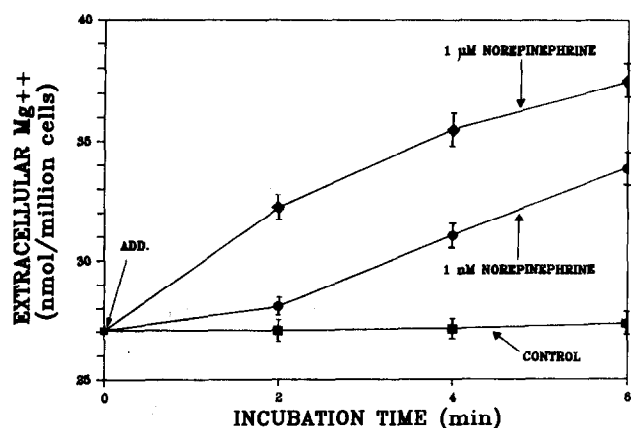


Fig. 2.  $\text{Mg}^{2+}$  efflux induced by different doses of norepinephrine on isolated hepatocytes. Where indicated 1 nM or 1  $\mu\text{M}$  norepinephrine was added to a suspension of isolated hepatocytes. The data are means  $\pm$  SE of 4 different preparations.

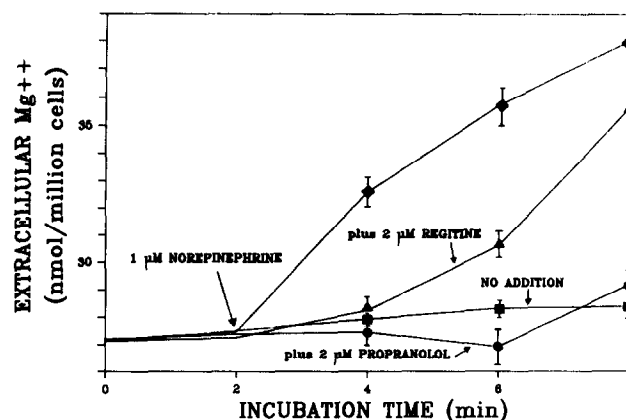


Fig. 3. Inhibition by  $\alpha$ - and  $\beta$ -adrenergic receptor blocking agents on the  $\text{Mg}^{2+}$  efflux induced by norepinephrine in isolated hepatocytes. Where indicated, 1  $\mu\text{M}$  norepinephrine (NE) was added in the absence or presence of 2  $\mu\text{M}$  regitine or 2  $\mu\text{M}$  propranolol. The data are the means  $\pm$  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 $\pm$ 0.53	27.40 $\pm$ 0.55	27.20 $\pm$ 0.65
1 $\mu$ M NE	32.78 $\pm$ 0.70	35.27 $\pm$ 0.67	37.50 $\pm$ 0.55
50 $\mu$ M forskolin	31.25 $\pm$ 0.75	33.75 $\pm$ 1.10	38.05 $\pm$ 1.80
100 $\mu$ M dBucAMP	30.78 $\pm$ 0.23	35.08 $\pm$ 1.40	38.73 $\pm$ 0.53
100 $\mu$ M 8-Cl-cAMP	33.53 $\pm$ 0.80	36.93 $\pm$ 0.65	39.10 $\pm$ 0.85
250 $\mu$ M 8-Br-cAMP	32.98 $\pm$ 0.48	36.70 $\pm$ 0.75	39.40 $\pm$ 0.35

Data are means  $\pm$  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  $\mu$ M 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  $\alpha_1$ -adrenergic receptor. Our data show that the  $Mg^{2+}$  efflux from perfused liver and liver cells is occurring

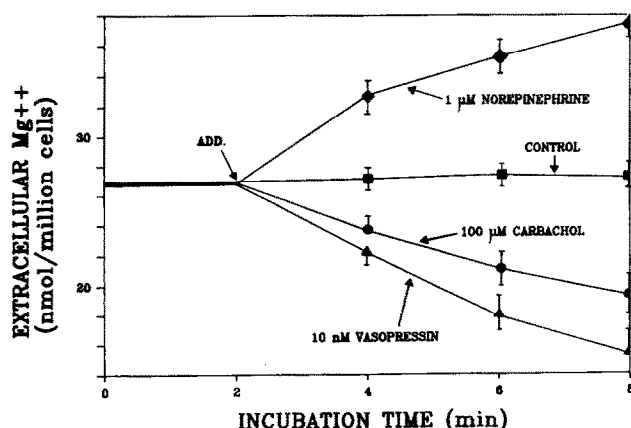


Fig. 4. Fluxes of  $Mg^{2+}$  across isolated hepatocytes: stimulation of efflux by norepinephrine and of uptake by carbachol and vasopressin. Where indicated carrier (as control), 1  $\mu$ M norepinephrine (NE), 100  $\mu$ M carbachol or 10 nM vasopressin were added. The data are the means  $\pm$  SE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

through the adrenergic stimulation of  $\beta$ -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  $\alpha$  receptors [17] or, more likely, to an absence of complete specificity of regitine towards the  $\alpha$ -receptors.

The increase in cellular cAMP concentration via  $\beta$ -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 ( $\mu$ M) 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.

**Acknowledgements:** This work was supported by NIH Grant 18708. We thank Ms M. Fatholahi for skillful help in the preparation of hepatocytes.

#### 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 Fritzsche, 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.