Regulation of Mg^{2+} homeostasis by insulin in perfused rat livers and isolated hepatocytes

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Abstract Several recent studies demonstrate that adrenergic receptor stimulation evokes marked changes in Mg^{2+} homeostasis. As insulin counter-regulates many of the metabolic consequences of adrenergic receptor stimulation, we evaluated the potential influence of insulin on Mg^{2+} movements in response to adrenergic stimulation. The data demonstrate that insulin is able to block the Mg^{2+} efflux from perfused rat livers stimulated by isoproterenol or 8-Br-cAMP, but has little or no effect on epinephrine or phenylephrine induced Mg^{2+} efflux. Thus, evidence is provided demonstrating that there are redundant adrenergic pathways regulating Mg^{2+} efflux from liver tissue. One of these pathways, the β-adrenergic component, is selectively blocked by insulin. Furthermore, these findings may provide a cellular explanation for hypomagnesemia associated with diabetes.

Key words: Mg^{2+}; β-Adrenergic receptor; Insulin; Liver; cAMP

1. Introduction

To date, adrenergic receptor agonists have been established as the preeminent endogenous mediator of Mg^{2+} homeostasis. In rat liver, a paradigm has emerged whereby stimulation of the β-adrenergic (β-AR) receptor results in the mobilization of an intracellular Mg^{2+} pool and simultaneous extrusion of Mg^{2+} across the plasma membrane into the extracellular space [1–4]. In vivo, a measurable increase in circulating Mg^{2+} level occurs following β-AR stimulation [5]. Thus, β-AR stimulation has profound effects on Mg^{2+} homeostasis at the cellular and extracellular level. In addition, there is some evidence indicating that α-adrenergic (α-AR) stimulation may also regulate extrusion of Mg^{2+} across the plasma membrane in the liver [6].

Insulin is known to antagonize many of the metabolic responses that occur secondary to either α-AR or β-AR stimulation in the liver [7–10]. Most notably, insulin inhibits both α-AR (Ca^{2+} mediated) and β-AR (cAMP mediated) induced glycogenolysis and glucose release. In addition, insulin has been shown to alter Mg^{2+} homeostasis in several tissues [11–13] where increases in intracellular Mg^{2+} following insulin treatment have been observed [14,15]. The potential role of insulin as an endogenous mediator of Mg^{2+} homeostasis is supported by evidence revealing that conditions of decreased insulin release are associated with alterations of blood and tissue Mg^{2+} level [14,16].

The current study was undertaken to elucidate the cellular mechanism(s) responsible for the insulin effect on cellular Mg^{2+} transport and to assess the possible inter-relationship between insulin, adrenergic stimulation and Mg^{2+} homeostasis in the liver.

2. Materials and methods

2.1. Mg^{2+} efflux from perfused rat livers

Rat livers from fed, male Sprague-Dawley rats (250–350 g body wt) were perfused with a hemoglobin-free buffer according to the method of Sugano et al. [17]. After cannulation of the portal vein, livers were perfused with a buffer containing (mM): Na^+, 144; K^+, 4.7; Cl^-, 110; HCO_3^-, 24; Ca^{2+}, 1.2; PO_4^{2-}, 1.2; HEPES, 10; glucose, 10; at a pH of 7.2 with no added Mg^{2+}. The Mg^{2+} present as contaminant in the buffer was 15 μM. The buffer was equilibrated with a O_2/CO_2 95:5 gas mixture and maintained at 37°C. The livers were removed from the abdomen, inverted and placed on a platform. After 10 min of washout (−20 → −10 min), the effluent was sampled at 1-min intervals. The first 10 min of sampling (−10 → 0 min) provided a baseline and was used to initiate pre-treatment with insulin (INS, 10 mU/ml). Epinephrine (EPI, 1 μM), phenylephrine (PHE, 1 μM), isoproterenol (ISO, 10 μM), 8-bromo-cAMP (8BrcAMP, 125 μM) or vehicle (CON) were added to the perfusate during the second 10 min of sampling (0 → 10 min). The last 10 min of sampling (10 → 20 min) allowed for recovery. Effluent (~1 ml) was collected in borosilicate glass culture tubes and the Mg^{2+} content was measured by atomic absorbance spectrophotometry (AAS) in a Perkin-Elmer 3100.

2.2. Mg^{2+} efflux from collagenase dispersed rat hepatocytes

Collagenase dispersed rat hepatocytes were isolated according to the method of Seglen [18] from fed, male Sprague-Dawley rats (225–300 g body wt). The hepatocytes were sedimented through a Percoll gradient resulting in a viability of ≥95% as measured by the trypan blue exclusion test. Cells were then suspended in a buffer identical to that used to perfuse livers except for a Mg^{2+} concentration of 1.2 mM. The cells were continuously equilibrated with a O_2/CO_2 95:5 gas mixture and maintained at 25°C until used. To measure Mg^{2+} extrusion across the hepatocyte plasma membrane, aliquots of cells were resuspended in a buffer identical to that described above but with no added Mg^{2+}. The cells were then incubated in 10 ml of the same Mg^{2+}-free buffer pre-warmed to 37°C. The cells were maintained in suspension by magnetic stirring, under continuous flow of O_2/CO_2 95:5. After 3 min of equilibration (−5.0 → −2.0 min), insulin or vehicle were added to the suspension and sampling was performed thereafter at 2.0-min intervals. The first sampling period (−2.0 → 0 min) allowed for the measurement of a baseline. Insulin (10 mU/ml ~6 nM) was added at −2.0 min and isoproterenol (10 μM), or vehicle (buffer), was added at 0 min. Sample aliquots were sedimented in microfuge tubes at 8000×*g* for 30 s and the Mg^{2+} concentration in the supernatant was measured by AAS.

2.3. Statistical analysis

Data are displayed as mean ± S.E.M. The data were first analyzed using ANOVA then Tukey's test for multiple comparisons with statistical significance designated as a p value of ≤0.05.

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3. Results

Fig. 1 shows the change in Mg^{2+} content in the effluent of perfused control livers (CON) and perfused livers following treatment with epinephrine in either the absence (EPI) or presence (INS/EPI) of insulin. As previously shown [2,6], epinephrine (EPI) added to the perfusate at 0 min resulted in a rapid increase in effluent Mg^{2+} from 12.3 ± 1.2 to 25.1 ± 5.1 μM during the first 3 min of perfusion. No statistically significant differences in effluent Mg^{2+} level were observed between any of the control and treated groups during the first 10 min (−10 to 0 min) of sampling. A persistent elevation of Mg^{2+} for the next 3 min was observed, decreasing thereafter despite the persistence of EPI in the perfusate. 10 min of perfusion of insulin alone had negligible effect on liver Mg^{2+} redistribution in that there was little change in the effluent Mg^{2+} between −10 (14.7 ± 3.9 μM) and 0 (15.4 ± 5.3 μM) min. When insulin was present in the perfusate for the 10 min prior to and during epinephrine treatment (INS/EPI), the extrusion of Mg^{2+} in response to epinephrine was decreased. The initial increase in effluent Mg^{2+} was quantitatively similar to that observed in the non-insulin treated group. However, at the latter time points the Mg^{2+} content in the effluent in the INS/EPI group was less than that observed in the EPI group (although this difference did not achieve statistical significance). Since it has been previously demonstrated that both α-AR and β-AR stimulation result in Mg^{2+} extrusion from perfused rat livers [2,6], we hypothesized that partial inhibition of epinephrine induced Mg^{2+} efflux by insulin may be due to the preferential blockade of either the α or β mediated pathway. Fig. 2A demonstrates the variations in effluent Mg^{2+} from livers perfused with phenylephrine (a selective α-AR agonist) in either the absence (PHE) or presence (INS/PHE) of insulin. Addition of PHE to the perfusate resulted in an increase in effluent Mg^{2+} from 12.13 ± 0.9 μM at 0 min to 17.3 ± 5.4 μM at 1 min. Effluent Mg^{2+} remained at this elevated level for the following 4 min before returning to baseline levels despite the persistence of PHE in the perfusate. In the presence of insulin, the Mg^{2+} measured in the effluent in response to phenylephrine was slightly higher at the first observation points and then declined towards values not significantly different than those observed with PHE alone. In Fig. 2B, the livers were perfused with isoproterenol (a selective β-AR agonist) in either the absence (ISO) or presence (INS/ISO) of insulin. The mobilization of Mg^{2+} in the effluent induced by ISO was qualitatively different from that observed following EPI or PHE in that there was a more gradual increase over time. Furthermore, in contrast to the previous observations from livers perfused with either a mixed adrenergic receptor agonist (EPI) or an α-AR agonist (PHE) in the presence of insulin, the increase in effluent Mg^{2+} following β-AR (ISO) stimulation was completely inhibited by the insulin pre-treatment. To determine whether insulin was inhibiting ISO stimulated Mg^{2+} efflux from the liver through an effect on the β-AR second messenger system cyclic AMP, livers were perfused with the cell-permeant cyclic AMP analogue 8-bromo-cAMP, in either the absence (8Br-cAMP) or presence (INS/8Br-cAMP) of insulin. Fig. 3 demonstrates that, after a delay of 5 min following the addition of 8Br-cAMP to the perfusate, the Mg^{2+} content of the effluent increased. The presence of insulin prior to and during the

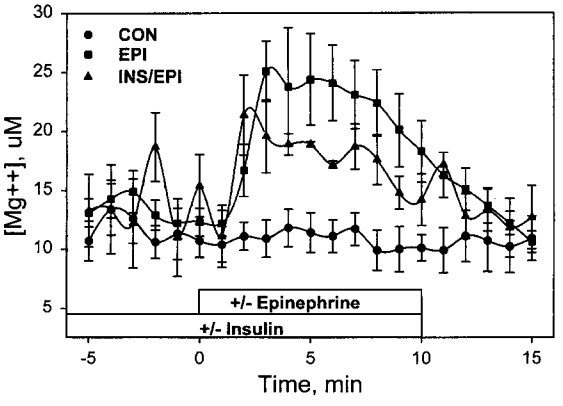

Fig. 1. Effect of insulin on epinephrine induced Mg^{2+} efflux from perfused rat liver. Concentration of Mg^{2+} in effluent vs. time. Rat livers were perfused as described in Section 2. During the designated time, epinephrine or vehicle were added to the perfusing buffer. For the insulin pre-treatment group, insulin was present in the perfusing buffer for the 10 min prior to and during agonist treatment. ANOVA and Tukey's test for multiple comparisons was performed at 0, 2, 4, 6, 8 and 10 min. Despite the partial inhibition, there were no statistically significant difference between EPI and ISO/EPI.

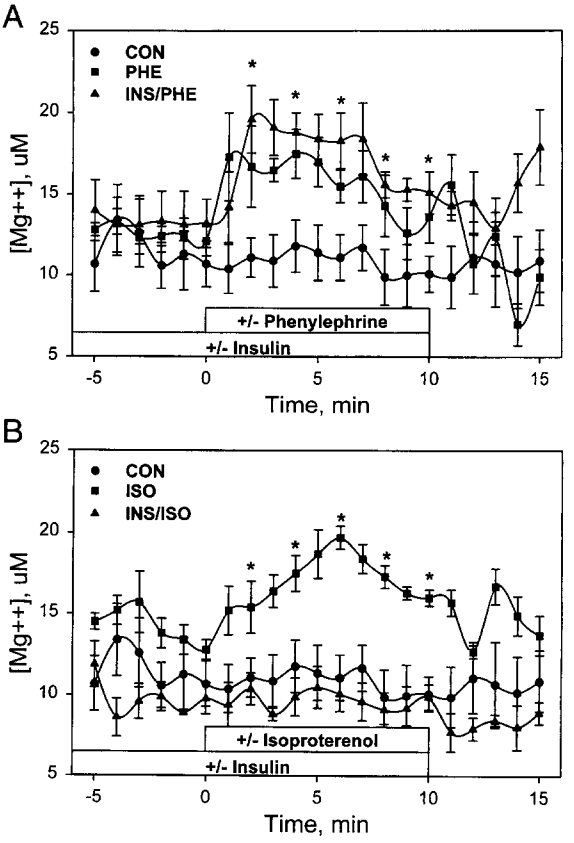

Fig. 2. Effect of insulin on (A) phenylephrine or (B) isoproterenol induced Mg^{2+} efflux from the perfused rat liver. Concentration of Mg^{2+} in effluent vs. time. Rat livers were perfused as described in Section 2. During the designated time, phenylephrine, isoproterenol or vehicle were added to the perfusing buffer. For the insulin pre-treatment groups, insulin was present in the perfusing buffer for the 10 min prior to, and during, agonist treatment. ANOVA and Tukey's test for multiple comparisons was performed at 0, 2, 4, 6, 8 and 10 min. There were no significant differences between the PHE and INS/PHE groups. $^*p \leq 0.05$ vs. control and vs. isoproterenol in the presence of insulin.

8Br-cAMP treatment completely abolished the Mg^{2+} efflux. As stated above, and as partially evident in the figures, the presence of insulin for the 10 min pre-treatment period (−10→0 min) had no effect on the Mg^{2+} content in the effluent. In data not shown, livers were perfused with insulin and buffer containing up to 70 μM Mg^{2+} and no detectable Mg^{2+} uptake was observed. Taken together, these results suggest that insulin antagonizes the effects of β-AR and 8Br-cAMP rather than activating a separate Mg^{2+} uptake process. Finally, Fig. 4 shows the effect of insulin pre-treatment on isoproterenol induced Mg^{2+} extrusion from collagenase dispersed rat hepatocytes. Also in this system, ISO resulted in a rapid extrusion of Mg^{2+} into the supernatant with over 90% of the effect already present at 2 min following the addition of agonist. Consistent with the data from perfused livers, treatment with insulin completely blocked the extrusion of Mg^{2+} into the supernatant.

4. Discussion

The current study illustrates a novel mechanism of hormonal control of intracellular Mg^{2+} homeostasis in liver cells. The data demonstrate that insulin completely inhibits β-AR mediated Mg^{2+} efflux from perfused livers or isolated hepatocytes, has a reduced effect, under similar experimental conditions, on inhibiting mixed adrenergic Mg^{2+} efflux, and has no effect on α-AR induced Mg^{2+} extrusion. Several important conclusions can be derived from these observations regarding Mg^{2+} handling under both physiological and disease states.

These findings support an interesting model in the liver whereby epinephrine, the endogenous circulating catecholamine, can induce Mg^{2+} extrusion, secondary to both α- and β-AR stimulation and insulin acts as the endogenous counter-regulatory hormone to the β-AR activated pathway. Under conditions where the β-AR mediated component is inhibited (i.e. insulin pre-treatment), mixed adrenergic stimulation is still capable of inducing a Mg^{2+} efflux, thus indicating that the α- and β-AR mediated pathways are distinct.

The mechanism by which insulin exerts an effect on β-AR mediated Mg^{2+} extrusion is speculative at this point. Insulin has been shown to interfere with β-AR signalling at several levels. Recent evidence has shown that insulin phosphorylates two tyrosine residues in the C-terminus of the β_2-AR [19,20]. In addition, insulin activates the calmodulin-dependent phosphodiesterase which degrades β-AR stimulated, adenylyl cyclase generated, cAMP to AMP in the cytosol of several cell types, including hepatocytes [21]. These observations are sufficient to explain the observed inhibitory effect of insulin on the β-AR mediated extrusion of Mg^{2+} from liver cells. Yet, based on these preliminary observations, it cannot be excluded that insulin has also a direct inhibitory effect on the plasma membrane Mg^{2+} extrusion mechanism(s), or that it affects intracellular Mg^{2+} redistribution. A schematic representation of these possibilities is illustrated in Fig. 5.

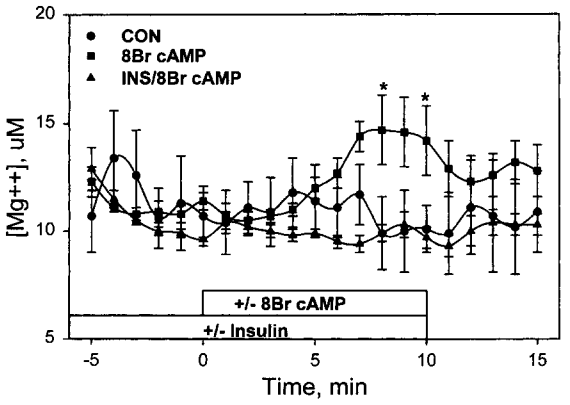

Fig. 3. Effect of insulin on 8-bromo-cAMP induced Mg^{2+} efflux from the perfused rat liver. Concentration of Mg^{2+} in effluent vs. time. Rat livers were perfused as described in Section 2. During the designated time, 8-bromo-cAMP or vehicle were added to the perfusing buffer. For the insulin pre-treatment groups, insulin was present in the perfusing buffer for the 10 min prior to, and during, agonist treatment. ANOVA and Tukey's test for multiple comparisons was performed at 0, 2, 4, 6, 8 and 10 min. $^*p \leq 0.05$ vs. control and vs. 8-bromo-cAMP in the presence of insulin.

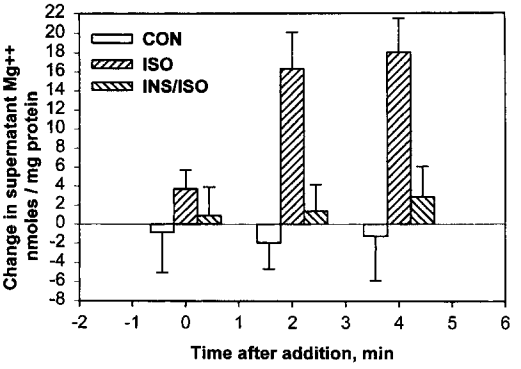

Fig. 4. Effect of insulin on isoproterenol induced Mg^{2+} efflux from collagenase dispersed rat hepatocytes. Concentration of Mg^{2+} in supernatant vs. time. Rat hepatocytes were prepared as described in Section 2. Cells were pre-treated with either insulin or vehicle at −2 min and then treated with either isoproterenol or vehicle at 0 min. ANOVA and Tukey's test for multiple comparisons was performed at 0, 2 and 4 min. $^*p \leq 0.05$ vs. isoproterenol alone.

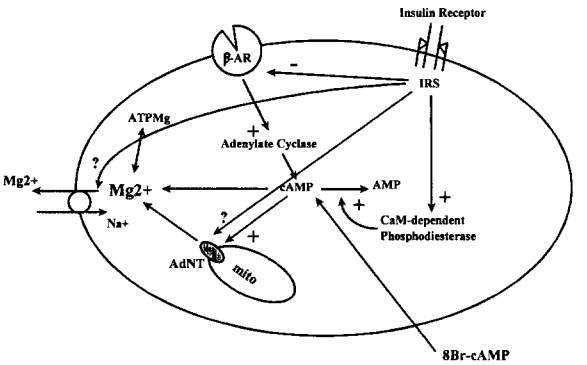

Fig. 5. The cartoon summarizes the mechanisms through which insulin can affect the extrusion of Mg^{2+} mediated by β-adrenergic receptors (β-AR). The stimulation of β-AR induces an increase in cytosolic cAMP which, in turn, mediates the extrusion of Mg^{2+} from mitochondria (MITO) via adenine nucleotide translocase (AdNT), an intrinsic protein of the inner mitochondrial membrane, likely by changing the substate of choice of this protein from ATP to ATPMg. The increase in cytosolic Mg^{2+} per se and/or the increase in cytosolic cAMP will probably activate the putative Na/Mg exchanger in the plasma membrane of liver cells and favour the extrusion of Mg^{2+} in the extracellular compartment. The possibility that insulin directly affects the operation of the Na/Mg exchanger in the plasma membrane and/or the operation of mitochondrial AdNT is also considered (question marks).

The physiological relevance of these observations is underscored by the acknowledged relationship between states of decreased insulin release or insulin-receptor activity and alterations in Mg^{2+} homeostasis. It is also relevant to the fact that diabetic patients [22] or animals [23] have significantly lower plasma Mg^{2+} levels than normal, healthy groups (1.2–1.3 vs 1.5–1.9 mEq/l). The data reported here suggest that insulin exerts a 'protective' effect on tissue Mg^{2+} stores which offsets the 'Mg^{2+} wasting' effect of β-AR stimulation. Prolonged periods of β-AR stimulation, unopposed by insulin activity, would then predispose to depleted tissue Mg^{2+} stores and eventually hypomagnesemia.

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References

[1] Romani, A. and Scarpa, A. (1990) FEBS Lett. 269, 37–40.
[2] Romani, A., Dowell, E. and Scarpa, A. (1991) J. Biol. Chem. 266, 24376–24384.
[3] Romani, A, Marfella, C. and Scarpa, A. (1992) FEBS Lett. 296, 135–140.
[4] Romani, A., Marfella, C. and Scarpa, A. (1993) Miner. Electrolyte Metab. 19, 282–289.
[5] Keenan, D., Romani, A. and Scarpa, A. (1995) Circ. Res. 77, 973–983.
[6] Jakob, A., Becker, J., Schottli, G. and Fritzsch, G. (1989) FEBS Lett. 246, 127–130.
[7] Pilkis, S.J. and El-Maghrabi, M.R. (1988) Annu. Rev. Biochem. 57, 755–783.
[8] Komjati, M., Astner-Kremsmayr, H., Waldhaus, I.H., Reitgruber, W., Breitenecker, F. and Troch, I. (1988) Endocrinology 123, 1798–1807.
[9] Pilkis, S.J. (1991) Annu. Rev. Nutr. 11, 465–515.
[10] Davis, D.J., Hickman, J.M., Lefebvre, C.A., et al. (1992) Am. J. Phys. 263, L562–L567.
[11] Ishijima, S., Sonoda, T. and Tatibana, M. (1991) Am. J. Phys. 261, C1074–C1080.
[12] Barbagallo, M., Gupta, R.K. and Resnick, L.M. (1993) Diabetologia 36, 146–149.
[13] Hwang, D.L., Yen, C.F. and Nadler, J.L. (1993) J. Clin. Endocrinol. Metab. 76, 549–553.
[14] Paolisso, G., Sgambato, S., Passariello, B., Giuglaino, D., Scheen, A.J., D'Onofrio, F. and Lefevbre, P.J. (1986) Diabetologia 29, 644–647.
[15] Paolisso, G., Scheen, A., D'Onofrio, F. and Lefevbre, P. (1990) Diabetologia 33, 511–514.
[16] White, J.R. and Campbell, R.K. (1993) Annu. Pharmacother. 27, 775–780.
[17] Sugano, T., Suda, K. and Shimada, M. (1978) J. Biochem. 83, 995–1007.
[18] Seglen, P.O. (1976) Methods Cell Biol. 13, 29–83.
[19] Karoor, V., Baltensperger, K., Paul, H., Czech, M.P. and Malbon, C.C. (1995), J. Biol. Chem. 270, 25305–25308.
[20] Baltensperger, K., Karoor, V., Paul, H., Ruoho, A., Czech, M.P. and Malbon, C.C. (1996) J. Biol. Chem. 271, 1061–1064.
[21] Smoake, J.A., Moy, G.M.-M., Fang, B. and Solomon, S.S. (1996) Arch. Biochem. Biophys. 323, 223–232.
[22] Lentner, C. (1984) Geigy Scientific Table, vol. 3, pp. 84–85, Ciba-Geigy, Basel, Switzerland.
[23] Wallach, S. and Verch, R.L. (1987) Magnesium 6, 302–306.