Effects of Insulin on Intracellular Magnesium of Platelets

JUNJI TAKAYA, HIROHIKO HIGASHINO, RIKI MIYAZAKI, AND YOHNOSUKE KOBAYASHI

Department of Pediatrics, Kansai Medical University, Osaka 570-8506, Japan

MAGNESIUM ($\text{Mg}^{2+}$), the second most abundant intracellular cation, is a critical cofactor in numerous enzymatic reactions and probably plays a role in cellular regulation [1]. The concentration of $\text{Mg}^{2+}$ inside cells is precisely regulated in spite of wide changes in the extracellular $\text{Mg}^{2+}$ concentration, which implies the existence of a specialized $\text{Mg}^{2+}$ transport system. A variety of growth factors act on quiescent mammalian cells, leading to an increase in the fluxes of $\text{Na}^+$, $\text{K}^+$ and $\text{H}^+$ across the plasma membrane and to stimulation of $\text{Ca}^{2+}$ mobilization [2]. Growth factors or hormones regulating $\text{Mg}^{2+}$ transport and its relation to platelet activity remain unclear. Our purpose is to clarify how $\text{Mg}^{2+}$ crosses the membrane and what factors would influence the $\text{Mg}^{2+}$ transport.

Materials and Methods

Platelet preparation

Human platelets were obtained from healthy donors with informed consent. The platelets were isolated as previously described [3]. Approximately 10 ml of venous blood was drawn into 3.8% (w/v) acid citrate buffer (10:1, v/v) and was centrifuged at 200 g for 10 min at room temperature. The platelet-rich plasma was centrifuged at 1,000 g for 10 min, and the cells were washed three times in Hepes buffer solution (HBS) containing (mM) NaCl 140, KCl 5, glucose 25, MgSO$_4$ 0.8, Na$_2$HPO$_4$ 1, Hepes 25 (pH 7.2), and EGTA 0.2. The platelets were studied within 4 h after blood drawing.

Measurements of intracellular calcium and magnesium concentrations

Intracellular ionic calcium ([Ca$^{2+}$]$_i$) and magnesium ([Mg$^{2+}$]$_i$) concentrations were measured with a Hitachi F-2000 fluorescence spectrophotometer as described by Grynkiewicz et al. [4]. Two $\mu$mol of Ca-fura-2/acetoxymethyl (for Ca measurement) or Mg-fura-2/acetoxymethyl (for Mg measurement) was added to the platelet suspension and incubated at 37 °C for 30 min. After removing the fura dyes by centrifugation, the platelets were resuspended in HBS. The excitation wavelengths were set at 340/380 nm (for Ca) or 335/370 nm (for Mg), and the emission wavelength was 510 nm. The intracellular ionic concentration was calculated as described [4] by using kilodalton$=224$ (nmol) for Ca and kilodalton$=1500$ ($\mu$mol) for Mg.

Results of the experiments were expressed as the mean ± SD. Statistical significance was assessed by Student's $t$-test. For intergroup comparisons, data were subjected to one-way ANOVA. A $P$ value of $<0.05$ was considered to be of statistical significance.

Results

Basal intracellular [Mg$^{2+}$]$_i$ in platelets was 412 ± 30 $\mu$M (n=40). Insulin increased [Mg$^{2+}$]$_i$ of fura-2-loaded platelets in a linear manner. The increase in [Mg$^{2+}$]$_i$ was rapid, reaching a plateau within a few seconds. After 60 sec of 100 $\mu$U/mL insulin stimulation, [Mg$^{2+}$]$_i$ was significantly increased to 1,020 ± 78 $\mu$mol (n=30, $P<0.0001$), i.e. 279 ± 11%
greater than the basal value. Insulin-like growth factor 1 (IGF-1) (1 µg/ml) also increased [Mg^{2+}]_i (639 ± 93 µM, P<0.05, n=5). GH had no effect on the increase in [Mg^{2+}]_i (Fig. 1). After the platelets were treated with EGTA, which chelates extracellular Mg^{2+} or Ca^{2+}, the insulin effect of the [Mg^{2+}]_i increase was not observed. Ouabain and wortmannin, both of which slightly decreased [Mg^{2+}]_i, had no effect on the increase in [Mg^{2+}]_i due to insulin. Amiloride, which increases [Mg^{2+}]_i, did not block the insulin effect. The addition of 1 mM MgCl_2 induced an increase in [Mg^{2+}]_i, and 1 mM CaCl_2 had the same effect. Insulin alone had no effect on the changes in [Ca^{2+}]_i. Glucose (5 mg/mL) increased [Mg^{2+}]_i but was not effective in altering [Ca^{2+}]_i. The addition of thrombin, which increases [Ca^{2+}]_i, induced a rapid decrease in [Mg^{2+}]_i, which, however, gradually recovered to the basal value (Fig. 1). The basal [Mg^{2+}]_i was 541 ± 68 µmol and significantly decreased to 185 ± 40 µmol after the addition of 0.1 U/mL thrombin (37.8 ± 6.4%, P<0.001, n=12). Serum or 0.1% BSA also decreased [Mg^{2+}]_i and it did not return to the basal level.

The addition of 1 mM ATP, which decreases [Mg^{2+}]_i, blocked the increase in [Mg^{2+}]_i due to insulin. ADP (0.1 mM) did not block the effect of insulin (Fig. 2). Insulin had no effect on [Mg^{2+}]_i when potassium was removed from the medium. The addition of either 1 mM NaCl or 1 mM KCl to the HBS medium had no effect on [Mg^{2+}]_i.

Fig. 1. Time-course of agonists effect on [Mg^{2+}]_i levels in platelets. GH had no effect on the increase in [Mg^{2+}]_i. Insulin (100 µU/mL) rapidly increased [Mg^{2+}]_i of Mg-fura 2-loaded platelets, which reached a plateau within a few seconds. Insulin-like growth factor 1 (IGF-1) (1 µg/ml) also increased [Mg^{2+}]_i, but to a lesser extent than with insulin.

Fig. 2. Time-course of agonists effect on [Mg^{2+}]_i levels in platelets. The addition of 0.1 mM ADP did not block the effect of insulin (upper panel). ATP (1 mM) decreased [Mg^{2+}]_i and blocked the increase in [Mg^{2+}]_i due to insulin (lower panel).
Discussion

Agonists induce an immediate increase in $[\text{Mg}^{2+}]_i$, which reached its peak within a few seconds. No increase in $[\text{Mg}^{2+}]_i$ was observed when extracellular Mg$^{2+}$ was chelated, which indicates that insulin can translocate Mg$^{2+}$ from the extracellular space to the intracellular space. Insulin and IGF-1 induced an increase in $[\text{Mg}^{2+}]_i$ more rapidly than has been reported previously [5]. This discrepancy may be due to the different type of medium in which the platelets were suspended. The increase in $[\text{Mg}^{2+}]_i$ was hardly observed at physiological insulin or IGF-1 concentration. The intra-accumulation of Mg$^{2+}$ caused by insulin occurs in the presence of both ouabain, which inhibits (Na$^+$-K$^+$)ATPase, and amiloride, which inhibits Na$^+$-H$^+$ antiport.

It was reported that magnesium regulates $[\text{Ca}^{2+}]_i$ in vascular smooth muscle cells [6]. Thrombin induced $[\text{Mg}^{2+}]_i$ withdrawal through the increase in $[\text{Ca}^{2+}]_i$. Mg$^{2+}$ may be co-transported with Ca$^{2+}$ following stimulation with thrombin. Intracellular Mg$^{2+}$ exists in two forms. Some is ionized and the rest is bound to such substances as ATP and RNA. BSA or serum may decrease $[\text{Mg}^{2+}]_i$ by binding intracellular Mg$^{2+}$. In membrane patches excised from insulin-secreting cells, K$^+$-ATP channels were inhibited by ATP but activated by ADP. In the presence of ATP, the insulin effect on $[\text{Mg}^{2+}]_i$ increase was blocked. We consider that Mg$^{2+}$ ions are transported through K$^+$-ATP channel in platelets.

In conclusion, insulin causes hyperpolarization of the platelets and Mg$^{2+}$ enters as a consequence of the change in electrical potential. Insulin-induced hyperpolarization may induce transduction of $[\text{Mg}^{2+}]_i$ not only in muscle and adipocytes, but also in platelets.

References