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Rapid communications

Use of protein kinase C inhibitors results in rapid $[Mg^{2+}]_i$ mobilization in primary cultured rat aortic smooth muscle cells: are certain protein kinase C isoforms natural homeostatic regulators of cytosolic free Mg^{2+}?

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Abstract

The effects of five different protein kinase C inhibitors—calphostin C, chelerythrine, bisindolylmaleimide I, staurosporine and Gö6979—on intracellular free magnesium ($[Mg^{2+}]_i$) content and mobilization were investigated in primary, cultured rat aortic smooth muscle cells. All these protein kinase C inhibitors significantly and rapidly increased $[Mg^{2+}]_i$ both in normal media (1.2 mM Mg^{2+}) and in Mg^{2+} free media. These data suggest that the increments of $[Mg^{2+}]_i$, induced by the diverse protein kinase C inhibitors, are derived from the release of bound intracellular Mg^{2+} and that activation of protein kinase C isozymes are normally responsible for helping to maintain basal levels of $[Mg^{2+}]_i$ in rat aortic smooth muscle cells.

Keywords: Mg^{2+}, intracellular free; Smooth muscle, vascular; Protein kinase C inhibitor

There is now considerable evidence for important roles of extracellular and intracellular free magnesium ($[Mg^{2+}]_i$) in vascular smooth muscle contraction, regulation of cytosolic free calcium ($[Ca^{2+}]_i$) levels, regulation of transmembrane Ca^{2+} and K^+ currents, vasomotor tone, blood pressure regulation, regulation of lipid second messengers and membrane fatty acid content, and regulation of nitric oxide biosynthesis and release (Altura and Altura, 1974, 1995; Delpiano and Altura, 1996; Morrill et al., 1997, 1998; Yang et al., 2000). However, the molecular basis for regulation of $[Mg^{2+}]_i$ is far less understood than that of other major cations. It has been suggested, previously, that activation of protein kinase C may be involved in catecholamine-induced increases in Mg^{2+} influx and rises in $[Mg^{2+}]_i$ in cardiac myocytes (Romani et al., 1992).

To consider the possibility, that protein kinase C activation might also be involved in homeostatic regulation of $[Mg^{2+}]_i$ mobilization and content in vascular muscle, we compared the effects of five different protein kinase C inhibitors on $[Mg^{2+}]_i$ in primary, cultured rat aortic smooth muscle cells. A highly specific protein kinase C inhibitor, calphostin C (2×10^{-6} M), which is Ca^{2+}/phospholipid-independent and competes with 1,2-diacylglycerol binding sites (Ogiwara et al., 1998), was chosen first. Other potent and selective inhibitors of protein kinase C, viz., chelerythrine (a competitive inhibitor with respect to the phosphate acceptor), two highly selective, cell-permeable but phospholipid/Ca^{2+}-dependent protein kinase C inhibitors, namely staurosporine, bisindolylmaleimide I, as well as Gö6979 (Ca^{2+}-dependent selective inhibitor of protein kinase C-α and protein kinase C-β_1) (Ogiwara et al., 1998) were also utilized. Measurements of $[Mg^{2+}]_i$ were made at 10–20 min after exposure of these cultured smooth muscle cells to each of the inhibitors. Primary aortic cultured smooth muscle cells were obtained and maintained as described previously (Yang et al., 2000). Values are means ± S.E.M. of at least 35–40 cells each.

The basal $[Mg^{2+}]_i$, as determined by digital image analysis with 2 μM mag-fura-2/AM, in rat aortic smooth

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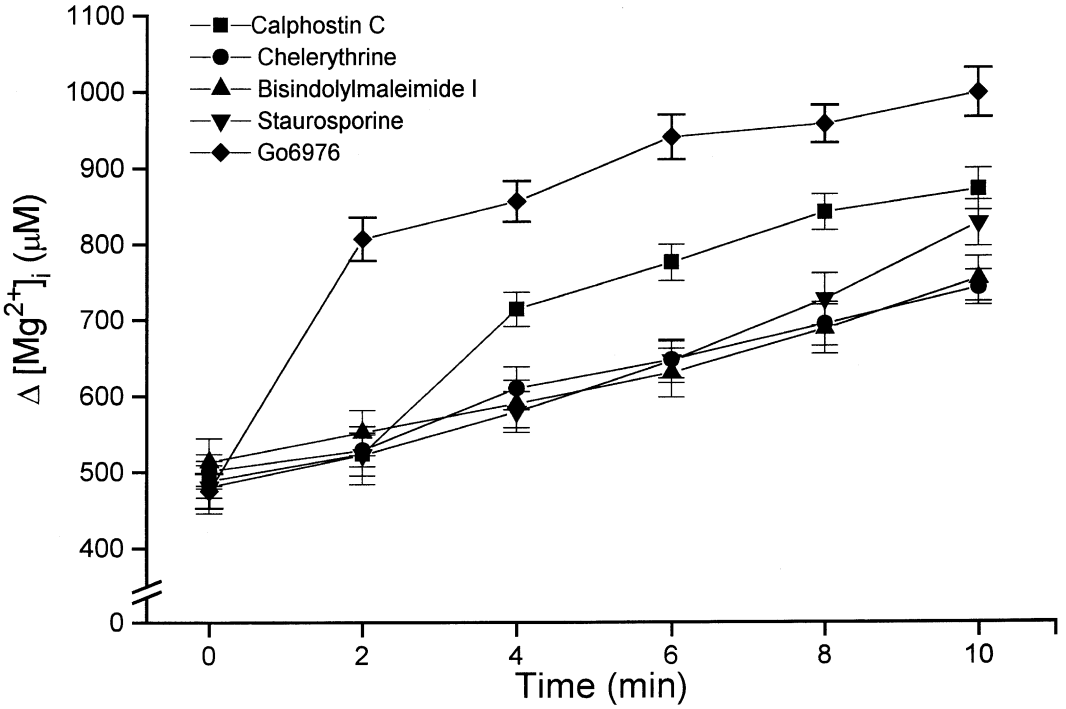

Fig. 1. Time-dependent effects of five different protein kinase C inhibitors, i.e., calphostin C (2×10^{-6} M), chelerythrine (10^{-6} M), bisindolymaleimide I (5×10^{-6} M), staurosporine (5×10^{-6} M) and Gö6979 (3×10^{-6} M), on intracellular free magnesium ($[Mg^{2+}]_i$) mobilization in primary cultured rat aortic smooth muscle cells. Values are the means ± S.E.M. of at least 40–45 cells. Measurements of $[Mg^{2+}]_i$ shown here were made at 10 min after the exposure of these cultured smooth muscle cells to each of the inhibitors.

muscle cells cultured in normal media (1.2 mM Mg^{2+}), was 530 ± 60 μM ($n = 45$); in Mg^{2+}-free media, for 10–20 min, it was 519 ± 49 μM ($n = 40$) ($P > 0.01$). We found that all the inhibitors of protein kinase C utilized significantly increased $[Mg^{2+}]_i$, i.e., calphostin C to 872 ± 65 μM ($P < 0.01$), chelerythrine to 743 ± 65.5 μM ($P < 0.01$), bisindolylmaeimide I to 754 ± 58.7 μM ($P < 0.01$), staurosporine to 825 ± 53.1 μM ($P < 0.01$) and Gö6979 to 999.4 ± 70 μM ($P < 0.01$) (Fig. 1). Moreover, when Mg^{2+}-free incubation media (for 20 min) was used, each of these protein kinase C inhibitors also increased $[Mg^{2+}]_i$ in cultured rat aortic smooth muscle cells, i.e., calphostin C to 868 ± 53 μM ($P < 0.01$), chelerythrine to 750 ± 66.2 μM ($P < 0.01$), bisindolylmaeimide I to 746 ± 49.7 μM ($P < 0.01$), staurosporine to 798 ± 69.1 μM ($P < 0.01$) and Gö6979 to 986.4 ± 59.0 μM ($P < 0.01$). Interestingly, use of the selective protein kinase C-α and protein kinase C-β_1 inhibitor, Gö6979, appears to result in the most rapid and greatest rises of $[Mg^{2+}]_i$ (Fig. 1), suggesting, possibly, that either one or both of these protein kinase C isozymes are the major enzymes keeping $[Mg^{2+}]_i$ at its basal cytosolic level. These results were not different from the data found in $[Mg^{2+}]_o$-containing media. These protein kinase C inhibitors induced rises of $[Mg^{2+}]_i$ in a concentration-dependent manner (10^{-8} to 10^{-5} M) and clearly did this in a $[Ca^{2+}]_o$-independent manner (data not shown).

In conclusion, this study suggests that the increments of $[Mg^{2+}]_i$, induced by the diverse protein kinase C inhibitors, are derived from the release of bound intracellular Mg and that activation of protein kinase C isozymes are normally responsible for helping to maintain basal levels of $[Mg^{2+}]_i$ in rat vascular smooth muscle cells. In addition, the results herein could be used to suggest a new, potential therapeutic approach for elevating cellular and tissue $[Mg^{2+}]_i$, which often becomes dangerously low, in clinical states of hypomagnesemia, particularly those known to be refractory to acute oral and systemic administration of Mg salts.

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