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SPECIAL REPORT

Protein kinase C-independent sensitization of contractile proteins to Ca^{2+} in α-toxin-permeabilized smooth muscle cells from the guinea-pig stomach

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Involvement of protein kinase C in receptor-operated Ca^{2+} sensitization of cell shortening was investigated by use of α-toxin-permeabilized smooth muscle cells from the fundus of the guinea-pig. Most of the isolated cells responded to 0.6 μM Ca^{2+} with a maximal shortening to approximately 65% of the resting cell length. Addition of acetylcholine (ACh) at a maximal concentration (10 μM) resulted in a marked decrease in the concentration of Ca^{2+} required to trigger a threshold response from 0.6 μM to 0.2 μM. The augmentation of Ca^{2+} sensitivity by ACh was not inhibited by specific protein kinase C inhibitors, calphostin C and K-252b at a concentration of 1 μM. These findings suggest that protein kinase C is not involved in the muscarinic receptor-operated augmentation of Ca^{2+} sensitivity.

Keywords: Ca^{2+} sensitivity; cell shortening; protein kinase C; smooth muscle cells; permeabilization; muscarinic receptor

Introduction We have recently shown the augmentation of Ca^{2+} sensitivity by stimulation of muscarinic receptors or guanosine 5'-triphosphate (GTP)-binding proteins in α-toxin-permeabilized single smooth muscle cells from the fundus of the guinea-pig stomach (Ono *et al.*, 1992). The involvement of protein kinase C in the increase of Ca^{2+} sensitivity has been proposed in studies on permeabilized smooth muscle tissues (Nishimura *et al.*, 1988; Itoh *et al.*, 1988). We, therefore, investigated the involvement of protein kinase C in receptor-operated Ca^{2+} sensitization of cell shortening in α-toxin-permeabilized single smooth muscle cells by use of specific protein kinase C inhibitors, calphostin C and K-252b.

Methods Cell isolation, measurement of cell shortening, and cell permeabilization were as described previously by Ono *et al.* (1992). Data shown in the figures are normalized by taking the baseline and the maximal shortening of each cell as 0 and 100%, respectively. Data are expressed as means ± s.e.means. Statistical significance was determined by Student's *t* test (paired). Calphostin C and K-252b were purchased from Kyowa Hakko Co. (Tokyo, Japan).

Results Most of the isolated cells responded to 0.6 μM Ca^{2+} with a maximal shortening (Figures 1a and 2a). Addition of acetylcholine (ACh) at a maximal concentration (10 μM) resulted in a marked decrease in the concentration of Ca^{2+} required to trigger a threshold response from 0.6 μM to 0.2 μM (Figures 1b and 2b). These results were consistent with previous observations (Ono *et al.*, 1992). When 1 μM calphostin C was present in the perfusion solution, ACh induced the sensitization to Ca^{2+} without any influence on the threshold concentration of Ca^{2+} (Figures 1a and 1b). The same finding was obtained with 1 μM K-252b (Figures 2a and 2b). These results suggest that protein kinase C is not involved in the muscarinic receptor-operated augmentation of Ca^{2+} sensitivity.

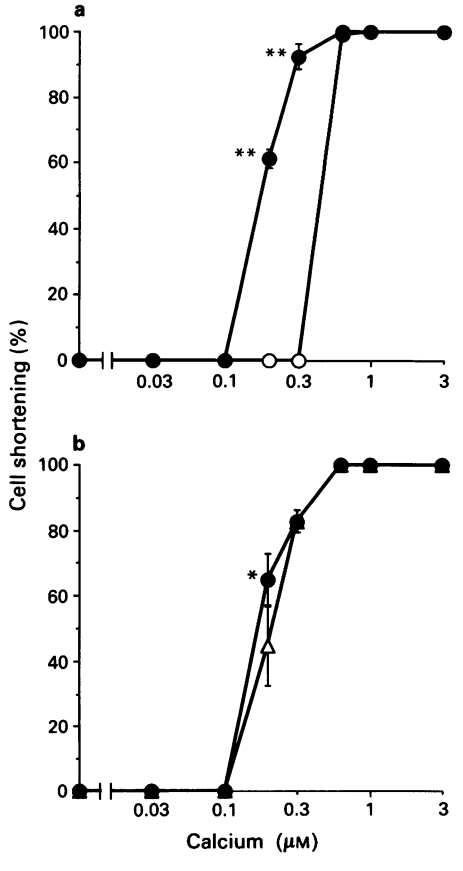

Figure 1 Effect of calphostin C on acetylcholine (ACh)-induced Ca^{2+} sensitization of cell shortening in α-toxin-permeabilized single smooth muscle cells. (a) The first and second shortenings were evoked by 10 nM to 3 μM Ca^{2+} in the absence (○) and presence (●) of 10 μM ACh plus 1 μM calphostin C, respectively. (b) The first and second shortenings were evoked by 10 nM to 3 μM Ca^{2+} plus 10 μM ACh in the absence (△) and presence (●) of 1 μM calphostin C, respectively. $n = 3$. $*P < 0.05$; $**P < 0.01$.

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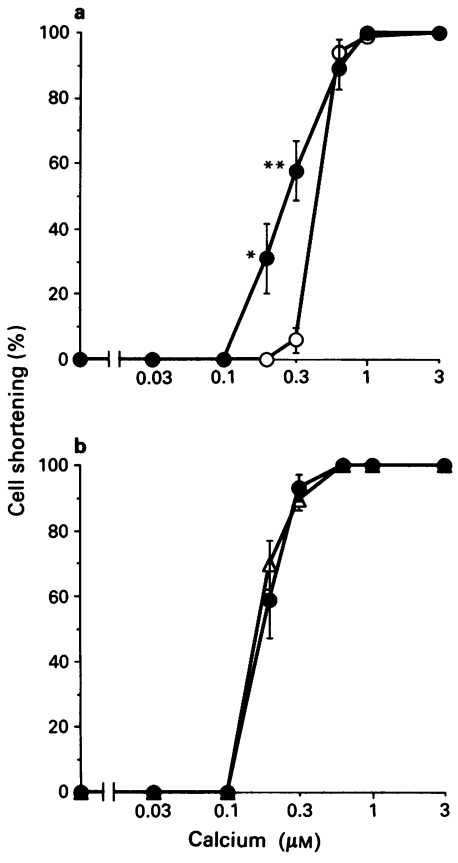

Figure 2 Effect of K-252b on acetylcholine (ACh)-induced Ca^{2+} sensitization of cell shortening in α-toxin-permeabilized single smooth muscle cells. (a) The first and second shortenings were evoked by 10 nM to 3 μM Ca^{2+} in the absence (○) and presence (●) of 10 μM ACh plus 1 μM K-252b, respectively. (b) The first and second shortenings were evoked by 10 nM to 3 μM Ca^{2+} plus 10 μM ACh in the absence (△) and presence (●) of 1 μM K-252b, respectively. $n = 3$. $^*P < 0.05$; $^{**}P < 0.01$.

Discussion In a previous paper, we have shown that GTP-binding proteins regulate the sensitivity of the contractile proteins to Ca^{2+} (Ono *et al.*, 1992). Two major superfamilies of GTP-binding proteins are present in mammalian tissues. One is the superfamily of heterotrimeric GTP-binding proteins which are now known to couple cell surface receptors to target enzymes such as phospholipase C (Freissmuth *et al.*, 1989). It has been shown that stimulation of various cell surface receptors including m1, m3, and m5 subtypes of muscarinic receptor leads to an activation of phospholipase C *via* a GTP-binding protein (Nathanson, 1987). We have shown here that specific protein kinase C inhibitors had no effect on the ACh-induced Ca^{2+} sensitization in the isolated single smooth muscle cell system. We previously showed that inositol 1,4,5-trisphosphate ($InsP_3$)-induced Ca^{2+} release is not involved in muscarinic receptor-operated shortening of the permeabilized cells (Ono *et al.*, 1992). Taken together, it is likely that heterotrimeric GTP-binding proteins which couple muscarinic receptors to phospholipase C are not involved in the augmentation of Ca^{2+} sensitivity.

In addition to such heterotrimeric GTP-binding proteins, the superfamily of monomeric GTP-binding proteins with molecular mass in the 20–30 kDa range has been implicated in the pathways of stimulus-response coupling. It has been shown that *rho* p21 families, which are thought to control cytoskeletal organization (Paterson *et al.*, 1990), are one of the most abundant small GTP-binding proteins present in bovine aortic smooth muscle (Kawahara *et al.*, 1990). Hirata *et al.* (1992) have clearly demonstrated that *rho* p21 is involved in the GTPγS-enhanced Ca^{2+} sensitivity of contraction in the rabbit mesenteric arterial smooth muscle and mentioned that *rho* p21 may be regulated by signal transduction pathways including protein kinase C-Ca^{2+} systems. Such a small GTP-binding protein could be another candidate for regulation of Ca^{2+} sensitivity, if located in the smooth muscle cells of the guinea-pig stomach. However, the possibility of regulation of *rho* p21 through protein kinase C system is ruled out by our present findings. Another novel pathway which regulates *rho* p21 remains to be clarified.

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