Gap junctional permeability is affected by cell volume changes and modulates volume regulation

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Isolated pancreatic acinar cell pairs became electrically uncoupled by exposure to a mild hypotonic shock. Reduction of bath osmolarity caused a delayed closure of gap junctional channels in the minute range. Dialysis of cell pairs by GTP[S] in the double whole-cell patch-clamp mode shortened the latency and shifted the hypotonically induced electrical uncoupling to lower osmolarity changes. Cellular treatment with cytochalasin B catalyzed electrical uncoupling by a hypotonic shock. In all cases, electrical uncoupling could be blocked completely by the protein kinase C (PKC) inhibitor polymyxin B. These results provide the first evidence suggesting that changes of cell volume and gap junctional permeability are correlated and that a G-protein dependent mechanism is involved. Evidence is presented that gap juntional coupling modulates volume regulation.

Cell volume; Gap junctional conductance; Hypotonic shock; G-protein

1. INTRODUCTION

Evidence has accumulated for the existence of a functional relationship between the phosphatidylinositol (PI) cycle [1] and cytoskeletal organization where PI breakdown causes cytoskeletal changes [2,3]. Here we present data which would indicate that an imposed change on the cytoskeleton affects the activity of the PI cycle and of PKC. A hypotonic shock, causing cell swelling and thus changing the cytoskeleton, increases the activity of the PI cycle. The hypotonically evoked decrease of g_j can be potentiated by GTP[S]. Recently it has been reported that the stability of the heterotrimeric G proteins depends on the cytoskeletal organization and that they have structures similar to cytoskeletal proteins [4,5]. Therefore it is suggested that cell swelling affects the coupling of the specific G proteins to their effector phospholipase C (PLC)[1]. To determine swelling we measure the cell diameter [6], and as a signal for the PKC activity we follow the gap junctional conductance (g_j) [7,8] measured with the double whole cell patch clamp method [9].

2. MATERIALS AND METHODS

Acinar cell pairs were isolated from the pancreas of 2-3 month-old male NMRI mouse by enzymatic dissociation [10]. The double whole cell patch clamp technique [9] was used to measure the gap junctional conductance of these exocrine gland cells [11,12]. Patch clamp data were evaluated as described previously [10]. The pipette control solution contained (in mM): 135 K^+, 10 Na^+, 119 Cl^-, 0.0001 Ca^{2+} (pCa7: 3.3 Ca^{2+} tot; pCa6: 4.9 Ca^{2+} tot), 1.0 Mg^{2+} (6.0 Mg^{2+} tot), 5.0 ATP^{2-}, 0.1 db-cAMP$^-$, 10 glucose, 10 HEPES, 5.0 EGTA, pH 7.4. Including cAMP and ATP in pipette control was sufficient in achieving stable coupling [10]. Composition of the isotonic (310 mOsm) bath medium, NaCl-BS (in mM): 145 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, pH adjusted to 7.4 with NaOH. Reduction of NaCl to 135 mM yielded 294.5 mOsm and to 120 mM, 279 mOsm as indicated. All experiments were carried out at room temperature (22-25°C).

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3. RESULTS AND DISCUSSION

A sudden reduction of the bath osmolarity (π) by 10% ($\Delta\pi$ = 310-279 mOsm = 31 mOsm) induced a decrease of the gap junctional conductance (g_j) of pancreatic acinar cell pairs (Fig. 1). The latency for the decrease of g_j was 13.5 ± 1.1 min ($n=4$). With a smaller hypotonic shock of $\Delta\pi$ = 15.5 mOsm a significant change of g_j could not be observed ($n=5$) during the typical recording time of about 40 min. Addition of GTP[S] to the pipette solution shortened the latency of electrical uncoupling (Fig. 2). In the presence of 10 μM GTP[S] the latency was 9.6 ± 0.7 min ($n=3$) and at 100 μM GTP[S] 3.2 ± 1.1 min ($n=3$) after reduction of the bath osmolarity by 31 mOsm. Supplementation of the pipette control solution with 100 μM GTP[S] caused a significant decrease of g_j, also after a smaller hypotonic shock of $\Delta\pi = 15$ mOsm which in the absence of GTP[S] did not affect g_j (compare the corresponding curves of Figs 1 and 2). These findings indicate a dose-dependent mechanism for the action of GTP[S] on the hypotonically evoked change of g_j.

Previously it had been shown that stimulation of this exocrine cell type by a cholinergic secretagogue (carbamoylcholine - CCh) reduced g_j, a reduction which

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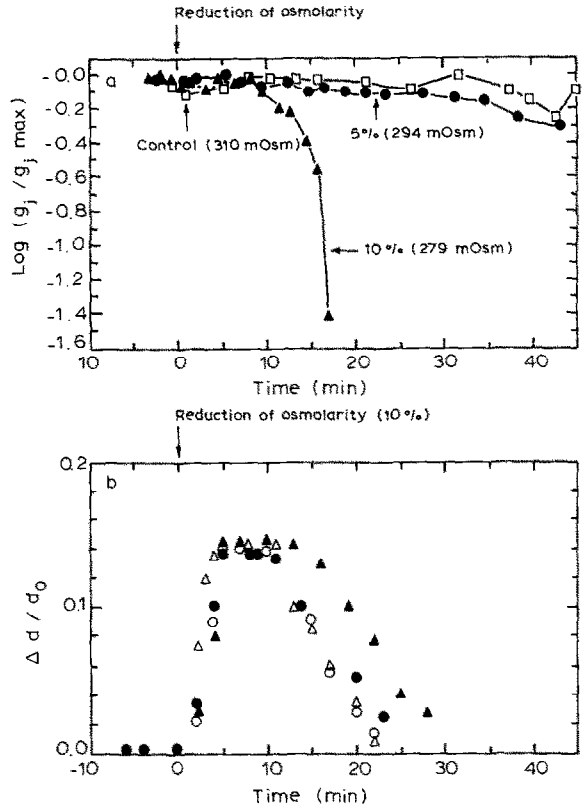

Fig. 1. Effect of reduction of bath osmolarity on gap junctional conductance g_j (a), and on the simultaneously measured relative change ($\Delta d/d_0$) of cell diameter (b). (a) At $t=0$ the bath osmolarity was reduced either by 5% to 294 mOsm (filled circles) or by 10% to 279 mOsm (filled triangles). Open squares denote a control experiment with isotonic bath (310 mOsm). For clearer presentation g_j was normalized to the maximal value of g_j (g_{jmax}) of the corresponding experiment. (b) $\Delta d/d_0$ was determined for isolated single cells (filled circles) and for single cells within coupled cell pairs (filled triangles), respectively. At $t=0$ the bath osmolarity was reduced by 10%. Open circles and open triangles show the corresponding results for the same selection of single cells, but after preincubation of the cell suspension in an isotonic bath containing 20 μM carbamoylcholine (CCh) for 15 min. Electrical uncoupling by CCh [8] accelerates the volume regulation of cells within cell pairs (compare open and filled triangles).

could be potentiated by GTP[S] in a dose-dependent manner [8]. No effect on g_j was observed in the absence of CCh for concentrations of GTP[S] up to 500 μM. Evidence had also been presented that the CCh-induced and GTP[S]-potentiated electrical uncoupling is mediated by PKC [7,8,13]. It is known that secretagogues cause a transient rise of PKC and $[Ca^{2+}]_i$ by activating the 'dual pathway' receptor → G-protein → phospholipase C (PLC) → IP_3 and DAG, the former releasing Ca^{2+} from intracellular stores and the latter activating PKC [14–16]. We propose here that hypotonic shock also modulates this dual pathway. Therefore we examined whether G-protein dependent stimulation of PI-breakdown, leading to an increase of PKC activity, is responsible for electrical uncoupling. We probed this hypothesis with the specific PKC inhibitor polymyxin B (PMB), a polycationic peptide an-

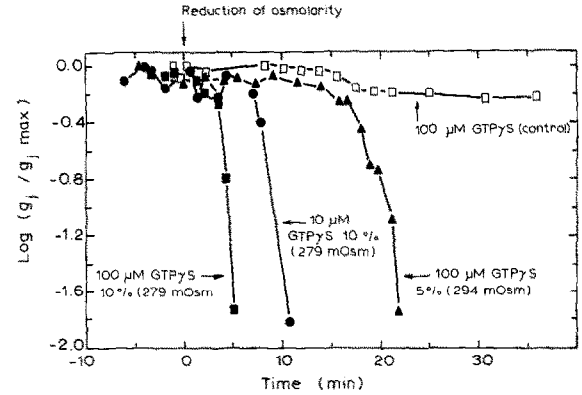

Fig. 2. Potentiation of osmotically-induced decrease of gap junctional conductance g_j by GTP[S]. Filled triangles: Addition of 100 μM GTP[S] to pipette control solution; reduction of bath osmolarity by 5% at $t=0$ (compare to filled circles of Fig. 1a). Filled circles (filled squares): Addition of 10 μM (100 μM) GTP[S] to pipette control solution; reduction of bath osmolarity by 10% at $t=0$. Open squares: Addition of 100 μM GTP[S] to pipette control solution; isotonic bath (310 mOsm) throughout the recording.

tibiotic [17], which competitively inhibits PKC with respect to phosphatidylserine, without affecting cyclic AMP or cyclic GMP-dependent protein kinases [18]. In pancreatic acinar cells, PMB has been shown to eliminate PKC-dependent substrate protein phosphorylation [19]. Recently, we have demonstrated that PMB suppresses 1-oleoyl-2-acetyl-*sn*-glycerol (OAG) – as well as CCh/GTP[S]-induced uncoupling [7]. Fig. 3 shows that addition of 10 μM PMB to the pipette solution completely suppressed GTP[S]-potentiated uncoupling by hypotonic shock. These findings support the hypothesis that activation of PKC might be involved in the hypotonically evoked uncou-

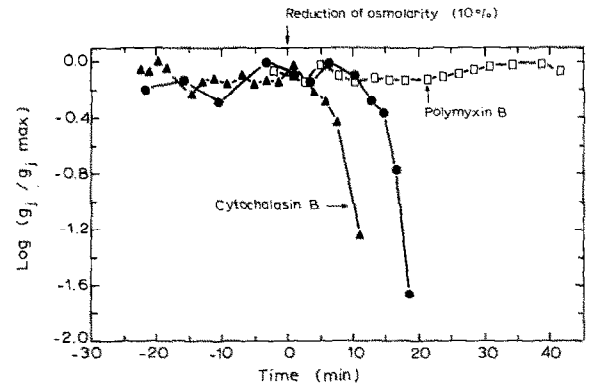

Fig. 3. Effects of polymyxin B (PMB) or cytochalasin B on osmotically-induced decrease of gap junctional coupling g_j. At $t=0$ the bath osmolarity was reduced by 10%. Open squares: 10 μM GTP[S] and 10 μM (PMB) were included in pipette control. PMB suppressed the GTP[S] potentiated decrease of electrical uncoupling. Filled triangles: Preincubation of an acinar cell pair in a control bath solution containing 5 μM cytochalasin B for 15 min; pipette control was used as filling solution. For comparison the filled circles present an experiment where pipette control was used as filling solution without additives to the bath.

pling. Enhanced PI-breakdown has indeed been observed during and after cell exposure to a hypotonic shock [20].

A second candidate for the mediator of gap junctional uncoupling is an increase of $[Ca^{2+}]_i$, since in many cases cell swelling by a hypotonic shock is linked to a Ca_i^{2+} increase [21,22]. In the foregoing experiments $[Ca^{2+}]_i$ in the pipette solution was buffered to 10^{-7} M with 3.3 mM $CaCl_2$ and 5 mM EGTA. By use of a nominally Ca-free pipette control solution, containing 5 mM EGTA ($[Ca^{2+}]_i \leq 10^{-8}$M), the hypotonically induced electrical uncoupling could be completely suppressed even for cells dialyzed with solutions containing 100 μM GTP[S]. In the latter case an increase of $[Ca^{2+}]_i$ has been reported [23]. In our experiments GTP[S] induced variations of $[Ca^{2+}]_i$ should have been suppressed by the presence of 5 mM EGTA. $Ca^{2+}{}_i$ thus is essential for uncoupling, but this requirement is met by the presence of resting levels of $[Ca^{2+}]_i$. This inhibition of GTP[S]-enhanced uncoupling by $[Ca^{2+}]_i \leq 10^{-8}$ M is in accordance with the observation that $[Ca^{2+}]_i$ levels below 10^{-7} M inhibit the stimulation of PLC activity by GTP[S] [24]. In addition, PKC requires Ca^{2+} for its activation, independently of other modulators [25,26]. When we buffered $[Ca^{2+}]_i$ to 10^{-6} M, uncoupling was not accelerated compared to those experiments with 10^{-7} M $Ca^{2+}{}_i$. This $Ca^{2+}{}_i$ concentration also had no effect on g_j in the absence of a hypotonic shock within an observation time of 40 min.

Finally, the question arises as to the nature of the signal transduction sequence leading to gap junctional channel closure after a hypotonic shock. To gain insight into the link cytoskeleton-PI cycle, we investigated the role of the microfilament structure of pancreatic acinar cell pairs by addition of 5 μM cytochalasin B (CB) to the isotonic bath. Cytochalasins are fungal metabolites that inhibit actin polymerization yielding to disruption of the actin network within 2–3 min [27]. Within a preincubation period of 15 min, no significant change of g_j and of cell diameter was observed. Thereafter, hypotonically induced electrical uncoupling became accelerated (Fig. 3). This catalytic action of CB suggests an involvement of microfilaments. Interestingly, two actin-binding proteins, gelsolin and profilin, found in brain, specifically interact with phosphatidylinositol 4,5-bisphosphate (PIP_2). CB affects this interaction [2,3]. With this in mind we propose the following signal transduction sequence: A hypotonic shock may affect the cytoskeletal organization, which is responsible for the stability of the heterotrimeric G-protein [4], such that $G_{\alpha\text{-GTP}}$ is liberated and can increasingly activate its effector PLC. The 'receptor' of hypotonically induced uncoupling would then be the network of the cytoskeleton and not of any specific receptor molecule. Consequently, hydrolysis of PIP_2 by PLC would increase PKC activity, which finally would lead to gap junctional channel closure. It is tempting to suggest that CB increases the substrate (PIP_2) availability [2,3], making it susceptible to hydrolysis and thereby catalyzing the PKC mediated junctional uncoupling.

To elucidate the possible physiological role of cell coupling for volume regulation, cell coupling and cell diameter were measured simultaneously (Fig. 1). After hypotonic shock the volume regulation of an isolated single cell occurs more rapidly than that of a cell within a cell pair. But after electrical uncoupling of a cell pair by CCh [8] a cell pair behaves like single isolated cells (see Fig. 1b). Therefore we suggest that gap junctional channel closure after e.g. a hypotonic shock, could facilitate the cell volume regulation of an individual cell within the cell syncytium.

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