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A G-protein mediates secretagogue-induced gap junctional channel closure in pancreatic acinar cells

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Using the double whole-cell patch-clamp technique, we determined that dialysis of cell pairs by GTP[S] potentiated electrical uncoupling induced by extracellular addition of carbamylcholine (CCh) An inhibitor of diglyceride lipase, RHC 80267, further potentiated CCh/GTP[S]-induced junctional channel closure, probably by accumulation of diacylglycerol Moreover, the protein kinase C inhibitor polymyxin B completely blocked uncoupling elicited by CCh/GTP[S] These results provide the first evidence suggesting that gap junction channel closure by cholinergic stimulation is mediated by a G-protein, which acts by increasing phosphatidylinositol biphosphate breakdown and protein kinase C activity

Gap junction, G-protein, Patch-clamp, Protein kinase C

1. INTRODUCTION

Secretagogues, such as cholinergic agonists, lead to a reduction of the gap junctional permeability between exocrine gland cells [1–3]. These agents are also known to enhance G-protein-mediated phospholipase C (PLC) activity. PLC causes breakdown of phosphatidylinositol bisphosphate (PI breakdown) to inositol trisphosphate (IP_3) (which increases intracellular Ca^{2+} [4,5]) and diacylglycerol (DAG) (which activates protein kinase C [6]). In previous electrophysiological experiments using the double whole-cell configuration [7], we have shown that introduction of protein kinase C (PKC) into murine pancreatic acinar cell pairs causes gap junction channel closure [8]. Here we report that addition of GTP[S] to the pipette filling solution potentiates the effect of cholinergic stimulation by the secretagogue CCh, leading to accelerated and complete gap junction channel closure.

2. MATERIALS AND METHODS

Acinar cell pairs were isolated from the pancreas of 2–3 month old male NMRI mice by enzymatic dissociation [9] The double whole-cell patch-clamp technique was used to measure the junctional conductance, g_j, as described previously [9] On the basis of diffusion times calculated for typically determined pipette resistances between 1 5 and 8 0 MΩ and cell diameters of 10–15 μm, it was estimated that 90% (50%) of the pipette concentration of substances of M_r 1200 (Polymyxin B) and M_r 549 (GTP[S]) will be reached in the cytosol within 1–15 min (0 3–5 min) and 1–11 min (0 3–3 4 min), respectively [10] Experiments were performed at room temperature The standard pipette control solution contained (in mM) 135 K^+, 10 Na^+, 119 Cl^-, 0 0001 Ca^{2+} (pCa 7, 3 3 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 Composition of the 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 For all figures, the quotients of the individual g_j values over the maximal starting value was determined for each experiment The lines connect the mean ± SE of these normalized values pooled from different experiments The time axis origin was calibrated to the exchange of the bath medium with NaCl-BS containing agonist To allow for the exchange of the pipette solution with the cytosol, carbachol was added 5–10 min after breaking both patch membranes

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3. RESULTS AND DISCUSSION

Fig.1 illustrates the potentiating effect of cell dialysis with GTP[S]-containing pipette electrolytes on CCh-induced electrical uncoupling of pancreatic acinar cell pairs. Application of CCh at concentrations above 1 μM caused a transient and incomplete decrease of electrical coupling in the absence of GTP[S]. The slope of the corresponding decline of the junctional conductance, g_j, and the time lag of action vary considerably from experiment to experiment. Addition of GTP[S] to the pipette solution led to a decrease of g_j by more than three orders of magnitude to total electrical uncoupling (g_j < 3 pS) within 6 min of stimulation with 10 μM CCh. At 1 μM CCh, the time lag of the decline of g_j decreased with increasing GTP[S] concentrations (fig.2). This indicates a dose-dependent mechanism of action of GTP[S] on g_j. The potentiating effect of GTP[S] on the cholinergic uncoupling response may be ascribed to sustained activation of a GTP-binding protein (G-protein) [11]. Since muscarinic receptors are known to interact with a number of different G-

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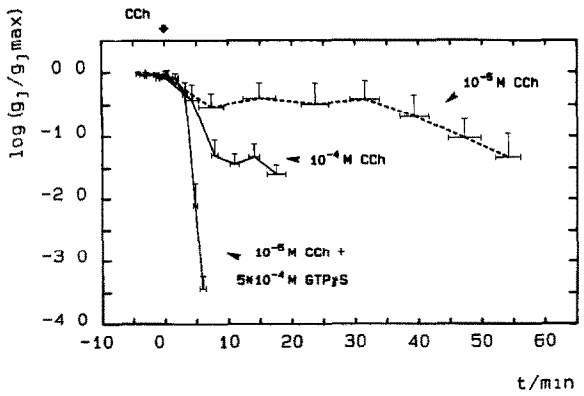

Fig.1 Potentiation of carbachol(CCh)-induced decrease of junctional conductance g_j by GTP[S] Unmodified pipette control was used in the pipette and 100 μM CCh (dotted line, n = 2), or 10 μM CCh (broken line, n = 2) were added to the bath Supplementation of pipette control by 500 μM GTP[S] resulted in potentiation of uncoupling induced by 100 μM CCh (solid line, n = 2)

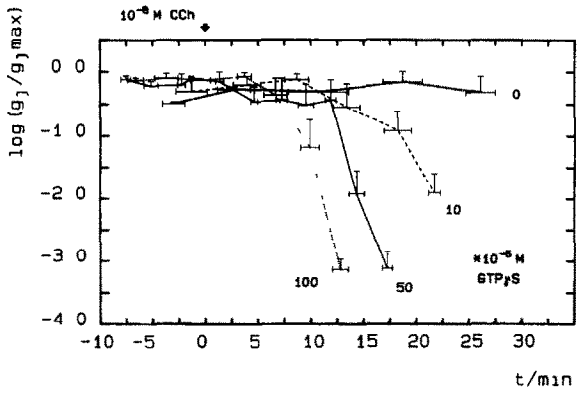

Fig.2. Effects of different intracellular concentrations of GTP[S] on CCh-induced change of g_j All experiments were performed adding 1 μM CCh to the bath GTP[S] in the pipette control solution is lowered from 100 μM (dotted line, n = 2) to 50 μM (solid line, n = 4) and 10 μM (broken line, n = 2) An experiment using pipette control solution in the pipette is shown for comparison (line interrupted by dots, also compare to fig 1).

proteins [12], there are several mechanisms possible for CCh/GTP[S]-induced uncoupling. Uncoupling could be due to direct interaction of a G-protein with the junctional channel, as has been described for plasma membrane ion channels [12,13]. Alternatively, the activation of a sequence of biochemical steps, initiated by the stimulated G-protein, might result in junctional channel closure [12].

Addition of non-hydrolysable analogues of GTP (e.g. GTP[S]) to permeabilized pancreatic acinar cell suspensions causes PLC activation [14], IP_3 production [15], and potentiates the effect of ACh on Ca_i^{2+} liberation [5,16]. It is therefore reasonable to assume that CCh-stimulated PLC activity is significantly enhanced by cell dialysis with GTP[S] in our experiments. An IP_3-mediated increase of $[Ca^{2+}]_i$ probably did not play a substantial role in modulating g_j, since $[Ca^{2+}]_i$ was strongly buffered to 10^{-7} M with 5 mM EGTA in the cell dialysate. This is in agreement with results obtained on lacrimal gland cells, in which the GTP[S]-induced potentiation of the muscarinic Ca^{2+}-dependent current response can be eliminated by buffering $[Ca^{2+}]_i$ with 5 mM EGTA [17]. On the other hand, CCh-stimulated PLC also leads to release of DAG [18] and concomitant activation of PKC [6]. CCh/GTP[S]-induced gap junction channel closure may possibly be mediated by a G-protein-induced activation of PKC.

Different steps in the cholinergic signal transduction pathway were investigated to determine the mechanism of CCh/GTP[S]-induced uncoupling. One of these steps is diglyceride lipase (DGL). This enzyme is involved in the elimination of DAG and generation of arachidonic acid (AA). AA is known to uncouple lacrimal gland cells [19]. It is also formed following secretagogue stimulation of pancreatic acinar cells [20]. An inhibitor of DGL in pancreatic acinar cells, RHC 80267 [20], was added to the pipette solution. RHC 80267 markedly enhanced CCh/GTP[S]-stimulated electrical uncoupling, causing a further reduction of the time lag of uncoupling and acceleration of the decrease of g_j (fig.3). This can readily be explained by an RHC 80267-induced accumulation of DAG and the resulting increase of PKC activity [21]. As has been concluded previously, AA apparently is not involved in mediating the effect of CCh [19]. Otherwise, an inhibition of uncoupling would have been expected on account of reduced AA production by RHC 80267.

The DAG branch of CCh-induced PI-breakdown terminates in activation of PKC. This step may be probed with a specific PKC inhibitor. Polymyxin B (PMB), a polycationic peptide antibiotic [22], competitively inhibits PKC with respect to phosphatidylserine, while not affecting cyclic AMP or cyclic GMP-dependent

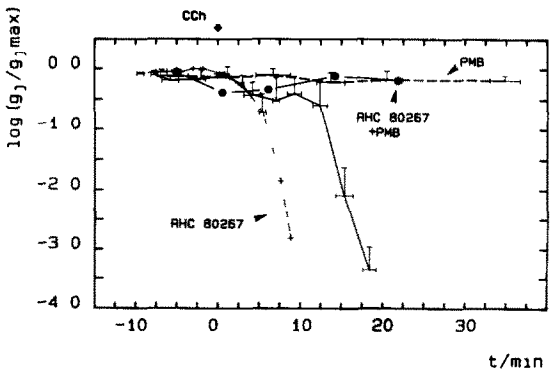

Fig 3. Effects of RHC 80267 and PMB on CCh/GTP[S]-induced uncoupling 50 μM GTP[S] was included in pipette control and 1 μM CCh was added to the bath. Dotted line (n = 2): addition of 50 μM or 100 μM RHC 80267 to pipette control Solid line (same as in fig 2). supplementation by 50 μM GTP[S] alone Broken line (n = 3)· 100 μM to 200 μM PMB were included in pipette control RHC 80267 was not effective in counteracting the suppression by PMB (line interrupted by dots, 50 μM RHC 80267 and 200 μM PMB pipette control)

protein kinases [23]. In pancreatic acinar cells, PMB has been shown to inhibit CCh and 12-*O*-tetradecanoylphorbol-13-acetate TPA-induced amylase release, and eliminate PKC-dependent substrate protein phosphorylation [24]. We have recently demonstrated that PMB suppresses 1-oleoyl-2-acetyl-*sn*-glycerol (OAG)-induced uncoupling of pancreatic acinar cells [8]. Here we found that addition of PMB to the pipette solution completely eliminated CCh/GTP[S]-induced uncoupling (fig.3). This inhibition could not be reversed by RHC 80267 (fig.3). These results support the hypothesis that the potentiating effects of GTP[S] and RHC 80267 on CCh-induced uncoupling are due to activation of PKC.

The results presented here suggest that the threshold for junctional channel closure induced by CCh is high in acinar cells. High levels of PKC activity may be required for uncoupling. Treatment of acinar cells with TPA is correlated with partial translocation of PKC, but causes only a weak reduction of the junctional permeability [25]. Activation of PKC by CCh instead of TPA is at least five-fold higher though [26], suggesting that CCh/GTP[S] may activate PKC sufficiently to surpass the threshold required for uncoupling.

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