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Direct activation of *trpl* cation channels by $G\alpha_{11}$ subunits

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G proteins of the $G_{q/11}$ subfamily functionally couple cell surface receptors to phospholipase C β (PLC β) isoforms. Stimulation of PLC β induces Ca^{2+} elevation by inositol 1,4,5-trisphosphate ($InsP_3$)-mediated Ca^{2+} release and store-dependent 'capacitative' Ca^{2+} entry through Ca^{2+}-permeable channels. The *Drosophila trp* gene, as well as some human *trp* homologs, code for such store-operated channels. The related *trp-like* (*trpl*) gene product also forms a Ca^{2+}-permeable cation channel, but is not activated by store depletion. Co-expression of the constitutively active G_q subfamily member $G\alpha_{11}$ ($G\alpha^*_{11}$) with *trpl* enhanced *trpl* currents 33-fold in comparison with co-expression of *trpl* with other Gα isoforms or Gβγ complexes. This activation could not be attributed to signals downstream of PLC β. In particular, $InsP_3$ infusion, modulation of protein kinase C activity or elevation of intracellular calcium concentration failed to induce *trpl* currents. In contrast, purified $G\alpha^*_{11}$ (but not other G protein subunits) activated *trpl* channels in inside-out patches. We conclude that *trpl* is regulated by G_{11} proteins in a membrane-confined manner not involving cytosolic factors. Thus, G proteins of the G_q subfamily may induce Ca^{2+} entry not only indirectly via store-operated mechanisms but also by directly stimulating cation channels.

Keywords: G proteins/ion channels/signal transduction

Introduction

Increases in the intracellular free calcium concentration ($[Ca^{2+}]_i$) trigger a plethora of cellular functions. Rises in $[Ca^{2+}]_i$ in response to many stimuli are characterized by a typical biphasic time course (Berridge, 1995; Clapham, 1995a, 1996). An initial increase is caused by release of calcium from internal stores. Subsequently, Ca^{2+} influx through the cell membrane leads to a sustained elevation of $[Ca^{2+}]_i$. A key role in these processes is attributed to G proteins of the $G_{q/11}$ subfamily. Numerous heptahelical receptors couple to $G_{q/11}$ proteins and thereby stimulate phospholipase C β (PLC β), resulting in the breakdown of phosphoinositides and production of inositol (1,4,5)-triphosphate ($InsP_3$) and diacylglycerine (Berridge, 1993). $InsP_3$ binds to intracellular receptors and leads to release of calcium from intracellular stores (Joseph, 1996). Calcium depletion of these stores generates a yet unknown signal that activates Ca^{2+}-permeable channels in the cell membrane (Berridge, 1995). This mechanism is named capacitative calcium entry (Putney, 1986, 1990) and the channels involved are classified as store-operated channels (SOC) (Clapham, 1995b). In the eye of *Drosophila*, the gene product *trp* codes for such a SOC (Hardie and Minke, 1993; Vaca *et al.*, 1994). *trp* appears to be a member of a gene family that includes *trpl* (*trp-like*) in *Drosophila* (Phillips *et al.*, 1992) as well as several recently identified invertebrate and mammalian homologs (Petersen *et al.*, 1995; Wes *et al.*, 1995; Zhu *et al.*, 1995, 1996; Zitt *et al.*, 1996). Like *trp*, the *trpl* gene product constitutes a Ca^{2+}-permeable cation channel when expressed in Sf9 cells. *trpl* currents are mainly carried by Na^+ or Cs^+ (Harteneck *et al.*, 1995) but also by Ca^{2+} (Vaca *et al.*, 1994). Stimulation of heptahelical membrane receptors co-expressed with *trpl* induced cation currents (Harteneck *et al.*, 1995). In contrast to *trp*, activation of *trpl* currents was not caused by store depletion (Vaca *et al.*, 1994). Here we present evidence that *trpl* channels are activated by the α subunits of G proteins of the $G_{q/11}$ subfamily in a membrane-confined way, independently of cytosolic factors. Thus, $G_{q/11}$-induced Ca^{2+} entry may be mediated by two distinct mechanisms, either by the store-operated pathway or by direct activation of cation channels by the G protein.

Results

trpl channels are activated by various heptahelical receptors, including the histamine H_1 receptor (H_1R) co-expressed with *trpl* in insect Sf9 cells (Harteneck *et al.*, 1995). To identify the G protein transducing the activating signal to *trpl*, we studied histamine-induced incorporation of a radiolabeled photoreactive GTP analog into membrane proteins of Sf9 cells expressing H_1R. The G protein isoform was identified with antisera known to specifically immunoprecipitate Gα subunits (Laugwitz *et al.*, 1994). Figure 1 shows autoradiograms of precipitates with AS 348 (α_s; lanes 1 and 2), AS 369 ($\alpha_{q/11}$; lanes 3 and 4), AS 266 ($\alpha_{i\ common}$; lanes 5 and 6) and AS 232 (α_{12}; lanes 7 and 8). Histamine activated only $G_{q/11}$-like insect G proteins.

Whole cell current recordings from Sf9 cells expressing the *trpl* gene product and H_1R revealed outward rectifying currents in response to histamine (n = 25 in 35 experiments) as described (Harteneck *et al.*, 1995). No such currents occurred in cells not infected with viruses coding for *trpl* (n = 19). Outward rectifying currents were also induced by infusion of either GTPγS (n = 7 out of 12

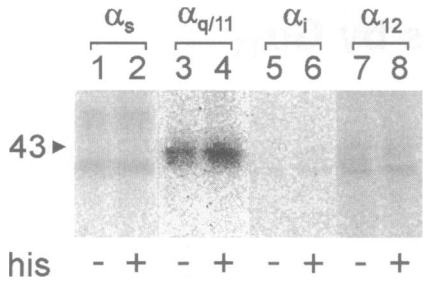

Fig. 1. [α-^{32}P]GTP–azidoanilide-photolabeling of membrane proteins of Sf9 cells expressing H_1R. Sf9 cells were infected for 28 h with viruses encoding guinea pig histamine H_1 receptor. For photolabeling and immunoprecipitation of Gα proteins, membranes were incubated with [α-^{32}P]GTP–azidoanilide in the absence (–) or presence (+) of histamine (his; 100 μM). The images shown are derived from the same gel. The gel was cut into strips and exposed for either 1 ($G\alpha_{q/11}$) or 3 days ($G\alpha_s$, $G\alpha_i$ and $G\alpha_{12}$). The apparent molecular mass of a marker protein is indicated on the left (kDa). Autoradiograms shown are representative of three independent experiments with similar results.

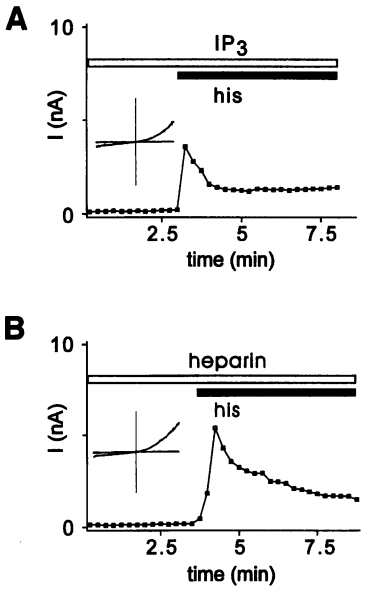

Fig. 2. Effect of $InsP_3$ (**A**) and heparin (**B**) on whole cell currents of Sf9 cells expressing *trpl* and H_1R. $InsP_3$ (10 μM) or heparin (0.5 mg/ml) were present in the pipette solution (I_1). Histamine (his, 40 μM) was present in the bath for the time indicated by the filled bars. Cells were held at 0 mV. Voltage ramps were applied every 15 s. The ordinates represent currents at 60 mV obtained during application of the ramps. The access resistance was 2–8 MΩ over the first 5 min. The inserts show the I–V relation of the currents, as obtained during voltage ramps from –60 to 60 mV, at the peak of the histamine effect. The range of the ordinates in these inserts is ±8 nA.

cells) or aluminium fluoride (AlF_4^-; n = 14 out of 24 cells, not shown).

No *trpl* currents were induced by infusion of $InsP_3$ (10 μM) into the cells via the patch pipette within 3 min (n = 24). Activation of *trpl* currents was frequently observed in cells kept in the whole cell configuration for a prolonged time (i.e. >3 min), in the absence (n = 6) as well presence of $InsP_3$ (n = 5). Hence, we cannot discriminate spontaneous activation of *trpl* from a delayed effect of $InsP_3$. However, cells infused with $InsP_3$ exhibited currents in response to histamine (n = 16; Figure 2A). Furthermore, the $InsP_3$ receptor blocker heparin (0.5 mg/ml; n = 7) did not prevent histamine-induced *trpl* currents (Figure 2B). We also tested other signals downstream of PLC β for activation of *trpl*, i.e. protein kinase C (PKC) and elevated cytosolic Ca^{2+} concentrations. Activation of *trpl* currents by histamine was not prevented (n = 4) by preincubation with phorbol myristate acetate (PMA; 1 μM) for 24–36 h, time sufficient to down-regulate PKC (Nishizuka, 1995). Furthermore, acute addition of PMA (0.1 μM, n = 3) did not activate *trpl* currents, whereas histamine-induced currents were not different from time-matched controls after preincubation with staurosporine (an inhibitor of PKC, 1 μM, 10–20 min) and with 1 μM staurosporine in the pipette solution (n = 3). Raising the intracellular (i.e. pipette) Ca^{2+} concentration to 0.3 or 1 μM did not induce *trpl* currents (n = 17; not shown).

To evaluate the capability of $G_{q/11}$ proteins to stimulate *trpl*, we co-infected Sf9 cells with *trpl* and $G\alpha^*_{11}$, a constitutively active $G\alpha_{11}$ mutant lacking intrinsic GTPase activity (Figure 3A; De Vivo *et al.*, 1992). These cells exhibited currents significantly larger than control cells co-expressing *trpl* and one of the following: H_1R, constitutively active $G\alpha_{12}$, wild-type $G\alpha_{i1}$ or $G\beta\gamma$ (i.e. $G\beta_2\gamma_2$). Co-infection of *trpl* and wild-type $G\alpha_{11}$ yielded currents larger than these controls but significantly smaller than co-infection with *trpl* and $G\alpha^*_{11}$ (see Figure 3A). Furthermore, co-infection of *trpl* and a constitutively active mutant of $G\alpha_q$ (n = 6) resulted in currents 4.2-fold larger than in cells co-infected with *trpl* and a G_q not constitutively active (n = 6). Infection of Sf9 cells with a virus coding for $G\alpha^*_{11}$ did not increase ion currents in the absence of *trpl* expression. Heparin infused into cells co-infected with *trpl* and $G\alpha^*_{11}$ did not change the time course of *trpl* currents (Figure 3B and C).

To assess the unitary current amplitude of *trpl* channels, we performed a fluctuation analysis of whole cell currents during histamine stimulation (Neher and Steven, 1977). The single channel amplitude calculated from the slope of the variance/mean current plot was 7.14 ± 1.1 pA (at +60 mV, n = 6; Figure 4). Channels with such a current amplitude were observed in excised inside-out patches from Sf9 cells expressing *trpl* (n = 198), but were absent in all excised inside-out patches from non-infected cells (n = 49). In single channel recordings, *trpl* channels had a linear current–voltage relation with a slope conductance of 110 pS (in 120 mM Cs^+; Figure 5A and B) but 3.5-fold (n = 6) higher open probability at positive than at negative holding potentials (Figure 5C). Figure 5D shows the distribution of open times of all channel events in three experiments. The monoexponential fit of the histogram yields a calculated mean open time of 0.6 ms.

Addition of AlF_4^- to inside-out patches of *trpl*-expressing cells evoked an immediate marked increase in channel activity (n = 5 out of 21 patches; Figure 6A). GTPγS (500 μM) was ineffective over 2–3 min (n = 12; not shown). However, when histamine was present in the pipette solution, channel acitivity was induced by addition of GTPγS to the cytosolic side of inside-out patches excised from cells expressing H_1R as well as *trpl* (4 out of 11 patches; Figure 6B). In inside-out patches excised from cells co-infected with *trpl* and $G\alpha^*_{11}$, the activity of *trpl* channels was preserved for >5 min (Figure 6C),

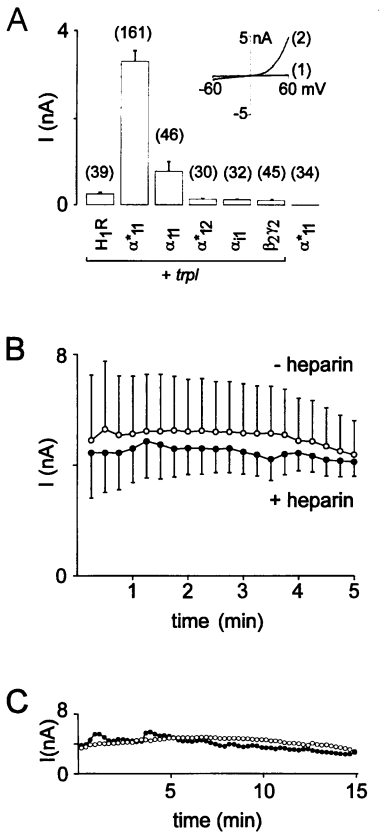

Fig. 3. Activation of *trpl* currents by Gα subunits in Sf9 cells. (**A**) Whole-cell currents (at 60 mV, ±SEM, for the numbers of cells indicated in parentheses, 30 s after break-in) in cells co-infected with *trpl* and either H_1R, constitutively active $G\alpha^*_{11}$ (α^*_{11}), wild-type $G\alpha_{11}$ (α_{11}), constitutively active $G\alpha^*_{12}$ (α^*_{12}), $G\alpha_{i1}$ (α_{i1}) or $G\beta_2\gamma_2$ ($\beta_2\gamma_2$). Additionally, cells were infected with a $G\alpha^*_{11}$-coding virus alone. The cells were infected for 24–40 h. Current values obtained after co-infection with viruses coding for *trpl* and $G\alpha^*_{11}$ were significantly different from all others ($P < 0.001$, Kruskal–Wallis one way ANOVA on ranks). No currents were detected in cells infected with $G\alpha^*_{11}$ alone. (Inset) I–V relation of the current in one cell infected with *trpl* and $G\alpha^*_{11}$ (2) and in one control cell (1), obtained during a voltage ramp. (**B**) Time course of mean currents in the presence (closed circles; $n = 6$) and absence (open circles; $n = 5$) of intracellular heparin (0.5 mg/ml heparin in the pipette) in cells co-infected with *trpl* and $G\alpha^*_{11}$. The pipette solution was I_2. The access resistance was <10 MΩ. (**C**) Whole cell currents over 15 min in two cells co-infected with *trpl* and $G\alpha^*_{11}$, one in the presence of intracellular heparin (closed circles) and one without intracellular heparin (open circles) at 60 mV. The access resistance was 5–8 MΩ. The pipette solution was I_2.

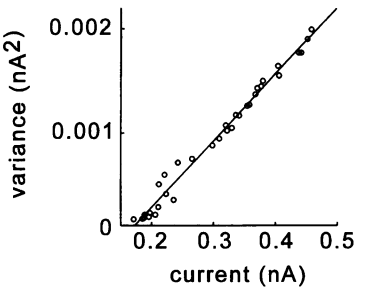

Fig. 4. Noise analysis of whole cell *trpl* currents: relation of current amplitude (at 60 mV) and current variance during activation with histamine (40 μM) in one cell expressing *trpl* and H_1R. The line is the linear regression line. The pipette solution was I_2.

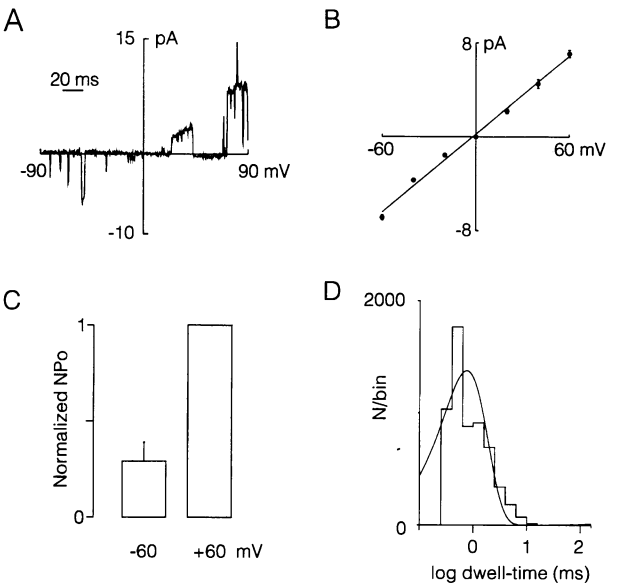

Fig. 5. Biophysical properties of *trpl* single channels. (**A** and **B**) Amplitude–voltage relation of *trpl* channels assessed from a single voltage ramp of –90 to 90 mV in a single patch (A) or from mean amplitudes at various holding potentials (B), where every point represents the mean from three to six experiments in which the amplitude at each holding potential was assessed from amplitude histograms. Data were filtered at 1 kHz. (**C**) Comparison of channel activity at –60 versus +60 mV. NP_o was normalized to the NP_o at 60 mV. NP_o values were calculated for each potential for 20 s periods. (**D**) Open time distribution. All channel events of three experiments were summed. The holding potential was 60 mV. Pipette and bath solutions were E and I_2 respectively. Data were filtered at 5 kHz and sampled at 10 kHz. The data were logarithmically binned and fitted to a monoexponential equation yielding a calculated mean open time of 0.6 ms ($n = 3$) (Sigworth and Sine, 1987). In this analysis we did not consider that channel openings typically occurred in bursts.

although cytosolic factors should be rapidly washed off under these conditions.

To obtain further evidence for a membrane-confined modulation of *trpl* channels, we partially purified $G\alpha^*_{11}$ from membranes of Sf9 cells expressing $G\alpha^*_{11}$. These preparations of isolated $G\alpha^*_{11}$ were confirmed to have G protein activity because they stimulated intracellular Ca^{2+} release after injection into RBL-2H3 cells (Harhammer *et al.*, 1996). Inside-out patches, excised from Sf9 cells expressing *trpl*, were exposed to solutions containing $G\alpha^*_{11}$ (20 nM). $G\alpha^*_{11}$-containing preparations activated *trpl* channels (Figure 7; $n = 8$ out of 32 patches). The success rate of experiments with $G\alpha^*_{11}$ was not different from that with AlF_4^- or GTPγS and histamine (see above). Several types of control experiments were performed to evaluate the specificity of $G\alpha^*_{11}$-induced *trpl* stimulation. Buffer with detergent alone (0.002% cholate) did not increase *trpl* channel activity ($n = 25$). Neither did membrane extracts from Sf9 cells that had been infected with a recombinant virus coding for a constitutively active $G\alpha^*_{12}$ mutant ($n = 11$). Likewise, Gβγ complexes (80 nM) purified from bovine brain subunits failed to activate *trpl* channels ($n = 20$), although the same protein preparation stimulated PLC β *in vivo* (Harhammer *et al.*, 1996). Furthermore, we did not observe an increase in channel activity by GTPγS-activated $G\alpha_{i1}$ (16 nM, $n = 23$). Finally, $InsP_3$ (3–10 μM) did not stimulate *trpl* channel activity in any inside-out patches ($n = 19$).

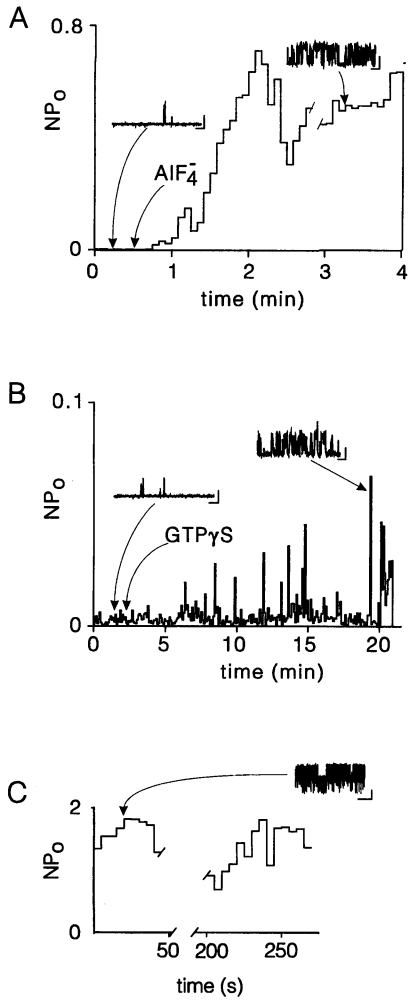

Fig. 6. Regulation of *trpl* channels in inside-out patches. (**A** and **B**) Channel activity was recorded in inside-out patches from Sf9 cells infected with *trpl*, before and after addition of AlF_4^- (30 μM $AlCl_3$ and 10 mM NaF; A) or GTPγS (500 μM, the pipette solution was E with 5 μM histamine; B). The holding potential was 60 mV. Inserts show single channel recordings at various times. The calibration bars denote 20 ms and 5 pA. (**C**) Time course of single channel activity (expressed as NP_o) of one inside-out patch from a cell co-infected with *trpl* and $G\alpha^*_{11}$. The insert illustrates a sample tracing from this patch at time 20 s. Channel activity was preserved over more than 5 min in all patches studied under the same conditions (n = 8). Solutions I_2 and E were used as bath and pipette solutions respectively. The calibration bars denote 1 s and 5 pA.

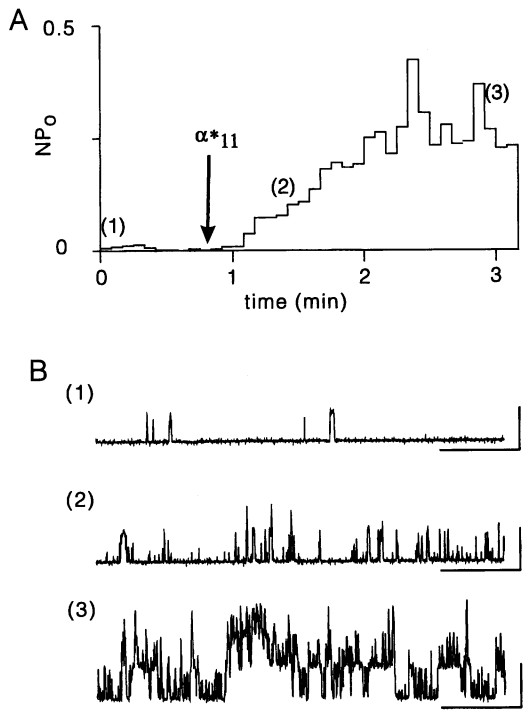

Fig. 7. Effect of isolated $G\alpha^*_{11}$ subunits on *trpl* channels. (**A**) Channel activity (expressed as NP_o) in an inside-out patch before and after exposure to an enriched $G\alpha^*_{11}$ preparation. Exposure to $G\alpha^*_{11}$ was started 46 s after excision of the patch and maintained. (**B**) Channel recordings at various time points. Calibration bars denote 100 ms and 10 pA. The holding potential was 60 mV. The patch was from a Sf9 cell infected with *trpl*. Solutions I_2 and E were used as bath and pipette solutions respectively.

Discussion

In the present study, we demonstrate that α subunits of the G protein G_{11} activate *trpl* channels expressed in Sf9 cells. The effect of $G\alpha_{11}$, a member of the G_q subfamily, does not require cytosolic factors but occurs in a membrane-confined way.

In a recent study, *trpl* currents were stimulated by $InsP_3$ and inhibited by heparin (Dong *et al.*, 1995). We cannot confirm these findings. We infused $InsP_3$ into the cytosol by dialysis of the cells with a pipette solution containing 10 μM $InsP_3$. From the size of the cells and the obtained access resistance, a rapid increase in the $InsP_3$ concentration in the cytosol would be expected, reaching 1–3 μM within 10–30 s when the pipette contains 10 μM $InsP_3$ (Pusch and Neher, 1988). However, $InsP_3$ infusion did not induce *trpl* currents within 3 min. After this time, spontaneous activation of *trpl* currents may occur, as we experienced in many control experiments. Therefore, we cannot exclude a delayed effect of $InsP_3$. Conversely, histamine rapidly activated *trpl* in the presence of $InsP_3$, as well as in the presence of heparin. Moreover, addition of $InsP_3$ to inside-out patches with *trpl* channels consistently failed to enhance channel activity. Hence, we conclude that stimulation of *trpl* by the histamine H_1 receptor does not require $InsP_3$. Activation of PKC or changes in $[Ca^{2+}]_i$ are not required either, although modulations of channel activity by these signals may well occur.

Channel activity in inside-out patches was stimulated by AlF_4^-, an activator of G proteins. GTPγS, in contrast, was effective only when histamine receptors were stimulated at the same time. These observations led us to suppose that activation of the *trpl* channel involves a G protein with a slow basal nucleotide exchange rate, such as a member of the $G_{q/11}$ familiy. Co-infection of *trpl* and $G\alpha^*_{11}$ induced significantly elevated currents in comparison with various controls. Remarkably, high levels of channel activity were preserved over long times in patches from cells co-infected with *trpl* and $G\alpha^*_{11}$. This finding makes it highly unlikely that soluble signals downstream of PLC β, accumulating in Sf9 cells during co-infection with *trpl* and $G\alpha^*_{11}$, maintain the high channel activity. The finding rather points to a membrane-confined action of $G\alpha^*_{11}$. To test this possibility in the most rigorous way presently possible, we exposed inside-out patches to $G\alpha^*_{11}$ isolated from membranes of Sf9 cell overexpressing recombinant $G\alpha^*_{11}$. Indeed, we observed a marked stimu-

lation of channel activity (see Figure 7). This effect was specific for $G\alpha^*_{11}$ and not mimicked by α subunits of other G proteins or by Gβγ complexes. The relatively low success rate in inside-out patches may indicate methodological problems. It may be difficult to deliver intact $G\alpha^*_{11}$ proteins to the putative site of interaction with *trpl*, presumably at or within the membrane. The geometry of inside-out patches in the pipette is uncertain, but probably highly variable. Conceivably, aqueous films around the patch and/or regions of high surface charges may constitute barriers not easily penetrated by proteins. We cannot exclude, however, that the stimulation of *trpl* by $G\alpha^*_{11}$ requires additional components present in only a minority of patches. This possibility may be supported by the similarly low success rate in inside-out patches stimulated with GTPγS or AlF_4^-. In conclusion, we demonstrate that the components involved in *trpl* channel activation by $G\alpha^*_{11}$ may be confined in inside-out patches. No signal pathways downstream of PLC β are required. Thus, we provide evidence for a membrane-confined action of $G\alpha^*_{11}$ on *trpl* channels, possibly involving a direct physical interaction with the channel protein.

These findings may have important implications for our understanding of how G proteins control ion flux and Ca^{2+} homeostasis. *trpl* is a member of the *trp* gene family that includes a growing number of mammalian homologs (Petersen *et al.*, 1995; Wes *et al.*, 1995; Zhu *et al.*, 1995, 1996; Clapham 1996; Zitt *et al.*, 1996). Members of this gene family may code for a wide range of cation channels thought to be regulated by a store-operated mechanism (Berridge, 1995, Clapham, 1995a, 1996). Store-operated activation has been shown for some of the human *trp* homologs (Zhu *et al.*, 1996; Zitt *et al.*, 1996). However, *trpl* is not controlled by the filling state of Ca^{2+} stores (Vaca *et al.*, 1994). The present study shows that *trpl* is directly stimulated by $G\alpha_{11}$ subunits. Possibly, more members of the *trp* family will be identified that share the regulatory mechanisms of *trpl*, in distinction to store-operated members of the family.

Furthermore, this study adds another ion channel to the group of G protein-regulated channels that includes voltage-gated calcium channels and inwardly rectifying potassium channels. All members are supposed to be modulated by Gβγ as well as Gα subunits (Wickman and Clapham, 1995; Herlitze *et al.*, 1996; Ikeda, 1996; Schreibmayer *et al.*, 1996). *trpl* is the first channel demonstrated to be modulated by a member of the G_q subfamily. Whereas most G proteins regulate multiple effectors, $G_{q/11}$ proteins were previously only shown to activate PLC β. We now provide evidence that G_{11} and probably G_q control another cellular effector. Thus, the ubiquitously expressed G_q family may have a general role in the regulation of cellular Ca^{2+} homeostasis by stimulating two different effector systems, PLC β as well as Ca^{2+}-permeable channels.

Materials and methods

Transient gene expression in Sf9 cells

Sf9 cells were cultured in TNM-FH medium (Sigma, Deisenhofen, Germany) supplemented with 10% (v/v) fetal calf serum and Lipid Concentrate (1:100; Life Technologies, Karlsruhe, Germany) at 27°C. Sf9 cells were grown in suspension. Thirty to forty eight hours before electrophysiological measurements, the cells were seeded at a density of ~5×10^4 cell/cm^2 on glass slides and infected with recombinant baculoviruses (multiplicity of infection 3–5). For construction of baculoviruses encoding $G\alpha_q$, $G\alpha_{11}$, constitutively active $G\alpha_q$, constitutively active $G\alpha_{11}$ ($G\alpha^*_{11}$) and constitutively active $G\alpha_{12}$ ($G\alpha^*_{12}$), we obtained cDNAs from M.Simon and cloned them into the baculovirus transfer vectors pVL1392 and pVL1393. For $G\alpha^*_{11}$ viruses, the sequence had been changed by site-directed mutagenesis, yielding an indicator *Bsu*36I restriction site and a mutated protein sequence (Gln206→Leu). For further details see Harteneck *et al.* (1995).

Photolabeling and immunoprecipitation of Gα subunits

Sf9 cells were infected with viruses encoding guinea pig histamine H_1 receptor for 28 h. Membranes were isolated and harvested by centrifugation at 2500 *g* for 15 min (4°C). Cells were resuspended and subjected to nitrogen cavitation (28 bar, 40 min). The homogenate was centrifuged at 700 *g* for 2 min (4°C) to remove nuclei and intact cells. Membranes were obtained by centrifugation at 80 000 *g* for 30 min (4°C) and stored at –80°C. Photolabeling and immunoprecipitation of receptor-infected Sf9 membrane proteins (200 μg protein) with [α-^{32}P]GTP azidoanilide were performed without and after stimulation with histamine (100 μM) according to published procedures (Laugwitz *et al.*, 1994). For optimal activation of the different G protein isoforms, membranes were incubated (30°C) over 30 min for G_{12}, 10 min for G_s and $G_{q/11}$ or 3 min for G_i with [α-^{32}P]GTP azidoanilide (4×10^6 c.p.m./tube). After addition of glutathione (final concentration 2 mM), proteins were irradiated for 15 s with a 254 nm UV lamp. After photolysis, solubilized membranes were incubated overnight with specific antisera AS 348 (anti-α_s), AS 369 (anti-$\alpha_{q/11}$), AS 266 (anti-$\alpha_{i\ common}$) and AS 232 (anti-α_{12}). Immunocomplexes were pelleted by addition of protein A–Sepharose beads and subjected to SDS–PAGE. Dried gels were analyzed with a BAS 1500 Fuji-Imager (Raytest, Straubenhardt, Germany).

Electrophysiological recording

The patch–clamp technique in the whole cell and inside-out configuration (Hamill *et al.*, 1981) was used to study currents through *trpl* channels. In whole cell experiments, the access resistance was <10 MΩ, series resistance compensation was set to 70–85%. The bath solution (solution E) contained: 90 mM NaCl, 10 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 70 mM sucrose, 10 mM glucose, 10 mM PIPES, pH 6.5. Pipette solutions contained: 120 mM CsCl, 1 mM $MgCl_2$, 0.5 mM EGTA, 30 mM glucose, 10 mM PIPES, pH 6.5 (solution I_1); or 120 mM CsCl, 1 mM $MgCl_2$, 10 mM EGTA, 30 mM glucose, 10 mM PIPES, pH 6.5 (solution I_2). The osmolality of all solutions was adjusted to 340–350 mosmol/l with mannitol. The bath solutions of all experiments were supplemented with the chloride channel blocker 5-nitro-2-(3-phenylpropyl-amino)benzoic acid (NPPB; 10 μM). For inhibition of $InsP_3$-mediated effects, low molecular weight heparin (mol. wt 3000; Sigma) was used.

Channel activity is expressed as NP_o, the product of the number (N) of channels and the open probability (P_o). NP_o values were calculated for consecutive 5 s periods after filtering at 1 kHz. An increase in channel activity was defined as a >2.5-fold increase in NP_o (calculated with the pCLAMP 6.0 program) over 1 min, in comparison with the time period of 2 min preceding application of a stimulus. Since activity appeared in bursts in some experiments, an increase in activity was also assumed if NP_o in at least two periods of 5–10 s within 1 min was at least four times higher than the maximal NP_o during the 2 min preceding application of a stimulus. All experiments were performed at room temperature.

Noise analysis

Currents were filtered at 1 kHz and records of 1 s were sampled at 10 kHz. The mean and variance of the whole cell current was computed using Puls 7.4 software (HEKA Electronic, Lambrecht, Germany). No series resistance compensation was used.

Purification of G proteins

$G\alpha^*_{11}$ was expressed in Sf9 cells and partially purified by DEAE–Sepharose anion exchange chromatography and heptylamine–Sepharose hydrophobic interaction chromatography. Sf9 cells (1.6 l, 1×10^6 cells/ml) were infected with viruses encoding $G\alpha^*_{11}$ for 43 h. Cells were harvested, resuspended in buffer A (20 mM Tris–HCl, pH 8.0, 1 mM EDTA, 20 mM β-mercaptoethanol, 3 mM $MgCl_2$, 100 μM GTP, 20 μg/ml phenylmethylsulfonyl fluoride, 20 μg/ml Nα-*p*-tosyl-L-lysine chloromethyl ketone, 20 μg/ml soybean trypsin inhibitor) and lysed by nitrogen cavitation (25 bar, 40 min). After removal of nuclei, cell membranes were prepared, resuspended in buffer A and solubilized with

sodium cholate (1% final concentration, w/v) for 1 h at 4°C. After centrifugation (200 000 *g* for 1 h), the clear supernatant was mixed with ethylene glycol to a final concentration of 30% (v/v) and applied to a 20 ml column of DEAE–Sepharose Fast Flow (Pharmacia, Freiburg, Germany). $G\alpha^*_{11}$-containing fractions eluting at NaCl concentrations between 300 and 450 mM were diluted in buffer A to reduce the cholate concentration to 0.2% and loaded onto a heptylamine–Sepharose column (15 ml). The column was washed and proteins were eluted with a reciprocal gradient of cholate (0.2–3%) and NaCl (500–0 mM). Fractions enriched with $G\alpha^*_{11}$ eluting at cholate concentrations between 2.5 and 3% were diluted to a cholate concentration of 0.9%, concentrated through an Amicon PM 10 filter and stored at –80°C until use. The final concentration of $G\alpha^*_{11}$ was 100 nM, as estimated from a silver stained SDS gel. Constitutively activate $G\alpha_{12}$ was extracted from membranes of baculovirus-infected Sf9 cells with 1% cholate. $G\beta\gamma$ dimers were purified as described (Nürnberg *et al.*, 1994). Before experiments, buffers were changed to solution I_2 with a final concentration of 0.002% cholate.

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