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ATP-operated calcium-permeable channels activated via a guanine nucleotide-dependent mechanism in rat macrophages

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1. To elucidate the possible involvement of a G protein in ATP-evoked Ca^{2+}-permeable channel activity, membrane currents of rat peritoneal macrophages were recorded using inside-out and cell-attached configurations of the patch clamp technique.

2. In inside-out experiments with a pipette solution containing 105 mM Ba^{2+}, application of 100 μM GTP or GTPγS to the internal surface of the membrane elicited a rise in channel activity. This effect was observed in 49% of the patches investigated ($n = 69$). The mean value of NP_o (N, number of open channels; P_o, channel open probability) was equal to 0·49 ± 0·27 (mean ± S.E.M.; $n = 16$). The delay in the activity development was 21 ± 8 s ($n = 18$) with 200 μM ATP added to the pipette solution and about 4 min ($n = 5$) without agonist in the pipette. Similar results were obtained with 10 mM Ca^{2+} as the only permeant cation.

3. Properties of GTPγS-evoked channels were identical to those of channels activated by extracellular application of ATP. The channels exhibited at least four conductance sublevels, the 4th one being the least frequent. With 105 mM Ba^{2+} as a permeant cation, sublevel conductances were 3·5, 7, 10 and 15 pS. Corresponding values for 10 mM Ca^{2+} were about 4, 9, 13 and 17 pS. Extrapolated reversal potential (E_r) values were about +40 and +25 mV for Ba^{2+} and Ca^{2+}, respectively.

4. The activity of channels with similar characteristics could be induced by the extracellular application of fluoride in cell-attached experiments without any agonist in the pipette solution.

5. Currents through non-selective cationic channels with properties much different from ATP-activated GTP-dependent Ca^{2+}-permeable channels were recorded with GTPγS added to the intracellular solution (inside-out experiments) and fluoride applied to the bath solution (cell-attached conditions). With isomolar concentrations of Ba^{2+} used extracellularly and K^+ intracellularly, the E_r appeared to be about 0 mV. These channels did not show determinable current sublevels. They were observed rarely (6 out of more than 100 experiments) and could not be attributed to activation by an agonist.

6. The data obtained suggest that an ATP-activated receptor is coupled with a Ca^{2+}-permeable channel via a GTP-dependent mechanism, presumably via a G protein.

Transient elevation of the free Ca^{2+} concentration in the cytosol is one of the first reactions of many types of cells to stimulation with hormones and growth factors. This elevation can be due either to Ca^{2+} release from intracellular stores or to Ca^{2+} influx from the external medium across the plasma membrane, or both. Though the mechanism underlying the Ca^{2+} release itself is fairly clear, data on Ca^{2+} entry through the plasma membrane are scarce. Results of tracer flux and Ca^{2+}-sensitive dye experiments suggest that the plasma membrane of many cells contains receptor-operated Ca^{2+}-permeable channels different from the well-known voltage-gated channels. In a number of studies, functional properties of receptor-operated Ca^{2+}-permeable channels in non-excitable cells have been investigated at the single channel level (Kuno, Goronzy, Weyand & Gardner, 1986; Kuno & Gardner, 1987; Benham & Tsien, 1987; Matsunaga, Nishimoto, Kojima, Yamashita, Kurokawa & Ogata, 1988; Mozhayeva, Naumov & Kuryshev,

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1989, 1991; Sasaki & Gallacher, 1990; Mahaut-Smith, Sage & Rink, 1990; Neuhaus, Reber & Reuter, 1991; Yamamoto, Chen, Miwa & Suzuki, 1992; Peppelenbosch, Tertoolen, den Hertog & de Laat, 1992).

Direct activation of ionic channels in excised patches suggests a direct coupling of agonist binding and channel opening, without the involvement of some readily diffusible messenger. Evidence has begun to accumulate suggesting activation of G protein-operated channels following the binding of an agonist to the receptors (Pfaffinger, Martin, Hunter, Nathanson & Hille, 1985; Breitwieser & Szabo, 1985; Kurachi, Nakajima & Sugimoto, 1986; Yatani, Codina, Brown & Birnbaumer, 1987*a*; Mozhayeva *et al.* 1991; Naumov, Kuryshev & Mozhayeva, 1993; Kuryshev, Naumov, Avdonin & Mozhayeva, 1993). The data on the possible involvement of G proteins in ATP-evoked activation of membrane currents is conflicting. Rat parotid acinar cells (McMillan, Soltoff, Lechleiter, Cantley & Talamo, 1988) respond normally to the application of ATP and not to carbachol, when the patch pipette is filled with GDPβS. In experiments in atrial cells under whole-cell conditions, the ATP-elicited conductance is not affected by the omission of GTP or by its replacement by GTPγS in the pipette solution (Friel & Bean, 1988). However, in lacrimal acinar cells (Sasaki & Gallacher, 1992) GTP enhances both the inward and outward current components of the ATP-evoked response. GTP increases the duration and reproducibility of the inward currents, while GTPγS produces even greater potentiation of the current amplitude and duration. The potentiation of the ATP-induced inward current responses by GTP and GTPγS suggests that G proteins couple an ATP receptor to cationic channels in the lacrimal acinar cell membrane.

In the present study, we have investigated the possible involvement of G proteins in the control of the ATP-activated pathway for Ca^{2+} influx in the plasma membrane of rat peritoneal macrophages. Channel activity was evoked when the non-hydrolyzable analogue of GTP (GTPγS) was applied to the internal surface of the membrane in inside-out experiments. The properties of these channels proved to be identical to those observed in cell-attached experiments in which channel activity was elicited by the addition of ATP to the external surface of the membrane (Naumov, Kaznacheyeva, Kiselyov, Kuryshev, Mamin & Mozhayeva, 1995). The data obtained are consistent with the supposition that G proteins are involved in signal transduction from the ATP receptor to the Ca^{2+}-permeable channel.

METHODS

Cells

Peritoneal resident macrophages from male Wistar rats (5–10 weeks old) were isolated and cultured essentially as described previously (Naumov *et al.* 1995). Rats were stunned and killed by cervical dislocation. A cell suspension from the peritoneal cavity of the animals was placed into plastic dishes containing 5 × 5 mm coverslip pieces. The cells on the coverslips were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma) supplemented with 10% fetal bovine serum, 100 units ml^{-1} penicillin and 100 mg ml^{-1} streptomycin in a 5% CO_2 humidified atmosphere for 1–10 days at 37 °C. The cultured macrophages were used in patch clamp experiments 2–10 days after plating. Cells were identified as macrophages using α-naphthyl acetate esterase staining (Monahan, Dvorak & Dvorak, 1981). At least 98% of cells in the monolayers were identified as macrophages. The identification was carried out in parallel with the patch experiments.

Electrical recording

Ionic currents were studied in cell-attached and inside-out configurations of the patch clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981).

The current signal was measured using a patch clamp amplifier with a conventional resistive feedback of 20 GΩ in the headstage, recorded at a bandwidth of 1·2 or 2·5 kHz using an analog FM recorder (EAM 340, Tesla, Czechoslovakia) and stored on magnetic tape. Data filtered using a 6-pole low-pass Bessel filter at a cut-off frequency of 0·6 kHz were digitized at 1 kHz and analysed off-line with an IBM-compatible computer using home-made software for standard single channel analysis (digital filtering, current amplitude histogram construction, open/closed time distribution and histogram fitting).

Measurements of the unitary current amplitudes were performed using either of two methods. In the first method, the difference between the averaged current amplitude during long channel opening and the baseline was considered to be the unitary current for that opening. In the second method, the unitary current was determined from the current amplitude distribution. Events longer than 1 ms were chosen for the construction of amplitude histograms. Data are given as means ± S.E.M. (number of experiments).

Experiments were carried out at room temperature (23–24 °C).

Solutions

In cell-attached experiments, after the gigaseal formation, the bath solution (comprising (mM): 145 KCl, 2 $CaCl_2$, 5 $Ca(OH)_2$–Hepes (pH 7·4)) was used to nullify the resting potential of the extrapatch membrane. The cytosol-like solutions for inside-out experiments contained (mM): 120 KCl (or potassium glutamate or potassium aspartate), 10 Hepes–KOH (pH 7·3), 10 EGTA–KOH, 0·2 $CaCl_2$ (free Ca^{2+} concentration $< 10^{-8}$ M), and 1 $MgCl_2$. Ca^{2+} concentrations were calculated using the stability constants taken from Fabiato & Fabiato (1979). The extracellular pipette solutions (cell-attached and inside-out experiments) contained 105 mM Ba^{2+} or 10 mM Ca^{2+}.

The isomolarity of the solutions containing Ca^{2+} was maintained by the addition of $Tris^+$. Calculation of the free ATP (ATP^{4-}) concentration was performed according to Fabiato & Fabiato (1979). To apply fluoride to the cell in cell-attached experiments the solution used differed from the control in that 10 mM KCl was replaced with 10 mM potassium fluoride. Prior to the experiments, the cells were sustained in a solution containing 50 μM $AlCl_3$. Small portions of the solutions containing ATP or GTPγS were kept frozen and thawed just before the experiment. Drugs were applied to cells or patches either by bath perfusion or by brief pressure ejection. In both cases, the duration of a complete change of the solution around the cell was < 300 ms.

ATP, GTPγS and Hepes were obtained from Sigma; EGTA was obtained from Fluka Chemie AG (Buchs, Switzerland).

RESULTS

Effect of GTP and GTPγS: inside-out experiments

Channel activity in cell-attached experiments has been observed when ATP is added to the pipette solution (Naumov *et al.* 1995). The activity lasted for 30–40 s and then disappeared almost completely, probably due to desensitization of the ATP receptor. Channel activity survived only briefly after the patch excision irrespective of its original value, and only a small number of pulse-like, inwardly directed events were recorded.

Figure 1 shows a representative experiment on an excised patch. The pipette solution contained 105 mM Ba^{2+} and 200 μM ATP. After the patch excision the current events were infrequent and short-term and were greatly augmented by the addition of GTP (100 μM). Washout of GTP resulted in the complete disappearance of the inward currents. The subsequent addition of GTPγS (100 μM) caused a resumption of the activity with a 25 s delay. The delay averaged 21 ± 8 s ($n = 18$). In experiments without agonist in the pipette solution the same results were

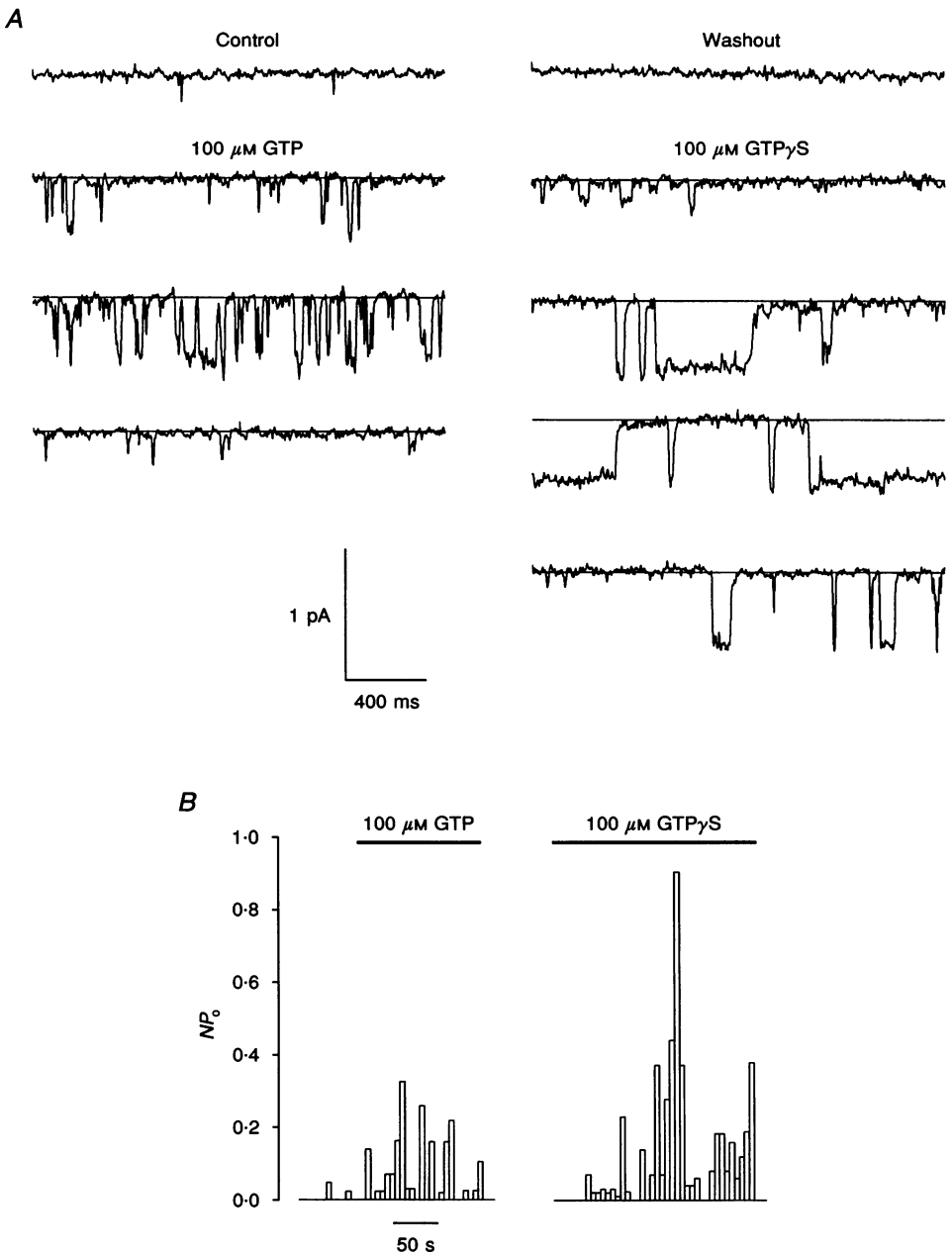

Figure 1. Inside-out single channel activity in the presence of GTP and GTPγS

A, single channel currents activated by GTP (100 μM; left traces) and GTPγS (100 μM; right traces). The pipette solution contained 105 mM Ba^{2+} and 200 μM ATP. Holding potential, −30 mV. *B*, plot of the time course of the channel activity measured in values of NP_o ($NP_o = I/i$, where I and i are the mean channel current and unitary current amplitude, respectively).

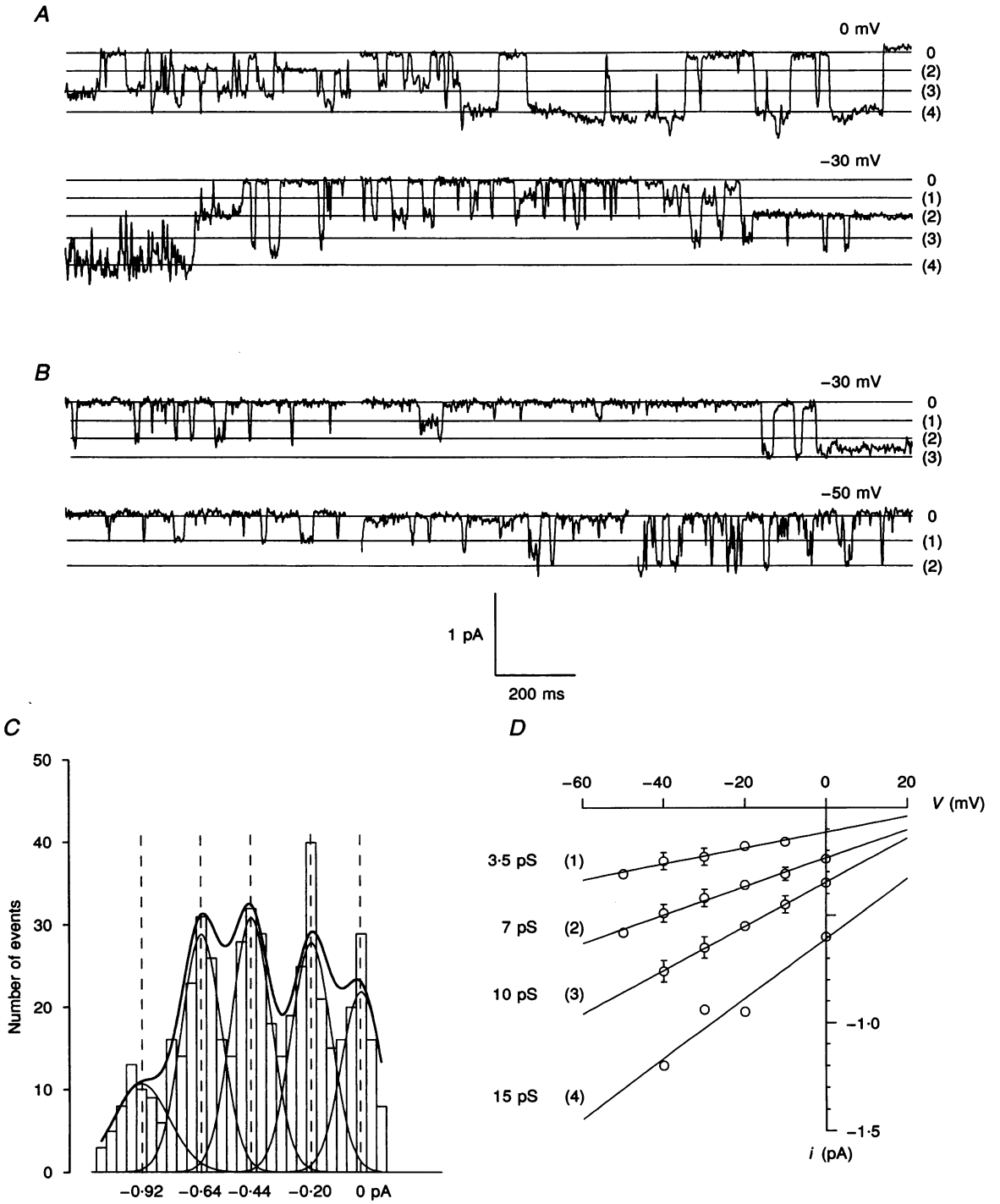

Figure 2. Current records obtained from two inside-out patches after the addition of GTPγS

A and *B*, current traces at different holding potentials shown on an expanded time scale (the experiment in *B* is that shown in Fig. 1). The pipette solution contained 105 mM Ba^{2+} and 200 μM ATP. Holding potential values are indicated above the traces. Horizontal lines pass at current sublevels indicated by the numbers in parentheses. *C*, amplitude histogram showing the current distribution, revealing the existence of current sublevels. The histogram was constructed at heavy filtration (50 Hz) from a 500 ms fragment of the current trace; bin width, 0·04 pA. The histogram was fitted by the sum of five Gaussian distributions with mean amplitudes of 0·20, 0·44, 0·64 and 0·92 pA. *D*, plot of mean values of current sublevels *vs.* membrane potential in experiments with 105 mM Ba^{2+} in the external solution. Each point corresponds to 1 experiment except those with error bars (± S.E.M.), which are the mean of 5 experiments. Numbers in parentheses correspond to the different current sublevels.

obtained but the delay in the appearance of the activity increased by up to 4 min ($n = 5$). The difference in the delay is consistent with prolonged coupling of GTPγS to the effector when the receptor is not occupied with an agonist (see Yatani, Codina, Imoto, Reeves, Birnbaumer & Brown, 1987*b*).

The current amplitudes did not change when glutamate was replaced by chloride in the internal solution. The positive

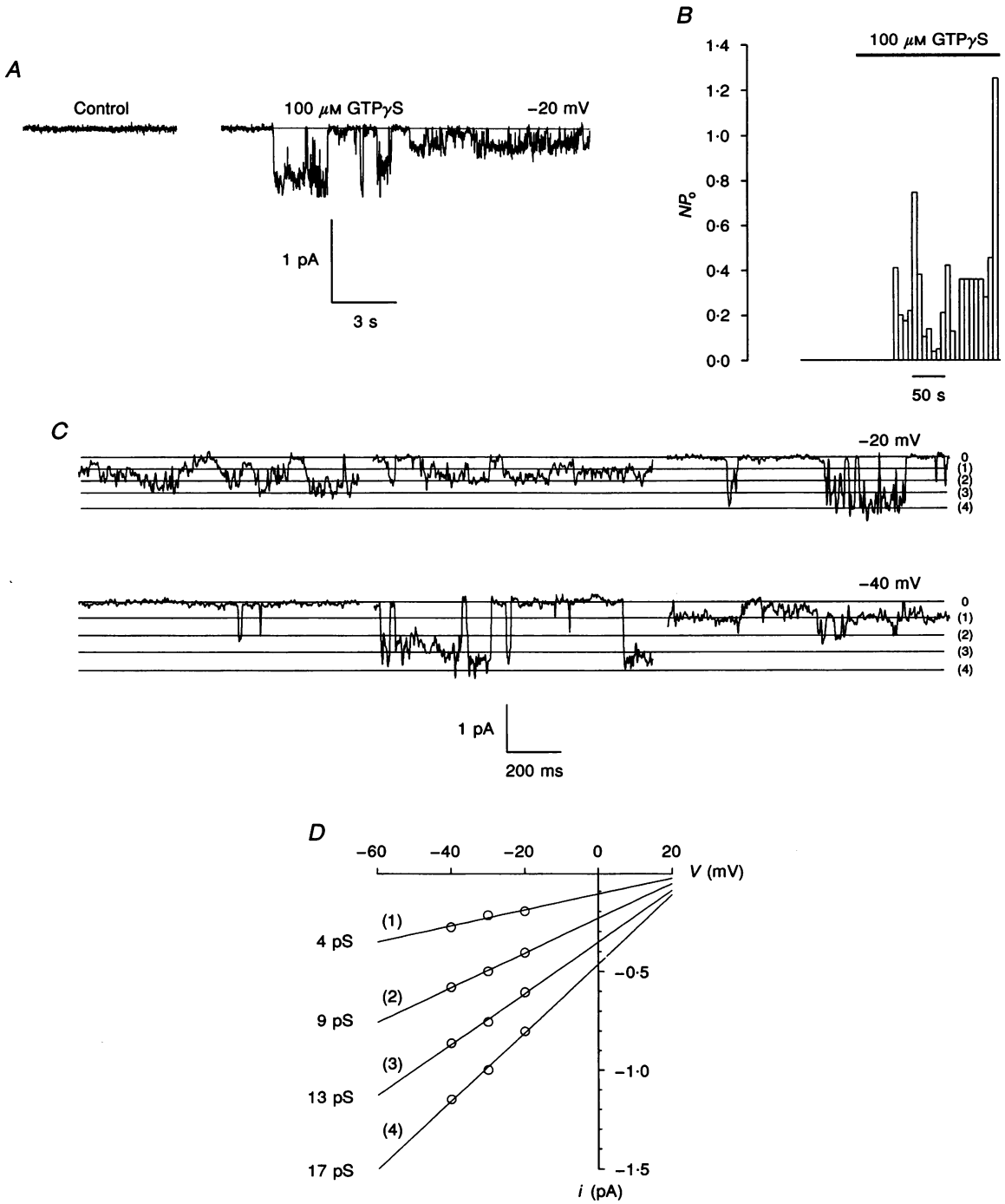

Figure 3. Channel activity in the excised patch after the addition of GTPγS to the internal solution

The pipette solution contained 10 mM Ca^{2+} and 50 μM ATP. *A*, current traces before and after the addition of GTPγS (100 μM). Holding potential, −20 mV. *B*, time course of the development of channel activity measured as values of NP_o. *C*, current traces recorded at different holding potentials shown on an expanded time scale. Holding potential values are indicated above the traces. Horizontal lines pass at current sublevels indicated by the numbers in parentheses. *D*, associated current–voltage relation. Numbers in parentheses correspond to the different current sublevels.

value of the extrapolated reversal potential (E_r; Fig. 2*D*) under these conditions indicates that the inward current was carried from the pipette solution by Ba^{2+}.

Channel activity was estimated as NP_o, where N is the number of open channels and P_o is the channel open probability. NP_o was determined from the following equation:

$$NP_o = I/i,$$

where I and i are the mean channel current and unitary current amplitude, respectively. I was estimated as the time integral of the patch current above the baseline and i was determined from current records. In most cases the NP_o values were calculated over a 5 s interval. The time course of the channel activity measured in values of NP_o after the addition of GTP and GTPγS is shown in Fig. 1*B*. When calculating NP_o, the value of i was determined to be −0·7 pA (see Fig. 2*D*). Similar results were obtained in twenty-nine out of fifty-nine experiments. The mean value of NP_o at the membrane potential of −30 mV was equal to 0·49 ± 0·27 ($n = 16$).

Current records from two excised patches after the addition of GTPγS are shown on an expanded time scale in Fig. 2. Figure 2*B* shows results from the same experiment shown in Fig. 1. It can be seen that the current amplitude at a given membrane potential is not constant; some current sublevels can be distinctly observed during the relatively long openings.

To support our visual measurements all-points amplitude histograms were constructed from 500 ms record traces and analysed statistically. An example of a histogram is shown in Fig. 2*C*. It was well fitted by the sum of five Gaussian distributions with close parameters and mean amplitudes of 0·20, 0·44, 0·64 and 0·92 pA, which are very close to values estimated visually.

Figure 2*D* shows mean sublevel values obtained in five inside-out experiments in which channel activity was induced by the application of GTPγS to the internal side of the membrane. In some patches two or three sublevel states only could be identified, whereas the others showed four sublevels.

Figure 3*A* and *B* illustrates the activation of channels by GTPγS in the inside-out experiment in which the pipette solution contained 10 mM Ca^{2+} and 50 μM ATP. Current recordings from the same experiment shown on an expanded time scale (Fig. 3*C*) enabled at least four subconductance levels expressed by GTPγS-activated channels to be seen, similar to Ba^{2+}-permeable channels. The plot of the current–voltage relation for current sublevels at different potentials is shown in Fig. 3*D*. Similar results were obtained in three additional experiments.

The properties of GTPγS-induced channels are similar to those of ATP-activated channels in cell-attached and outside-out configurations (Naumov *et al.* 1995) recorded under the same ionic conditions. As with ATP-activated channels, the channels recorded in excised patches have four current sublevels with conductances of 3·5, 7, 10 and 15 pS and extrapolated E_r values of about +40 mV, when Ba^{2+} was the only permeant cation. The 4th conductance level has the lowest probability; it was not detected in all

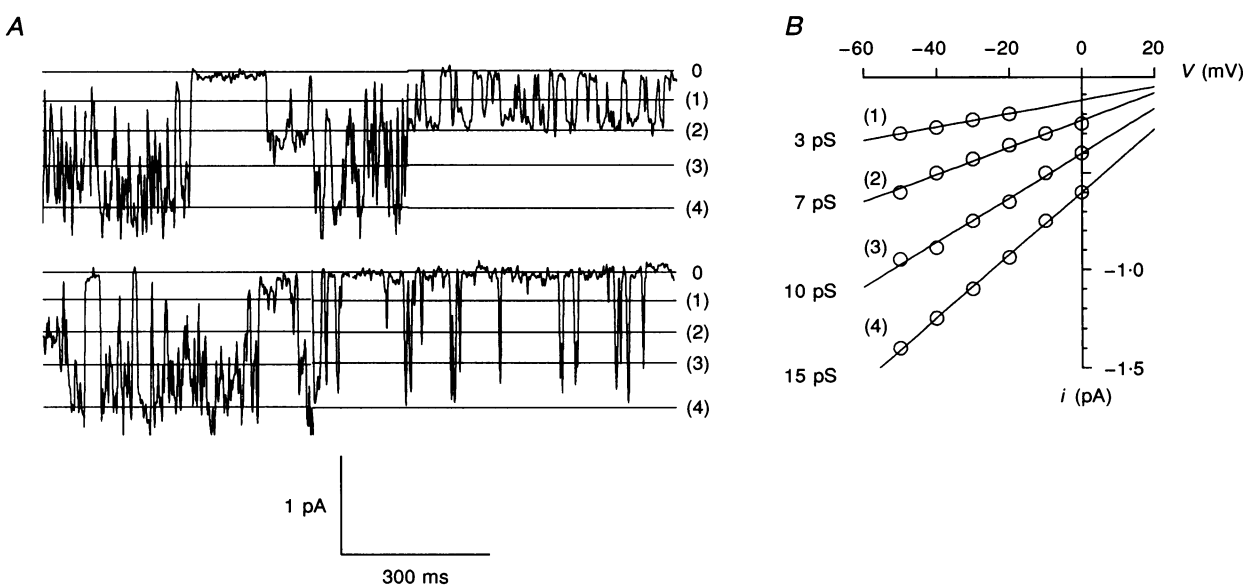

Figure 4. Cell-attached single channel activity recorded 1 min after the addition of fluoride (10 mM) to the bath solution

The pipette solution contained 105 mM Ba^{2+}, without agonist. *A*, current traces recorded at a holding potential of −50 mV. Different current sublevels are indicated by the numbers in parentheses. *B*, associated current–voltage relation for the different current sublevels.

patches and at all potentials. With 10 mM Ca^{2+} as the charge carrier, the values of the sublevel conductances were 4, 9, 13 and 17 pS and the extrapolated E_r was +25 mV. Thus, GTP and its non-hydrolyzable analogue, GTPγS, may increase the activity of the same channels as those activated by the extracellular application of ATP in cell-attached and outside-out configurations.

Effect of fluoride: cell-attached experiments

The aluminium fluoride complex (AlF_4^-) is a universal activator of heterotrimeric G proteins (Gilman, 1987; Bigay, Deterre, Pfister & Chabre, 1987). It activates a number of G protein-dependent responses, including the Ca^{2+} signal, in different cell systems (Bokoch & Gilman, 1984; Graier, Schmidt & Kukovetz, 1990; Woods, Dixon, Cuthbertson & Cobbold, 1990; Siffert, Jakobs & Akkerman, 1990). Figure 4 shows a cell-attached experiment performed with a pipette solution containing 105 mM Ba^{2+} without agonist present. No channel activity was observed for 3 min when $AlCl_3$ was present in the control solution (see Methods). After 10 mM potassium fluoride was added to the solution, channel activity appeared with a delay of approximately 1 min. The delay in the appearance of the effect seems to depend on the time taken for the fluoride ions to enter the cell and reach the internal side of the patch membrane. The conductance characteristics of fluoride-induced channels are similar to those of channels recorded after ATP and/or GTPγS application to the outside and inside surfaces of the membrane, respectively. Similar results were obtained in four experiments.

Non-selective cationic channels

In six out of more than 100 experiments, activity of another type of channel was observed. Figure 5 shows channel recordings obtained in one such experiment performed on an inside-out patch with 105 mM Ba^{2+} in the pipette solution and 145 mM K^+ in the bath solution (glutamate was the main anion). The properties of the channels differed strongly from those of the channels described above: the E_r of the currents was about 0 mV, the unitary conductance was estimated to be about 15 pS and the open time was significantly greater. No current sublevels were revealed in this channel. Replacement of the anion (glutamate by chloride) in the bath solution had no influence on the current amplitude, indicating the cationic nature of the channel with relatively equal permeability to Ba^{2+} and K^+.

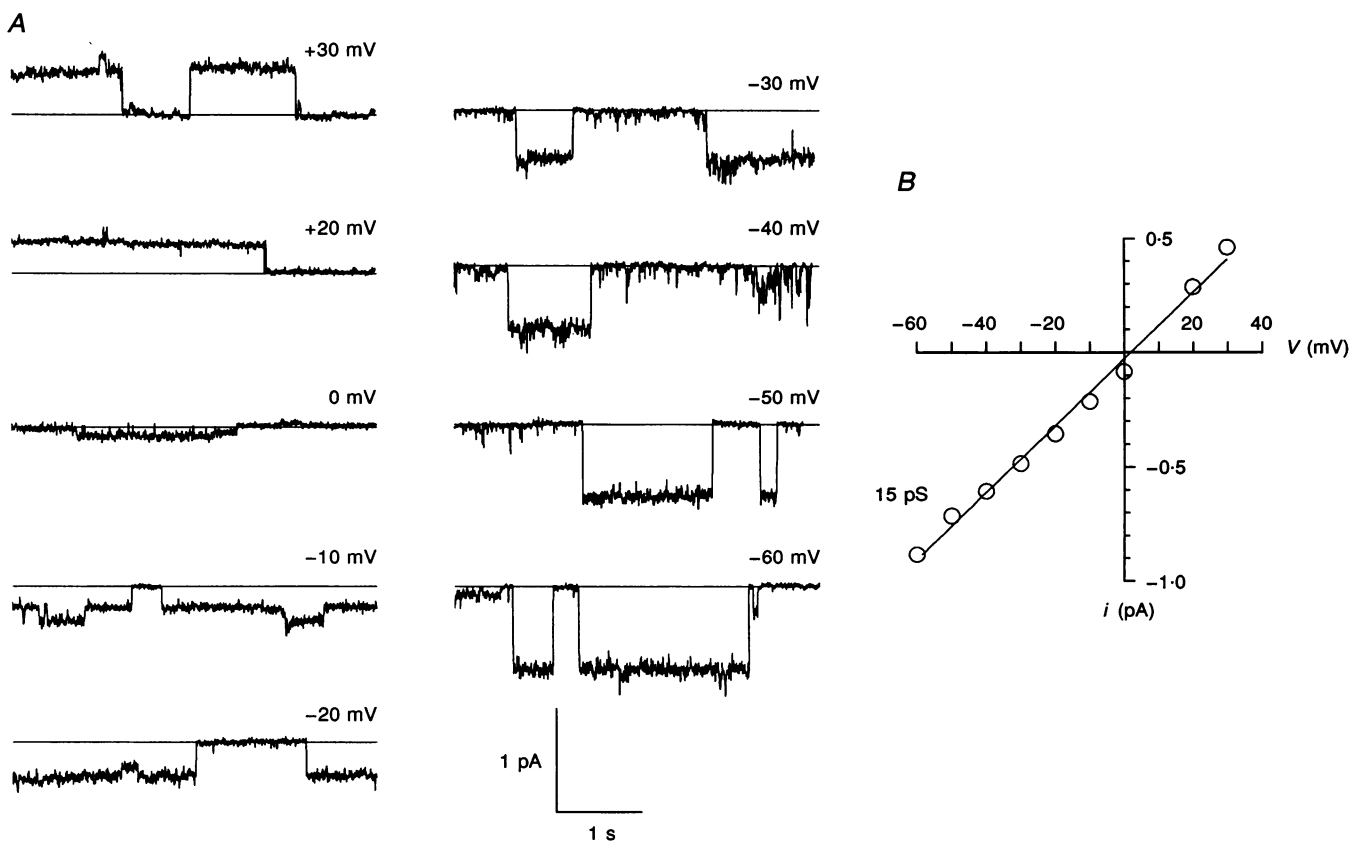

Figure 5. Activity of non-selective channels in the inside-out patch after the addition of GTPγS (100 μM) to the internal solution

The pipette solution contained 105 mM Ba^{2+} without agonist; the bath solution contained 145 mM K^+ (glutamate was the main anion). *A*, current traces recorded at the different holding potentials indicated above the traces. *B*, associated current–voltage relation.

Non-selective cationic channel activity was induced by GTPγS in four inside-out experiments, once in a cell-attached experiment with fluoride in the bath solution and once in a cell-attached experiment without ATP in the pipette solution. However, the kind of receptor responsible for the channel activation could not be concluded from the the results of these experiments.

DISCUSSION

In experiments under whole-cell recording conditions on rat parotid acinar cells (McMillan *et al.* 1988) and atrial cells (Friel & Bean, 1988), the ATP-elicited response was shown to be unaffected by the omission of GTP or the addition of GTPγS to the pipette solution. In lacrimal acinar cells (Sasaki & Gallacher, 1992), GTP and GTPγS produced a marked potentiation of the ATP-evoked response; this suggests coupling of the ATP receptor to the cationic channel. The results of our experiments confirm this supposition. We have shown that channel activity can be resumed at a single channel level in inside-out experiments on the application of GTP or GTPγS to the internal side of the membrane; channel activation by fluoride in cell-attached configuration with no agonist present in the pipette solution has also been demonstrated. The results of the experiments point directly to the involvement of a G protein in signal transduction from an ATP receptor to a Ca^{2+}-permeable channel. However, properties of ATP-activated Ca^{2+}-permeable channels in lacrimal acinar cells and in macrophages differ considerably. The results of this study and our previous data (Naumov *et al.* 1995) point to a rather high selectivity of the ATP-activated channels for divalent (Ba^{2+} and Ca^{2+}) *vs.* monovalent (K^+) cations and a somewhat higher single channel conductance.

One of the properties of ATP-sensitive channels is that they are essentially silent in excised patches in the absence of stimulation by GTPγS. Hence, these channels can be referred to as G protein-gated channels (Yatani *et al.* 1987*a*) in contrast to the channels that are merely regulated by G proteins (Holz, Rane & Dunlap, 1986; Yatani *et al.* 1987*b*; Dolphin, Scott & Wooton, 1988; Schubert, VanDongen, Kirsh & Brown, 1989; Mathie, Bernheim & Hille, 1992). The G protein-gated Ca^{2+}-permeable channels dependent on activation of the epidermal growth factor receptor have been characterized in our previous studies on A431 cells (Mozhayeva *et al.* 1991; Naumov *et al.* 1993; Kuryshev *et al.* 1993).

In rat macrophages (Naumov, Kuryshev, Kaznacheyeva & Mozhayeva, 1992) the currents conducted by channels permeable both to mono- and divalent cations have been recorded in outside-out conditions when GTPγS was added to the pipette solution with no agonist applied to the outer surface of the membrane. The single channel conductance with 105 mM Ba^{2+} as the only permeant cation was 10 pS on average. This value is close to that of the 3rd, most probable, conductance sublevel of the ATP-activated GTP-dependent channel. It remained valid for conditions in which 10 mM Ca^{2+} was the only permeant cation; the respective value was 13 pS. There are good reasons for concluding that the GTPγS-induced channels recorded in outside-out experiments (Naumov *et al.* 1992) are identical to those activated under normal conditions by ATP bound to the receptor.

In a number of inside-out and cell-attached experiments, currents were observed which entered through non-selective cation channels, the activity of which seemed to be dependent on G protein activation. As shown in a number of cell types (Benham & Tsien, 1987; Nakazawa & Matsuki, 1987; Friel & Bean, 1988; Neuhaus *et al.* 1991; Sasaki & Gallacher, 1992; Vincent, 1992) under whole-cell conditions, the ATP-induced integral current could be either inwardly or outwardly directed, depending on the membrane potential, with a E_r of about 0 mV. One could suggest that the non-selective channels observed in our study on rat macrophages were the only channels providing the ATP-induced current. Nevertheless, two points are inconsistent with this suggestion. First, such channels were rarely (< 6% of cases) observed during the present and previous studies (Naumov *et al.* 1995); second, no relation has been found with ATP receptor activation. The GTP dependence of the channels suggests the involvement of a G protein in their activation, with the participation of an as yet unidentified receptor.

Thus, there are strong grounds for assuming that the ATP-dependent GTP-gated Ca^{2+}-permeable channels allow ATP-induced Ca^{2+} entry in rat macrophages under normal physiological conditions.

Benham, C. D. & Tsien, R. W. (1987). A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. *Nature* **328**, 275–278.

Bigay, J., Deterre, P., Pfister, C. & Chabre, M. (1987). Fluoride complexes of aluminium or beryllium act on G proteins as reversibly bound analogues of the γ-phosphate of GTP. *EMBO Journal* **6**, 2907–2913.

Bokoch, G. M. & Gilman, A. G. (1984). Inhibition of receptor-mediated release of arachidonic acid by pertussis toxin. *Cell* **39**, 301–308.

Breitwieser, G. E. & Szabo, G. (1985). Uncoupling of cardiac muscarinic and β-adrenergic receptors from ion channels by a guanine nucleotide analogue. *Nature* **317**, 538–540.

Dolphin, A. C., Scott, R. H. & Wooton, J. F. (1988). Photorelease of GTPγS inhibits the low threshold channel current in cultured rat dorsal root ganglion (GRG) neurones. *Pflügers Archiv* **411**, 19P.

Fabiato, A. & Fabiato, F. (1979). Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *Journal de Physiologie* **75**, 463–505.

Friel, D. D. & Bean, B. P. (1988). Two ATP-activated conductances in bullfrog atrial cells. *Journal of General Physiology* **91**, 1–27.

GILMAN, A. G. (1987). G proteins: transducers of receptor-generated signals. *Annual Review of Biochemistry* **56**, 615–649.

GRAIER, W. F., SCHMIDT, K. & KUKOVETZ, W. R. (1990). Effect of sodium fluoride on cytosolic free Ca^{2+}-concentration and cGMP-levels in endothelial cells. *Cellular Signalling* **2**, 369–375.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

HOLZ, G. G., RANE, S. G. & DUNLAP, K. (1986). GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels. *Nature* **319**, 670–672.

KUNO, M. & GARDNER, P. (1987). Ionic channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. *Nature* **326**, 301–304.

KUNO, M., GORONZY, J., WEYAND, C. M. & GARDNER, P. (1986). Single channel and whole-cell recordings of mitogen-regulated inward currents in human cloned helper T-lymphocytes. *Nature* **323**, 269–272.

KURACHI, Y., NAKAJIMA, T. & SUGIMOTO, T. (1986). On the mechanism of activation of muscarinic K^+ channels by adenosine in isolated atrial cells: involvement of GTP-binding proteins. *Pflügers Archiv* **407**, 264–274.

KURYSHEV, YU. A., NAUMOV, A. P., AVDONIN, P. V. & MOZHAYEVA, G. N. (1993). Evidence for involvement of a GTP-binding protein in activation of Ca^{2+} influx by epidermal growth factor in A431 cells: effects of fluoride and bacterial toxins. *Cellular Signalling* **5**, 555–564.

MCMILLAN, M. K., SOLTOFF, S. P., LECHLEITER, J. D., CANTLEY, L. C. & TALAMO, B. R. (1988). Extracellular ATP increases free cytosolic calcium in rat parotid acinar cells. Differences from phospholipase C-linked receptor agonists. *Biochemical Journal* **255**, 291–300.

MAHAUT-SMITH, M. P., SAGE, S. O. & RINK, T. J. (1990). Receptor-activated single channels in intact human platelets. *Journal of Biological Chemistry* **265**, 10479–10483.

MATHIE, A., BERNHEIM, L. & HILLE, B. (1992). Inhibition of N- and L-type calcium channels by muscarinic receptor activation in rat sympathetic neurones. *Neuron* **8**, 907–914.

MATSUNAGA, H., NISHIMOTO, I., KOJIMA, I., YAMASHITA, N., KUROKAWA, K. & OGATA, E. (1988). Activation of a calcium-permeable cation channel by insulin-like growth factor II in BALB/c 3T3 cells. *American Journal of Physiology* **255**, C442–446.

MONAHAN, R. A., DVORAK, H. F. & DVORAK, A. M. (1981). Ultrastructural localization of nonspecific esterase activity in guinea pig and human monocytes, macrophages and lymphocytes. *Blood* **58**, 1089–1099.

MOZHAYEVA, G. N., NAUMOV, A. P. & KURYSHEV, YU. A. (1989). Epidermal growth factor activates calcium-permeable channels in A431 cells. *Biochimica et Biophysica Acta* **1011**, 171–175.

MOZHAYEVA, G. N., NAUMOV, A. P. & KURYSHEV, YU. A. (1991). Variety of Ca^{2+}-permeable channels in human carcinoma A431 cells. *Journal of Membrane Biology* **124**, 113–126.

NAKAZAWA, K. & MATSUKI, N. (1987). Adenosine triphosphate-activated inward current in isolated smooth muscle cells from rat vas deferens. *Pflügers Archiv* **409**, 644–646.

NAUMOV, A. P., KAZNACHEYEVA, E. V., KISELYOV, K. I., KURYSHEV, Y. A., MAMIN, A. G. & MOZHAYEVA, G. N. (1995). ATP-activated inward current and calcium-permeable channels in rat macrophage plasma membranes. *Journal of Physiology* **486**, 339–353.

NAUMOV, A. P., KURYSHEV, YU. A., KAZNACHEYEVA, E. V. & MOZHAYEVA, G. N. (1992). ATP-activated Ca^{2+}-permeable channels in rat peritoneal macrophages. *FEBS Letters* **313**, 285–287.

NAUMOV, A. P., KURYSHEV, YU. A. & MOZHAYEVA, G. N. (1993). Multiple conductance levels of calcium-permeable channels activated by epidermal growth factor in A431 carcinoma cells. *Biochimica et Biophysica Acta* **1145**, 273–278.

NEUHAUS, R., REBER, B. F. X. & REUTER, H. (1991). Regulation of bradykinin- and ATP-activated Ca^{2+}-permeable channels in rat pheochromocytoma (PC12) cells. *Journal of Neuroscience* **11**, 3984–3990.

PEPPELENBOSCH, M. P., TERTOOLEN, L. G. J., DEN HERTOG, J. & DE LAAT, S. W. (1992). Epidermal growth factor activates calcium channels by phospholipase A_2/5-lipoxygenase-mediated leukotriene C_4 production. *Cell* **69**, 295–303.

PFAFFINGER, P. J., MARTIN, J. M., HUNTER, D. D., NATHANSON, N. M. & HILLE, B. (1985). GTP-binding proteins couple cardiac muscarinic receptors to a K channel. *Nature* **317**, 536–538.

SASAKI, T. & GALLACHER, D. V. (1990). Extracellular ATP activates receptor-operated cation channels in mouse lacrimal acinar cells to promote calcium influx in the absence of phosphoinositide metabolism. *FEBS Letters* **264**, 130–134.

SASAKI, T. & GALLACHER, D. V. (1992). The ATP-induced inward current in mouse lacrimal acinar cells is potentiated by isoprenaline and GTP. *Journal of Physiology* **447**, 103–118.

SCHUBERT, B., VANDONGEN, A. M., KIRSH, G. E. & BROWN, A. M. (1989). Modulation of cardiac Na channels by β-adreno receptors and the G protein, G_s. *Biophysical Journal* **55**, 229A.

SIFFERT, W., JAKOBS, K. H. & AKKERMAN, J. W. N. (1990). Sodium fluoride prevents receptor- and protein kinase C-mediated activation of the human platelet Na^+/H^+ exchanger without inhibiting its basic pH-regulated activity. *Journal of Biological Chemistry* **265**, 15441–15448.

VINCENT, P. (1992). Cationic channels sensitive to extracellular ATP in rat lacrimal cells. *Journal of Physiology* **449**, 313–331.

WOODS, N. M., DIXON, C. Y., CUTHBERTSON, K. S. R. & COBBOLD, P. H. (1990). Fluoroaluminate mimics agonist application in single rat hepatocytes. *Biochemical Journal* **265**, 613–615.

YAMAMOTO, Y., CHEN, G., MIWA, K. & SUZUKI, H. (1992). Permeability and Mg^{2+} blockade of histamine-operated cation channel in endothelial cells of rat intrapulmonary artery. *Journal of Physiology* **450**, 395–408.

YATANI, A., CODINA, J., BROWN, A. M. & BIRNBAUMER, L. (1987*a*). Direct activation of mammalian atrial muscarinic potassium channels by GTP regulatory protein G. *Science* **235**, 207–211.

YATANI, A., CODINA, J., IMOTO, Y., REEVES, J. P., BIRNBAUMER, L. & BROWN, A. M. (1987*b*). A G protein directly regulates mammalian cardiac calcium channels. *Science* **238**, 1288–1292.

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