Journal of Physiology (1991), **436**, *pp.* 711–724
With 7 figures
Printed in Great Britain

NOVEL CHLORIDE CONDUCTANCE IN THE MEMBRANE OF BOVINE CHROMAFFIN CELLS ACTIVATED BY INTRACELLULAR GTPγS

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(*Received 17 July 1990*)

SUMMARY

1. The effects of introducing the non-hydrolysable GTP analogue guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS) into perfused bovine chromaffin cells were studied by a combination of the tight-seal whole-cell patch-clamp technique and Fura-2 fluorescence $[Ca^{2+}]_i$ measurements.

2. GTPγS (5–300 μM) induced a slowly developing transient current (inwardly directed at the holding potential −60 to −70 mV) and $[Ca^{2+}]_i$ oscillations. The current activated with a 10–50 s delay after the start of whole-cell dialysis, peaked at 70–120 s and decayed almost to its initial level during the next 150–300 s. Calcium oscillations were observed within the first 100–150 s of cell perfusion.

3. GTP competitively lowered the probability of current activation by GTPγS. At low GTPγS/GTP ratio (5 and 300 μM, respectively) activation of the current was observed only rarely.

4. The activation of the current was accompanied by an increase in conductance but not by changes in the current reversal potential. The changes in the conductance did not depend on the membrane potential; no time-dependent relaxation of the current was induced by steps in the membrane voltage.

5. The current reversal potential was close to the Cl^- equilibrium potential; changes in the extracellular Cl^- concentration induced corresponding changes in the current amplitude and shifted its reversal potential. The permeability to larger anions – aspartate, glutamate and isethionate – was about one-tenth of that for chloride.

6. Single-channel conductance, estimated from the ratio of the mean current and its variance, was about 1–2 pS.

7. The current could be reversibly blocked by 4,4′–diisothiocyanatostilbene-2,2′-disulphonate (DIDS, 10 μM), chlorpromazine (5 μM) and tolbutamide (0·5–5 mM).

8. It is suggested that the GTPγS-induced increase in the permeability to Cl^- ions is due to a G protein-mediated production of an as yet unidentified second messenger.

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INTRODUCTION

Adrenal medullary cells (chromaffin cells) synthesize, store and secrete a complex mixture of hormones, the most important of which is adrenaline. Voltage-activated ion channels (sodium, calcium, and potassium; Fenwick, Marty & Neher, 1982*b*; Marty & Neher, 1985), and receptor-operated ion channels (cholinergic nicotinic and $GABA_a$; Fenwick, Marty & Neher, 1982*a*; Bormann & Clapham, 1985) have been identified in the membranes of these cells.

Thus, chromaffin cells are capable of responding to neurotransmitters and to generate action potentials as part of excitation–secretion coupling (Brandt, Hagiwara, Kidokoro & Miyazaki, 1976). Apart from ion channels directly activated by electrical signals or neurotransmitters a number of channel types have been shown to respond to hormones via the interaction with G proteins or second messengers (see Rosenthal & Schultz, 1987, for review). In bovine chromaffin cells these types of channels or interactions have not yet been described. There is, however, evidence for the presence of specific receptors for neurotransmitters and hormones which are known to be coupled to G proteins (muscarinic cholinergic, bradykinin, substance P, neurotensin, neuropeptide Y; Clapham & Neher, 1984*a*; Misbahuddin, Isosaki, Houchi & Oka, 1985; Noble, Bommer, Liebisch & Herz, 1988; Plevin & Boarder, 1988; Bommer & Herz, 1989; O'Sullivan & Burgoyne, 1989). The experiments described in the present communication were designed to find out whether G protein-activated channels are present in the membrane of bovine chromaffin cells. This was tested by using the non-hydrolysable GTP analogue, GTPγS, introduced intracellularly to achieve generalized activation of G proteins.

METHODS

Bovine chromaffin cells in primary culture (see Fenwick *et al.* 1982*a* for technical details) were used 1–4 days after plating. Single cells were voltage clamped using the 'whole-cell patch-clamp' method (Marty & Neher, 1983). The experimental set-up allowed us to record membrane current and membrane potential and to calculate the current variance (in the frequency range 2–500 Hz). The concentration of intracellular free Ca^{2+} ions was measured with the fluorescent indicator dye Fura-2. Cells were loaded with the dye by diffusion of its pentapotassium salt from the recording pipette. The calibration procedure and details of the measurements are described elsewhere (Neher, 1989). Intracellular introduction of different subtances was achieved by including them in the pipette-filling solutions. Extracellular application, directly onto the cell under investigation, was accomplished by pressure ejection from a puffer pipette with a large (about 3 μm) tip positioned near the cell. All experimental data (including the readings of the pressure applied to the puffer pipette) were sampled every 0·5 s by a laboratory computer.

The standard extracellular solution used (standard saline) contained (in mM): 140 NaCl, 2·8 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES-NaOH at pH 7·2 (290 mosM). The intracellular solutions (standard internal solution) contained (in mM): 145 potassium glutamate, 8 NaCl, 1 $MgCl_2$, 0·5 MgATP, 10 HEPES-KOH at pH 7·2, or (Tris-based internal solution) 140 Tris-HCl, 1 $MgCl_2$, 10 HEPES-NaOH at pH 7·2. In some cases 10 mM-EGTA (replacing 10 mM-potassium glutamate or Tris-HCl in the corresponding solution) was added. The intracellular solutions also contained 100 μM-Fura-2. Compounds used and their sources were: DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid), HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulphonic acid)), EGTA (ethylene glycol-bis-(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid) from Sigma; Fura-2 pentapotassium salt from Molecular Probes, Eugene, OR, USA; GTP (guanosine-5′-triphosphate, dilithium salt) and GTPγS (guanosine-5′-*O*-(3-thiotriphosphate), tetralithium salt) from Boehringer, Mannheim; chlorpromazine-HCl and tolbutamide from Serva.

The membrane potential values given in the paper are corrected for liquid junction potentials developing at the tip of the recording pipette. Junction potentials were measured by comparing the voltage at which the pipette current was zero in symmetrical solutions (i.e. when the bath and pipette solutions were identical), and the voltage developed after replacing the bath solution with the solution used in the actual experiment. During this procedure, a 3 M-KCl bridge was employed in the bath to eliminate changes in reference electrode potential. The junction potentials were −8 mV with standard saline in the bath and standard internal solution as the intrapipette solutions. They were 1–2 mV with standard saline in the bath and Tris-based internal solution as the intrapipette solution. Replacement in the bath, during the reversal potential measurements, of the standard saline by low-Cl^- solution produced an additional junction potential of 11 mV; with the high-Cl^- solution this was −4 mV. These potential changes were also corrected for.

Previous studies on bovine chromaffin cells have shown a resting membrane potential of −60 to −80 mV (Fenwick *et al.* 1982*a*). Most of the experiments reported here were performed with the membrane voltage held near the resting potential, without any command voltage displacement.

RESULTS

Perfusion of cells with control solutions

In control experiments, cells were bathed in standard saline solution and the recording pipette was filled with either standard internal solution or Tris-based internal solution. Except for the presence or absence of potassium outward current, there was no marked difference during the intracellular perfusion with these two solutions. An example of data obtained in control experiments is demonstrated in Fig. 1*A*. The cell input resistance was usually larger than 10 GΩ. Establishment of a direct contact between the intrapipette solution and the cytoplasm after break-in, and the subsequent process of cell dialysis could be monitored by measuring changes in the Ca^{2+}-independent fluorescence of Fura-2 as the dye diffused into the cell from the patch pipette; other substances added to the perfusing solution are assumed to enter the cell in a similar manner. In the vast majority of control experiments the cells displayed stable, very small membrane currents. Currents changed at most by a few picoamperes, current variance (in the 2–500 Hz frequency bandwidth) was also very small, rarely exceeding 1 pA^2. There were no abrupt changes in the intracellular concentration of free Ca^{2+} ions (see the lower record in Fig. 1*A*). Addition of GTP (300 μM) to the intracellular solution did not affect the behaviour of the membrane current or the intracellular calcium.

Under control conditions, a small percentage of cells (in some preparations up to 5–10%) developed shallow transients of an inward current several tens of picoamperes in amplitude. In most of these experiments, the cells swelled or produced blebs before the current appeared.

Effects of intracellular introduction of GTPγS

The incidence of inward current activation increased dramatically when GTPγS (100–300 μM), a non-hydrolysable analogue of GTP, was added to the intracellular solution: more than 75% of cells investigated (about 300 cells) showed activation. The transmembrane current started to increase with a 10–50 s delay after the break-in (see upper trace in Fig. 1*B*). Its activation could be very abrupt so that within a few seconds the current increased from near zero level to several tens of picoamperes (see, for example, Fig. 1*C*). The current reached a maximum in 70–120 s (average

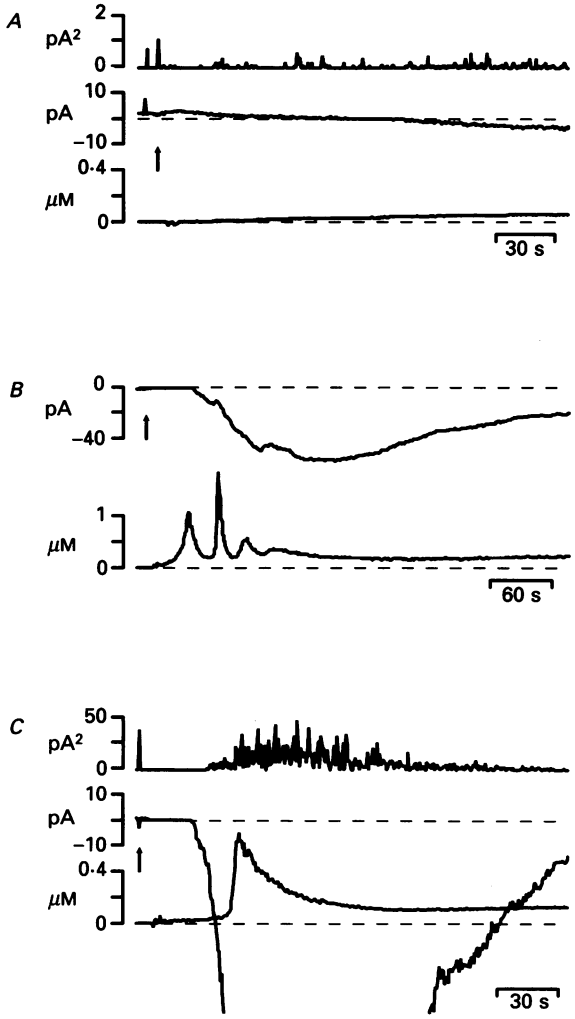

Fig. 1. Changes in the membrane current and intracellular Ca^{2+} concentration during cell perfusion with different intracellular solutions. *A*, an example of the control experiment in which the cell was bathed in standard saline solution and perfused with standard internal solution. The traces shown here and in some of the next figures were obtained after the tight-seal contact between the patch pipette and the cell membrane was established. Time of break-in is indicated by an arrow. The upper trace shows the current variance, middle trace shows membrane current, and lower trace shows intracellular Ca^{2+} concentration. Holding potential, −60 mV. *B*, intracellular GTPγS-activated transmembrane current and $[Ca^{2+}]_i$ transients. Ionic conditions are as in part *A* except that 100 μM-GTPγS has been added to the internal solution. The variance was not recorded in this experiment. *C*, activation phase of the GTPγS-activated current. Standard saline and Tris-based intracellular solution. The current trace has been truncated at the level of 80 pA, in this experiment it reached the maximum value of 300 pA at 80 s.

value 85 ± 35 s) and declined afterwards. Within 150–300 s it decreased to almost the initial value and stayed unchanged for the rest of the recording period (several minutes). At the holding potentials specified, the currents were inwardly directed.

The peak amplitude of the current varied significantly (from tens of picoamperes to about 1 nA) among the cells studied under the same experimental conditions. There was no correlation between the amplitude of the current and the concentration of GTPγS. There was, however, an effect on the probability of appearance of the current: with lower concentrations of GTPγS the current was observed in a smaller proportion of cells. As little as 5 μM-GTPγS was sufficient to activate the current in some preparations.

In parallel with the current activation, intracellular introduction of GTPγS elicited also $[Ca^{2+}]_i$ transients. Their amplitude could reach micromolar levels; usually the changes in $[Ca^{2+}]_i$ were observed within the first 100–150 s of perfusion (lower traces in Fig. 1*B* and *C*). The relationship between the current and the $[Ca^{2+}]_i$ transients is discussed in the accompanying paper in more detail.

Competition between GTPγS and GTP

From the general scheme of GTPγS action, it has been suggested that a decrease in the relative quantity of a non-hydrolysable *versus* a hydrolysable analogue of GTP should lead to a decrease in the former's ability to activate the current because of competition between the two analogues of GTP (Breitwieser & Szabo, 1988). A paired comparison of cells growing on the same cover-glass was made by alternately applying 5 μM-GTPγS alone and 5 μM-GTPγS plus 300 μM-GTP (see Fig. 2). Each bar in the figure represents a single-cell experiment in chronological order: filled bars correspond to cells perfused with Tris-based internal solution containing 5 μM-GTPγS alone, open bars represent experiments with 300 μM-GTP plus 5 μM-GTPγS added to the intracellular solution. It was found that those cells perfused with the solution containing GTPγS alone produced the current in seven out of eight experiments, while those perfused with the (GTPγS + GTP)-containing solution produced the current in only one out of five experiments.

Membrane conductance

In order to monitor changes in the membrane conductance which underlies current development, we used ramp-voltage stimuli applied at different times (Fig. 3). In this particular experiment, the ramps were applied during the activation phase of the current (1), its maximum (2), and during the decay phase (3 and 4 in Fig. 3*A*). The corresponding *I–V* curves are shown in Fig. 3*B*. It is apparent that the slope conductance changed in a manner similar to that of the current: the largest corresponding to the maximum of the current, the smallest to the small residual current after decay. These experiments show that the current activation is due to an increase in the membrane conductance and that the reversal potential of the current changes very little during the current development. The results suggest that the current development is based on changes in a single type of membrane conductance.

Single-channel conductance

To estimate the conductance of single channels which produce the inward current, we made use of current variance measurements. A typical example of changes in current and current variance is shown in Fig. 1*C*. (See also Fig. 7*C* and *D*). The

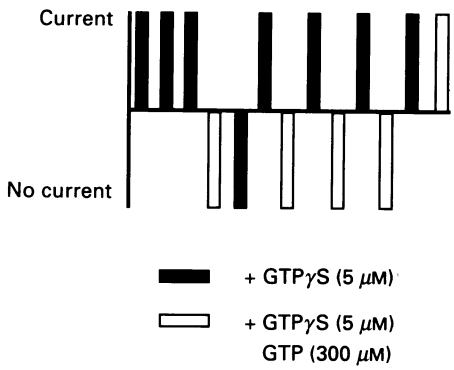

Fig. 2. Effect of GTP on activation of the current by GTPγS. An overview of a series of standard successive experiments carried out on cells from the same cover-glass over a period of about 2 h. The cells bathed in standard saline solution were intracellularly perfused with Tris-based internal solution plus either 5 μM-GTPγS (filled bars) or 5 μM-GTPγS + 300 μM-GTP (open bars). Each bar represents a single-cell experiment; the bar is directed upward when the current was activated, downward when the current was not observed. The sequence of bars corresponds to the sequence of experiments.

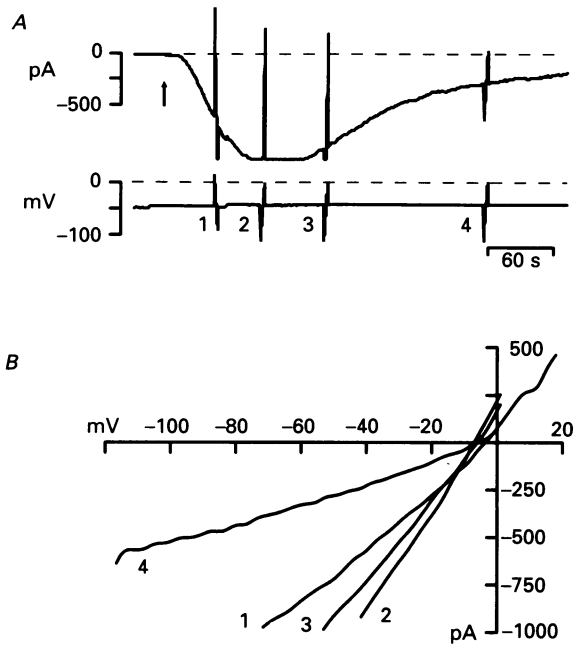

Fig. 3. Changes in membrane conductance during development of the GTPγS-activated current. *A*, the GTPγS-activated current recorded in a cell bathed in standard saline and perfused with Tris-based internal + 300 μM-GTP + 100 μM-GTPγS solutions. Deflections seen in the current and voltage traces represent episodes (1–4) of ramp-voltage commands (30–35 mV/s) used to measure *I–V* relationships. During the first ramp, the membrane voltage changed from positive to negative voltages, opposite to that of the next three ramps. Holding potential, −60 mV; *B*, *I–V* curves corresponding to the ramps shown in part *A*.

magnitude of the variance was very small when compared with that of other ionic currents in these cells (for example, with the variance of GABA-activated chloride current). Estimates of the single-channel conductance as obtained from the ratio between the mean current and its variance and the known zero-current potential

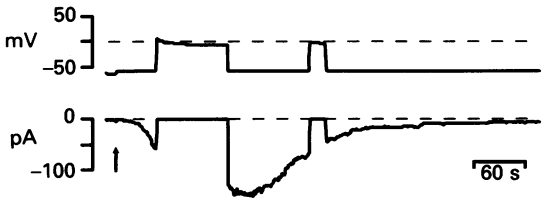

Fig. 4. Independence of current kinetics and membrane voltage. The GTPγS-activated inward current is shown in an experiment where voltage-clamp and current-clamp modes were alternated several times. During the second current-clamp episode, the membrane conductance decayed to a low level. The cell was bathed in standard saline and perfused with Tris-based internal solution + 300 μM-GTP + 100 μM GTPγS.

were in the range 1–2 pS. The variance was calculated in a limited frequency range (2–500 Hz). For this reason, the value given is probably an underestimate.

Voltage dependence

The time course of conductance changes did not noticeably depend on membrane voltage. It remained very similar when the membrane potential was made positive; in this case the current reversed in direction and became outward (see, for example, Figs 5*A* and 7*A*). Also, when the measuring mode was switched alternately between the voltage clamp and the current clamp (see Fig. 4) the overall time course of the current development was close to that of experiments at normal resting potential, although the membrane voltage was very low during a significant portion of the experiment. The absence of a clearly defined potential dependence of the conductance is also supported by the observation that step-like changes in the membrane potential applied while the current was activated did not reveal any time-dependent relaxations of the current (not shown). The *I–V* relationship during the current development showed only a slight outward rectification at positive potentials (see Figs 3*B*, 5*B* and 7*B*).

Ionic nature of GTPγS-activated current

The reversal potential values reported above suggest that the current is carried by ions present at equal concentrations in both the extracellular and intracellular solutions or that the conductance is very non-selective. Circumstantial evidence made it seem unlikely that the current was non-specific cationic in nature. Thus, we considered the possibility that the current is carried by anions. The most abundant anion present in the solutions used is Cl^-; its equilibrium potential in the experiment of Fig. 3 is close to 0 mV, which coincides with the measured reversal potential. The following experiments showed that changes in the extracellular Cl^- concentration were accompanied by changes in the current amplitude and by shifts of the current reversal potential in a way consistent with chloride selectivity. (1) Application of a

low-Cl^- solution (with 11 mM-Cl^- instead of 150 mM-Cl^- in the control standard saline solution) caused a decrease in outward current amplitude (at +40 mV holding potential, Fig. 5*A*). There was also a shift of the reversal potential in the positive direction (Fig. 5*B*). (2) Increasing the extracellular Cl^- concentration to 250 mM (see

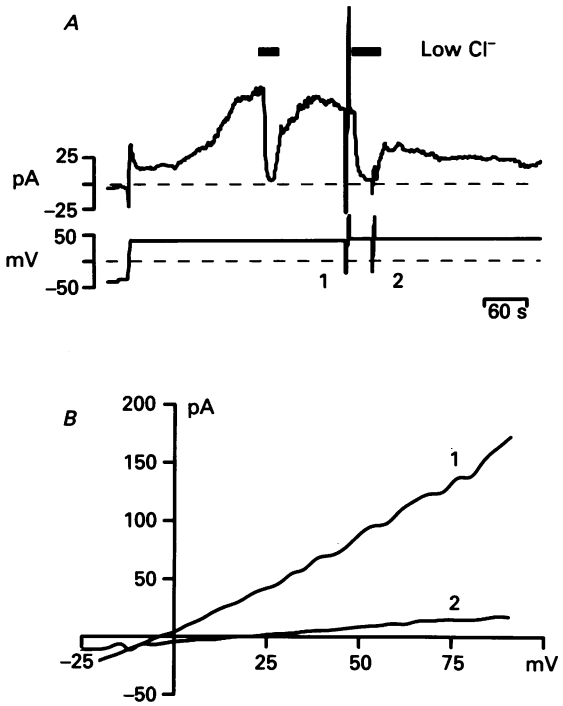

Fig. 5. Changes in the current induced by extracellular applications of a low-Cl^- (11 mM) solution. *A*, the upper trace shows the outward current during perfusion of the cell with Tris-based internal solution + 100 μM-GTPγS. The experiment was started at a holding potential of −40 mV (lower trace) which was switched to +40 mV after the current started to develop (the outward current peak seen at the moment of switching is probably due to potassium current). Low-Cl^- solution applications are marked with horizontal bars. Ramp-voltage commands were applied before and during the second application. The corresponding *I–V* curves are shown in part *B*, the two curves cross over at negative potentials in this particular experiment. This phenomenon, however, was not found in all cases.

next section) led to a decrease in the inward current and a shift of the reversal potential to the left (not shown).

Selectivity of the channels

In order to obtain quantitative information on the ion selectivity of the conductance underlying the current, we performed reversal potential measurements while changing the transmembrane gradient of Cl^- ions. For this purpose, a set of three extracellular and three intracellular solutions with different concentrations of Cl^- ions was used (see Table 1). In the low-Cl^- (11 mM-Cl^-) extracellular solution,

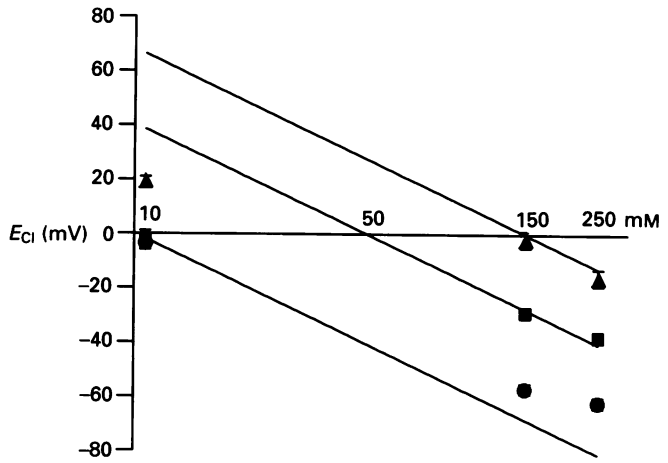

Fig. 6. Reversal potentials of the GTPγS-activated currents. Experimental values corrected for liquid-junction potentials (ordinate) are plotted *versus* $[Cl^-]_o$ (abscissa). Symbols show means ± S.D. values ($n = 3$ for low-Cl^- and high-Cl^- extracellular solutions; $n = 6$ for standard saline solution) obtained with different intracellular Cl^- ion concentrations: 10 mM (●), 50 mM (■) or 150 mM (▲) (see Table 1). Continuous lines indicate the predictions according to the Nernst equation for chloride ions. Deviations from these predictions are discussed in the text.

TABLE 1. Composition of extra- and intracellular solutions used in the reversal potential measurements (in mM)

Solution	NaCl	Sodium isethionate	Choline chloride	KCl	$CaCl_2$	$MgCl_2$	NaOH-HEPES	Tris-Cl	Tris-aspartate	[Cl]
Standard saline	140	—	—	2·8	2	2	10	—	—	150·8
High Cl^-	140	—	100	2·8	2	2	10	—	—	250·8
Low Cl^-	—	140	—	2·8	2	2	10	—	—	10·8
Tris (150 mM)	—	—	—	—	—	—	10	150	—	150
Tris (50 mM)	—	—	—	—	—	—	10	50	100	50
Tris (10 mM)	—	—	—	—	—	—	10	10	140	10

$[Cl^-]$ = total concentration of Cl^- ions in the solution.

chloride was replaced with isethionate; for the high-Cl^- (250 mM-Cl^-) solution, 100 mM-choline chloride was added to the standard saline solution. In intracellular solutions, Cl^- ions were replaced with aspartate. Each experiment was started with a cell bathed in the standard saline and perfused with one of the test intracellular solutions. Having measured the reversal potential by application of a voltage ramp under steady-state ionic conditions, we then applied the test extracellular solutions (one on each cell) from a puffer pipette and measured the reversal potential under these new ionic conditions. The values obtained were corrected for junction potential changes (as described in Methods). The corrected data are presented in Fig. 6 as a function of external chloride concentrations. The continuous lines show the theoretical chloride equilibrium potentials and different symbols indicate different internal chloride concentrations. The experimental values follow the trend of the theoretical predictions; however, there are two areas of serious discrepancy. The

deviations seen at high extracellular Cl^- concentrations can be accounted for by assuming that another anion in the intracellular solutions (aspartate) can also contribute to the total transmembrane current: reasonably good corrections can be obtained assuming an aspartate permeability of about 0·05–0·1 of that for chloride.

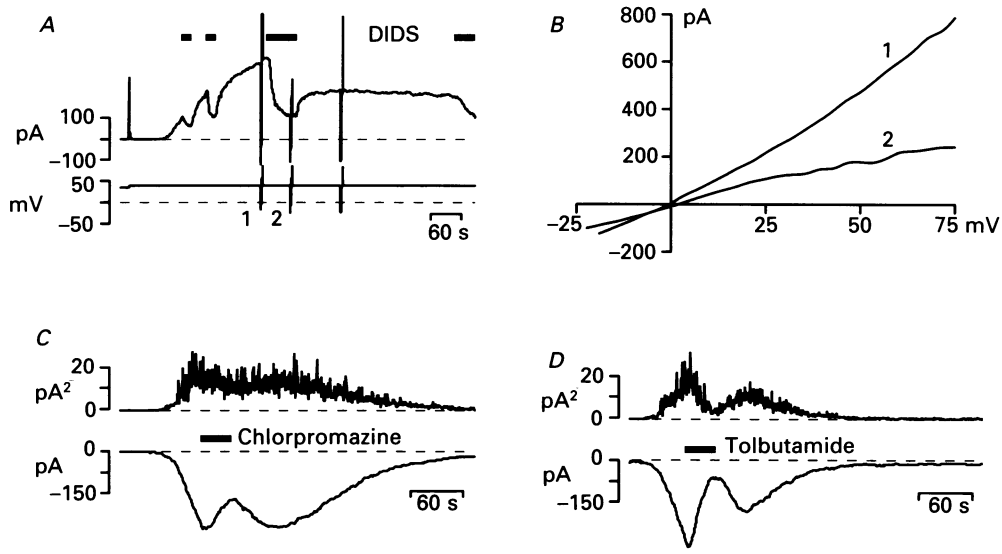

Fig. 7. Block of the GTPγS-activated current. *A*, inhibition of the current during extracellular applications of 10 μM-DIDS. Before, during, and after the third application, a ramp-voltage command was applied to the cell membrane. The corresponding *I–V* curves are shown in *B*. *C* and *D*, inhibition of the current with extracellularly applied 5 μM-chlorpromazine (*C*) or 5 mM-tolbutamide (*D*). The drugs were added to standard saline and applied from puffer pipettes. The applications are marked with horizontal bars. The cells were perfused with Tris-based internal solution + 100 μM-GTPγS + 10 mM-EGTA. Holding potentials: +40 mV in *A*, −60 mV in *C* and *D*. The upper traces in *C* and *D* show the current variance. Dashed lines show the corresponding zero levels.

The discrepancies seen at low extracellular Cl^- concentrations suggest that the membrane may be permeable to the second anion (isethionate) present in the extracellular solution under these conditions. When both extracellular and intracellular chloride concentrations were low, with the extracellular solution containing 140 mM-isethionate and the intracellular solution containing 140 mM-aspartate, the reversal potential was close to 0 mV. This may indicate that the permeabilities to aspartate and isethionate are roughly equal. Nevertheless, correcting for the isethionate permeability still cannot account for the discrepancies between the theoretical predictions and experimental values for the current reversal potential at low extracellular chloride concentration. An additional source of error could be the contribution of potassium outward current activated by the ramp-command at positive membrane potentials. The intracellular solutions used contained at least 1 mM-KCl. (The Fura-2 solution is another source of K^+ since the stock solution was prepared in 100 mM-KCl.) The relative contribution of the potassium current is expected to be larger at positive potentials because of its voltage

dependence and also because, at these potentials, the chloride current would be small due to a diminished electromotive force for Cl^- ions. When cells were perfused with the standard internal solution containing 145 mM-potassium-glutamate, the reversal potential for the GTPγS-activated current was −40 to −50 mV. This indicates that the membrane permeability for glutamate is close to that of aspartate: about 10-fold lower than for chloride.

Pharmacology of the current

The GTPγS-activated current in bovine chromaffin cells could be blocked by DIDS applied extracellularly (Fig. 7*A*). The outward current which developed at the positive holding potential was markedly depressed during applications of 10 μM-DIDS. The block developed rapidly, was readily reversible and could be reproduced several times during the same experiment (Fig. 7*A*). The changes in the current amplitude caused by application of DIDS were not accompanied by significant changes in the reversal potential (Fig. 7*B*). When added to the intracellular solutions 10 μM-DIDS did not prevent current activation (not shown). Because of the large variability in the current amplitude from cell to cell, it is difficult to say whether the effect of intracellular DIDS on current amplitude was substantially different from the extracellular DIDS.

It was found that the current could also be blocked by extracellularly applied chlorpromazine. At 5 μM-chlorpromazine, the block was rapid enough to be effective during the time course of the current activation; thus block by chlorpromazine and its reversal could be followed during current development (Fig. 7*C*). Smaller concentrations of the drug (as low as 0·5 μM) were also effective, but the block developed much more slowly. Another drug which reversibly inhibits the GTPγS-activated current is tolbutamide; it is, however, a less effective blocker than chlorpromazine or DIDS. A concentration of 5 mM was required to block current development (Fig. 7*D*). At lower concentrations, e.g. 0·5 mM, there was noticeable reduction of the current, but, as with low chlorpromazine concentration, the block developed very slowly.

DISCUSSION

The transmembrane current observed in bovine chromaffin cells during intracellular perfusion with GTPγS-containing solutions appears to be a novel type of ionic current in these cells. It differs from voltage-activated ion currents described earlier (Fenwick *et al.* 1982*b*; Marty & Neher, 1985) as follows: (1) it could be activated at very negative holding potentials without depolarizing shifts of the membrane voltage, and (2) its kinetics did not depend on membrane potential. There was no time-dependent relaxation of the current during step-like changes in the membrane voltage. Agonist-activated ion channels in the membrane of bovine chromaffin cells (nicotinic cholinergic receptors, Fenwick *et al.* 1982*a*, and Cl^--selective channels coupled to $GABA_a$ receptors, Bormann & Clapham, 1985) are also different from the GTPγS-activated chloride current: they activate and desensitize on a millisecond time scale and their single-channel conductances are at least one order of magnitude larger. The estimated single-channel conductance of the GTPγS-current in this study is 1–2 pS.

The channels which underlie the GTPγS-activated current in the membrane of bovine chromaffin cells appear to be permeable mainly to Cl^- ions; their permeabilities to the larger anions used in the substitution experiments – aspartate, glutamate and isethionate – are about 10 times smaller. The GTPγS-activated current could be blocked by micromolar concentrations of DIDS, a drug which has been shown to block Cl^--selective channels in various tissues (Miller & White, 1984; Gögelein, 1988). Chlorpromazine and (to a lesser extent) tolbutamide were also effective blockers. Both of these drugs were shown earlier to block other types of channels, with different selectivity (Clapham & Neher, 1984*b*; Trube, Rorsman & Ohno-Shosaku, 1986); their actions on Cl^- channels are probably not specific.

Among the Cl^--selective channels which have so far been described, those which most closely resemble to the GTPγS-activated channel studied here are the channels which may be involved in volume regulation in different cell types (e.g. lymphocytes, Cahalan & Lewis, 1988; human epithelial cells, Hazama & Okada, 1988), as well as Cl^- channels activated by certain agonists in rat mast cells (Matthews, Neher & Penner, 1989). In the cases listed above, the Cl^--selective channel is the predominant type seen. Similarly, the GTPγS-activated chloride channels appear to be the most abundant type of channel in the membrane of bovine chromaffin cells, some of which produced an inward current exceeding 1 nA at a driving force for Cl^- ions of 50–60 mV. To produce an inward current of this magnitude about 20000 channels should be activated. For a cell with a membrane capacitance of 5 pF, this corresponds to 40 channels/μm^2 – a density 150 times greater than that of ACh-activated channels (Fenwick *et al.* 1982*a*) or about 50 times more dense than voltage-activated sodium channels (Fenwick *et al.* 1982*b*) in these cells.

What could be the mechanism of the current activation? Numerous investigations using GTPγS have had as their premise that this non-hydrolysable analogue of GTP acts by activating G protein(s) in the cell membrane. Moreover, the ability of GTPγS to affect cellular processes is often taken as evidence (or even proof) for the involvement of G protein(s) (Evans & Marty, 1986; Rosenthal & Schultz, 1987; Wessling-Resnick, Kelleher, Weiss & Johnson, 1987; Dolphin, McGuirk & Scott, 1989; Torelli & Lux, 1989). This activation of G protein(s) provides the simplest explanation for the action of GTPγS on bovine chromaffin cells. Such a mechanism is supported by the appearance of $[Ca^{2+}]_i$ oscillations, which probably result from the generation of inositol trisphosphate by phospholipase C activity. This mechanism is also consistent with the observed competition between GTPγS and GTP.

Direct action of G proteins on channels (Brown & Birnbaumer, 1988) seems less probable; the kinetics of current development argues against this mechanism. In our view, it is more reasonable to suggest that it is the result of the action of some unknown second messenger generated in response to activation of G protein(s). The considerable delay between the beginning of intracellular perfusion and the current activation can be envisioned as a period of time needed to attain the necessary concentration of a second messenger. The presence of a delayed Cl^- current which is activated by agonists in rat mast cells and whose activation could be successfully mimicked by exogenous cyclic AMP (Matthews *et al.* 1989) supports this suggestion.

What can be the physiological role of the GTPγS-current? The evidence that its activation is mediated by a G protein suggests that there may exist membrane

receptors whose activation by agonist(s) induces long-lasting modulation of electrical excitability. This, in turn, would result in altered secretion of catecholamines. Alternatively, similarities to channels involved in volume regulation (Cahalan & Lewis, 1988; Hazama & Okada, 1988) may indicate that the GTPγS-induced chloride current is part of this mechanism.

This work was supported in part by the Alexander von Humboldt-Stiftung and the Deutsche Forschungsgemeinschaft (Grant 243/3-1). We thank Ruth Cahalan for revising the paper and Michael Pilot for excellent technical assistance.

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