Biochimica et Biophysica Acta, 1145 (1993) 273–278

BBAMEM 75864

Multiple conductance levels of calcium-permeable channels activated by epidermal growth factor in A431 carcinoma cells

Alexander P. Naumov, Yuri A. Kuryshev and Galina N. Mozhayeva

Institute of Cytology, Russian Academy of Sciences, St. Petersburg (Russia)

(Received 16 July 1992)
(Revised manuscript received 16 October 1992)

Key words: Patch clamp; Carcinoma cell; Calcium ion channel; EGF; Conductance sublevel

Single Ca^{2+}-permeable channels were studied in membrane patches from A431 carcinoma cells. Amplitudes of channel openings fell into three major groups with mean unitary conductances of 1.3, 2.4 and 5.1 pS (105 mM Ca^{2+} in the pipette as charge carrier). All three groups of events were activated with epidermal growth factor (EGF) from the outside and by GTP non-hydrolyzable analogues from the inside of the patch membrane. As a rule, channel openings were uniform in amplitude in each individual patch but sometimes transitions between openings of different conductance levels were seen. It is concluded that the plasma membrane of A431 cells contains a single type of EGF- and GTP-dependent Ca^{2+}-permeable channel (or channel complex) that can display, at least, three conductance levels.

Introduction

Agonist-stimulated calcium influx plays an important role in transmembrane signalling [1,2]. This influx seems to be the result of an activation of Ca^{2+}-permeable channels controlled by membrane receptors. Several types of receptor-dependent Ca^{2+}-permeable channels in plasma membrane of different cells [3–6] have been revealed using patch clamp technique.

Recently, we have found Ca^{2+}-permeable channels in plasma membrane of human carcinoma A431 cells [7,8]. The most frequently observed in the patches were channels with mean unitary conductance of 2.4 pS with 105 mM Ca^{2+} as charge carrier. In cell-attached patches the activity of these channels was considerably enhanced when epidermal growth factor (EGF) was added to the solution bathing the external side of the membrane patch. In cell-free patches their activity could be supported by non-hydrolyzable analogues of guanosine triphosphate (GTP) added to the artificial intracellular solution. It was suggested that these channels are activated by agonist-occupied EGF receptor via guanine nucleotide-dependent transducing mechanism (presumably a GTP-binding protein). Additionally, calcium channel currents with mean unitary conductances of 1.3 and 5.1 pS were observed in a number of experiments. Openings with conductance of 1.3 pS, like those with 2.4 pS, were shown to be dependent on the presence of GTP analogues in the intracellular solution. It remained uncertain at the time whether these openings were responsive to EGF. As to 5.1 pS currents, neither extra- nor intracellular activators were determined in those experiments.

The appearance of events with conductances of 1.3, 2.4 and 5.1 pS was interpreted as indication that three different channel types are present in the membrane. On the other hand, the data available did not exclude the possibility that openings with different conductances correspond to conductive states of the same channel.

The data presented in this paper suggest that 1.3, 2.4 and 5.1 pS conductance levels result from the gating of the same EGF- and GTP-dependent channel or channel complex.

Materials and Methods

Human carcinoma cells A431 (Cell Culture Collection, Institute of Cytology, Russia) were cultured as described earlier [8].

The pipettes were filled with 100 mM $CaCl_2$ plus 5 mM $Ca(OH)_2$/ Hepes buffer (pH 7.4). When necessary EGF (3.7 to 8.3 nM) was added to this solution. To nullify cell resting potential, when working in cell-attached configuration, a bath solution with a high con-

Correspondence to: A.P. Naumov, Institute of Cytology, Russian Academy of Sciences, Tikhoretsky Ave. 4, St. Petersburg 194064, Russia.

centraion of potassium was used; its composition (in mM) was 145 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 Tris-HCl (pH 7.4). The control 'intracellular' solution contained (in mM) 140 KCl (or K glutamate or sulfate), 10 Hepes/KOH (pH 7.4), 5 EGTA/KOH, 1.5 $CaCl_2$ (pCa 7.5), 1 $MgCl_2$. GTPγS (guanosine 5'-O-(3-thiotriphosphate)) and GppNHp (5'-guanylyl imidodiphosphate) were from Boehringer. EGF from mouse submaxillary glands was a gift of Dr. A.D. Sorkin [9].

Ionic currents were measured in cell-attached or inside-out configurations [8,10] at 30–33°C. The current signal recorded on magnetic tape was filtered with six pole Bessel filter at corner frequency 100 Hz. For current openings with single amplitude, the mean channel current expressed in unitary current amplitudes (NP) was calculated as $\langle I \rangle / i$ (N = number of open channels, P = probability for the channel to be open, $\langle I \rangle$ = mean current and i = unitary current). The values of $\langle I \rangle$ were calculated from amplitude histograms as the time integral of patch current above baseline. The maximum NP value over 20 s interval (NP^{20}) was taken as a measure of channel activity for given experimental situation (control, presence of activator, etc.).

Averaged data are given as the means ± S.E. (number of experiments).

Results and Discussion

Small-amplitude calcium currents, referred to as SG currents, with mean unitary conductance of 1.3 pS were observed in 19 cell-attached and excised patches. Earlier [7.8] they were shown to be activated by intracellular application of non-hydrolyzable GTP analogues. To address whether these currents are activated by EGF, channel activity in cell-attached patches with and without EGF in the pipette solution was compared. In these experiments the currents were seen only when EGF was present in the pipette solution. NP^{20} values for SG events were calculated in five cell-attached experiments with EGF in the pipette; mean value was equal to 0.71 ± 0.11. In eight experiments without EGF there was no SG activity in the cell-attached configuration although the channels were present in the patches as revealed by application of GTPγS to the internal side of the patch membrane after the patches had been excised. Thus, SG events like those of 2.4 pS (designated G events) are dependent on the same extra- and intracellular activators i.e. EGF and GTP analogues, respectively.

The similarity can be explained in two ways: either there are two different channels with similar mechanism of activation or both types of events are generated by the same channel with two or more conductance levels. In most experiments either G or SG openings were seen separately in each individual patch; in some, however, the pattern of channel activity was more complicated giving support to the second possibility. One such experiment is illustrated in Fig. 1. Selected current records show that there are both G (2.4 pS) and SG (1.3 pS) current levels in the same patch. Because the 1.3 pS level appeared sometimes as separate openings, one of the possible explanations of the appearance of two current levels is the activity of two independent channels with 1.3 pS conductance. In this case, current jumps from zero to the larger (G) level (or in reverse direction) would occur extremely rarely, because of low probability for two independent channels to gate synchronously. But such current jumps accounted for most open-closed transitions in this experiment. Another possibility is that there were two channels in the patch with conductances of 1.3 and 2.4

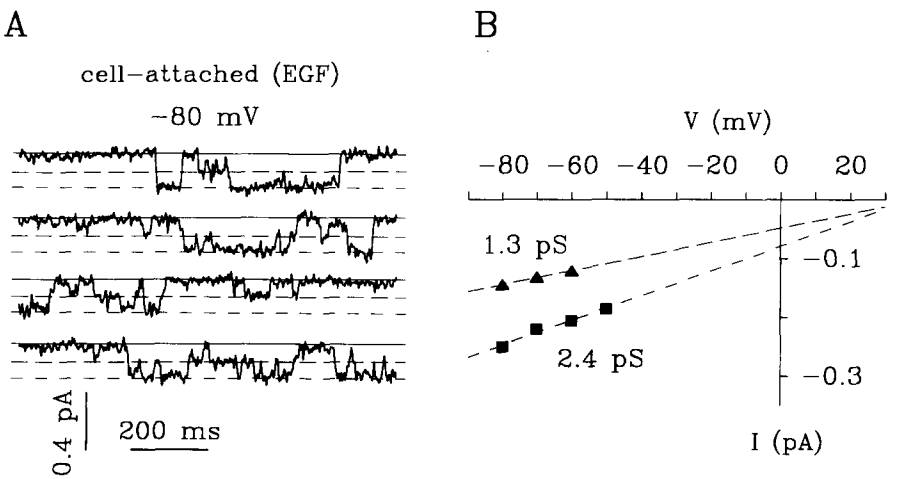

Fig. 1. Two levels of channel openings in the patch. Cell-attached configuration, EGF (8.3 nM) in the pipette. (A) Selected current traces at −80 mV. (B) Current–voltage relation for both current levels; the main (most probable in this experiment) level corresponds to 2.4 pS and the lower one to 1.3 pS.

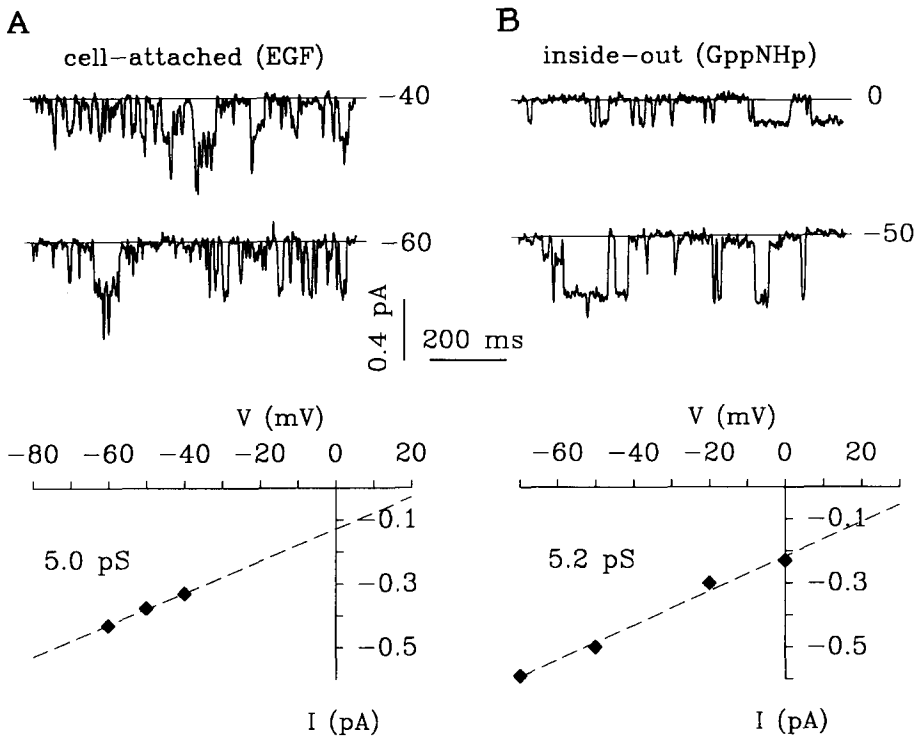

Fig. 2. Channel openings of BG type recorded in two separate experiments. (A) Cell-attached patch, EGF (6.6 nM) in the pipette. Top: current traces at −40 and −60 mV; bottom: current–voltage relation. (B) Inside-out patch, GTPγS (100 μM) in the intracellular solution with SO_4^{2-} as major anion. Top: current traces at 0 and −50 mV; bottom: current–voltage relation.

pS. In this case, superpositions of two types of events would have been observed. There were, however, no conductance levels exceeding 2.4 ps in this patch. The appearance of current jumps from zero to the 2.4 pS level or in reverse direction followed or preceded by the 1.3 pS level is hardly consistent with two independent channel hypothesis.

Similar examples of transitions between G and SG conductance levels were observed in three more experiments. Thus, it can be inferred that the same channel can have, at least, two conductance levels: SG and G ones. Hence, it would be reasonable to suggest that both SG and G events seen in the rest of our experiments are manifestation of the activity of the same

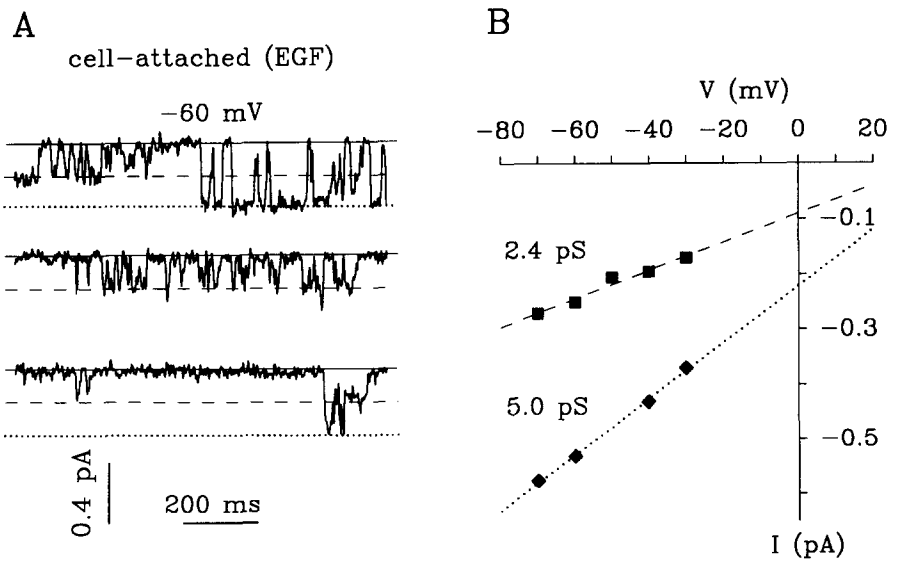

Fig. 3. Events of G and BG types in the same patch. (A) Consecutive current traces at −60 mV. Cell-attached patch, EGF (8.3 nM) in the pipette. (B) Unitary current–voltage relations for G and BG events with slope conductances 2.4 and 5.0 pS, respectively.

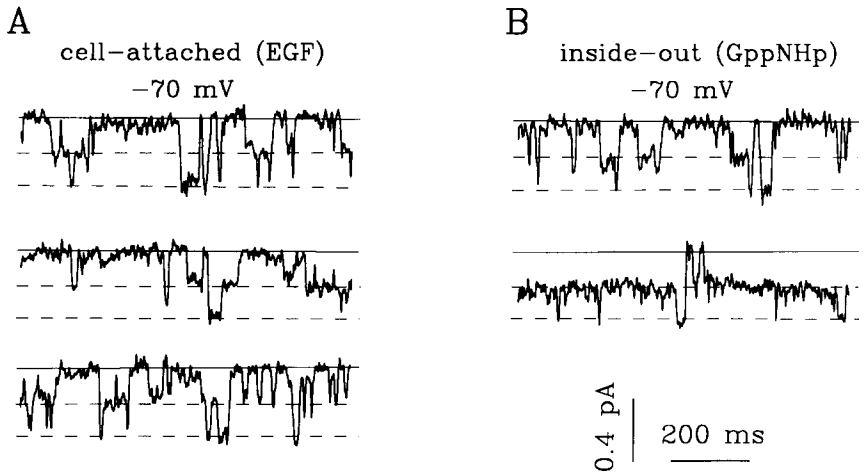

Fig. 4. Transitions between G and BG levels. Selected current traces in the patch firstly in cell-attached (A) and then in inside-out (B) configurations. (A) EGF (8.3 pS) is added to the pipette solution. Currents at −70 mV. (B) GppNHp (100 μM) is added to the bath solution. Currents at −70 and −80 mV.

channel (or channel complex) type which can operate in two (or more) conductance modes.

Measurements at high negative potentials (more than −90 mV) indicated that there is a conductance level even lower than SG which could not be, however, characterized quantitatively because of technical limitations.

Fig. 2 demonstrates one more type of channel openings referred to as BG type. Measurements in cell-attached patch are presented in part A. Observed unitary channel conductance is 5.0 pS, extrapolated value of reversal potential is positive suggesting again that currents are carried by Ca^{2+} ions. EGF was present in the pipette solution in the experiment. Openings with conductances from 4.6 to 5.6 pS (mean 5.1 pS) were observed in 7 more cell-attached patches, when EGF was added to pipette solution. The mean NP^{20} value was calculated to be equal to 0.195 ± 0.07 in these experiments. We have never seen such events in cell-attached patches in the absence of EGF. Channel events with similar conductances were seen also in inside-out patches (four experiments) in the presence

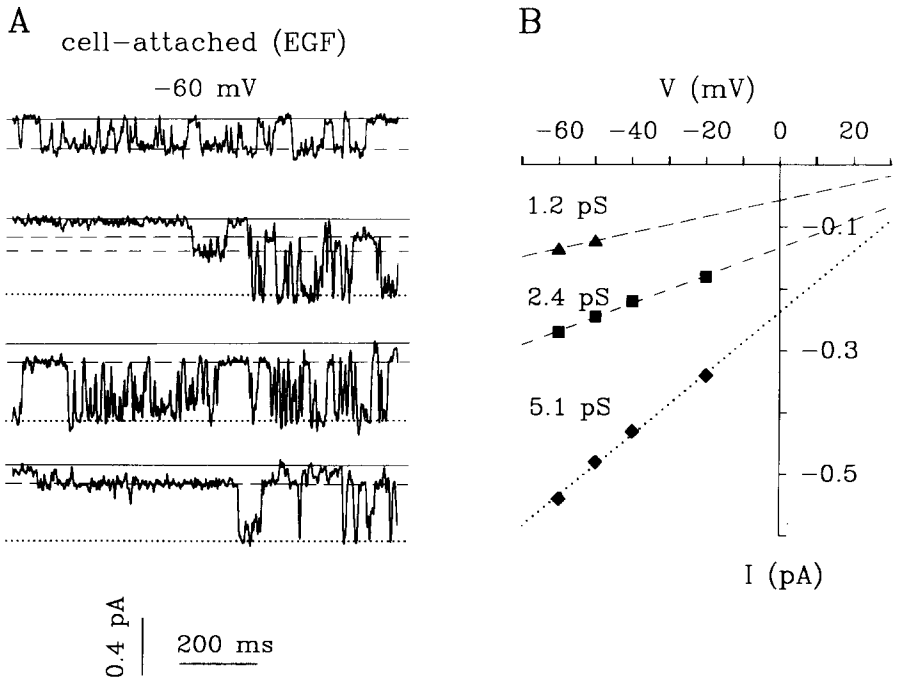

Fig. 5. Three levels of channel openings and transitions between them. Cell-attached experiment, EGF (6.3 nM) was added to the pipette solution. (A) Selected fragments of 10 s current record at −60 mV. Upper trace presents G level; second one: G, BG and SG levels; third and bottom ones: SG and BG levels. (B) Current–voltage relations for SG (1.2 pS), G (2.4 pS), and BG (5.1 pS) levels.

of GTPγS or GppNHp in internal solution. One of these experiments is illustrated by Fig. 2B. Thus, events of BG type, like events of G and SG types, are dependent on EGF from the outside and on GTP analogues from the inside. We suggest that all three types of events should reflect openings of the same channel type to different conductive levels. Some observations support this suggestion. Single-channel records in Fig. 3 show that events of G and BG types coexist in the same patch. If they corresponded to openings of different independent channels, superpositions of both types of events would be seen. However, no superpositions were seen in this experiment. Moreover, the appearance of transition between BG and G levels (bottom trace) is more readily consistent with the suggestion that both levels occurred in the same channel.

Fig. 4 shows transitions between G and BG levels, observed in one experiment firstly in cell-attached and then in inside-out configurations. The majority of events in this experiment were G type (2.4 pS) but sometimes current jumps of approximately double amplitude were seen.

Fig. 5 shows an experiment in which all three mentioned levels were seen.

Other conductance levels in the EGF-activated channel were observed as well. A level intermediate between 5.1 and 2.4 pS was observed in some patches, but its occurrence was much lower than for G and BG levels. In one experiment with an inside-out patch we observed channel activity with conductance of 10 pS after addition of GTPγS to intracellular solution. In two experiments along with 5.1 pS events there were several events of double amplitude. Thus, it is possible that the same channel can adopt conductance level higher than 5 pS. Additional experiments are necessary to establish this point with certainty.

The observations presented above enable us to conclude that the membrane of A431 cells contains one EGF- and GTP-dependent channel type which can operate, at least, in three conductance modes: 1.3, 2.4 and 5.1 pS.

Earlier [7,8] we estimated levels of EGF-induced channel activity in terms of probability for each channel in the patch to be open. This approach is good for single amplitude channels, however, it cannot be applied to channels with multiple conductance levels. Therefore we choose to estimate channel activity by value of mean channel current expressed in units of the minimal current (NP_m), which was calculated as $NP_m = \langle I \rangle / i_m$, where $\langle I \rangle$ is the mean channel current in the patch (see Materials and Methods) and i_m is the current corresponding to the lowest (SG) conductance level. In cases when this level was absent, it was taken to be 50% of G or 25% of BG levels, respectively. When the activity is presented by SG level only, NP_m becomes simply identical to NP value, commonly used

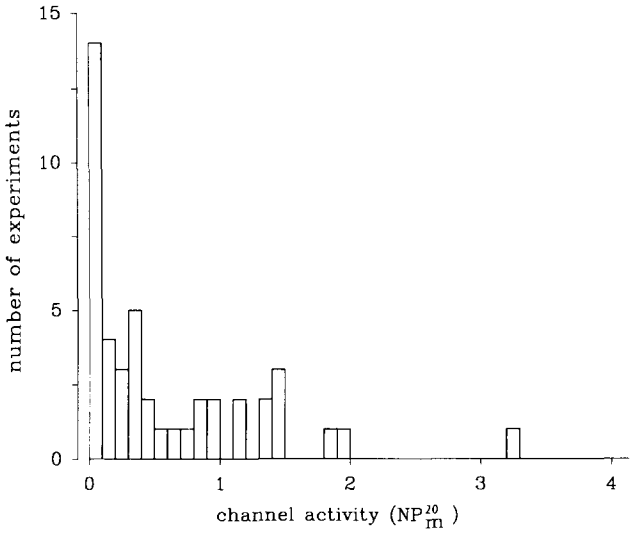

Fig. 6. The histogram of the EGF-induced channel activity in patches. *X* axis: maximal mean channel current for 20 s interval expressed in SG unitary current (see text for details). *Y* axis: the number of patches tested. The data collected from 45 non-blank on-cell patches.

for estimation of channels activity with single amplitude. By analogy with NP^{20} (see Materials and Methods) maximal NP_m value for 20 s interval is designated NP_m^{20}.

Fig. 6 shows the distribution of NP_m^{20} values from 45 cell-attached experiments with EGF in the pipette. Only patches with channels were included. The level of the channel activity varied considerably from patch to patch. In 8 experiments with EGF no activity was seen in cell-attached configuration though the channels were present in patches as revealed by GTP analogues applications after patch excision. On the other hand, in three patches NP_m^{20} values were larger than 1.5. The average NP_m^{20} was 0.567. This value corresponds to approximately 0.06–0.07 pA per patch at −60 mV.

Without EGF in the pipette channel activity was detected in 35 experiments; in seven of them channel openings were seen in cell-attached configuration with the highest NP_m^{20} value being equal to 0.01; in the rest of experiments channel activity in cell-attached configuration was not seen at all, and the presence of channels in the patches was revealed in inside-out configuration by addition of GTP analogues. Thus, EGF causes more than 50-fold increase in the channel activity.

The dependence of channel activity on intracellular GTP suggests the involvement of GTP-binding proteins (heterotrimeric [11,12] or small [13,14]) into signal transduction from the receptor to the channel. On the other hand, one of the obligatory events in the development of EGF-induced cell signal is activation of intrinsic tyrosine kinase activity of EGF receptor [15–

17]. One may think that both mechanisms are involved in the activation of channels, with GTP-binding protein activation being downstream from the phosphorylation of a yet unknown intermediate transducer at tyrosine. Additional evidence for involvement of GTP-binding proteins in EGF-induced signal was obtained recently [18] in fluorescent Ca^{2+}- sensitive dyes experiments on A431 cells.

The presence of multiple conductance levels seems to be a common property of many channel types [19–22]. The calcium-permeable channels are not unique in this respect. Ca^{2+}-permeable channels from reticulum of skeletal muscle [23] and endoplasmic reticulum of cerebellum [24] show at least four conductance levels. Recently, several conducting levels were reported for L-channels in GH_3 cells [25].

The simplest explanation for multiple conductance levels is the 'subunit' model in which a functional channel is viewed as composed of two or more conducting units (protochannels) which open, as a rule, in concerted way [19,20]. Following this model one can suggest that Ca^{2+} permeable protochannels in A431 cells either operate independently or form oligomers. If protochannel conductance is 1.3 pS, conductance levels of G and BG types correspond to di- and tetramers. Alternatively, multiple conductance levels may result from minor structural changes in a single ionic pathway.

The transitions between conductance levels are similar to changes in open probability in that both changes in channel behaviour would affect the amount of ions flowing through the channel. Thus, providing that there is a cellular mechanism regulating single channel conductance status, the multiplicity of channel conductance levels may offer an additional way to control ion fluxes, in particular Ca^{2+} influx, through the cell membrane.

References

1 Meldolesi, J. and Pozzan, T. (1987) Exptl. Cell Res. 171, 271–276.
2 Hallam, T. and Rink, T. (1989) Trends Pharmacol. Sci. 10, 8–10.
3 Von Tscharner, V., Prod'hom, B., Baggiolini, M. and Reuter, H. (1986) Nature 324, 369–372.
4 Kuno, M., Goronzy, J., Weyand, C.M. and Gardner, P. (1986) Nature 323, 269–272.
5 Matsunaga, H., Nishimoto, J., Kojima, J., Yamashita, N., Kurokawa, K. and Ogata, E. (1988) Am. J. Physiol. 255, C442–C446.
6 Luckhoff, A. and Clapham, D.E. (1992) Nature 355, 356–358.
7 Mozhayeva, G.N., Naumov, A.P. and Kuryshev, Yu.A. (1990) FEBS Lett. 277, 227–229.
8 Mozhayeva, G.N., Naumov, A.P. and Kuryshev, Yu.A. (1991) J. Membr. Biol. 124, 113–126.
9 Sorkin, A.D., Teslenko, L.V. and Nikolsky, N.N. (1988) Exptl. Cell Res. 175, 192–205.
10 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
11 Gilman, A.G. (1987) Annu. Rev. Biochem. 56, 615–649.
12 Birnbaumer, L., Abramowitz, J. and Brown, A.M. (1990) Biochim. Biophys. Acta 1031, 143–260.
13 Santos, E. and Nebreda, A.R. (1989) FASEB J. 3, 2151–2163.
14 Satoh, T., Endo, M., Nakafuku, M., Akiyama, T., Yamamoto, T. and Kaziro, Y. (1990) Proc. Natl. Acad. Sci. USA, 87, 7926–7929.
15 Chen, W., Lazar, C.S., Poenie, M., Tsien, R.Y., Gill, G. and Rosenfeld, M.D. (1987) Nature 328, 820–823.
16 Carpenter, G. and Cohen, S. (1990) J. Biol. Chem. 265, 7709–7712.
17 Ullrich, A. and Schlessinger, J. (1990) Cell 61, 203–212.
18 Kuryshev, Yu. A., Savokhina, G.A., Avdonin, P.V. and Naumov, A.P. (1991) Biol. Membr. 8, 1082–1088 (in Russian).
19 Miller, C. (1982) Phil. Trans. R. Soc. Lond. B. 299, 401–411.
20 Geletyuk, V.J. and Kazachenko, V.N. (1985) J. Membr. Biol. 86, 9–15.
21 Fox, J.A. (1987) J. Membr. Biol. 97, 1–8.
22 Meves, H. and Nagy, K. (1989) Biochim. Biophys. Acta 988, 99–105.
23 Liu, Q.-Y., Lay, A., Rousseau, E., Jones, R.V. and Meissner, G. (1989) Biophys. J. 55, 415–424.
24 Watras, J., Bezprozvanny, J. and Ehrlich, B. (1991) J. Neurosci. 11, 3239–3245.
25 Kunze, D.L. and Ritchie, A.K. (1990) J. Membr. Biol. 118, 171–178.