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Single K^+ channels in the apical membrane of amphibian peritoneum

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Application of the patch-clamp technique to the apical membrane of amphibian peritoneum revealed a K^+ permeability governed by voltage-dependent, K^+-permeable channels ($P_K/P_{Na} = 3.5$) that are reversibly blocked by 20 mmol/l internally applied tetraethylammonium. The channels show discrete and multiple conductance levels with elementary conductance of about 22 pS (in 120 mmol/l KCl solution on both sides of the membrane). The channels' behaviour is consistent with an aggregation of channel-forming subunits into clusters with cooperative gating mechanism.

In basic studies on improving techniques for treating patients with renal failure by peritoneal dialysis, an important element is the evaluation of physiological characteristics of the peritoneum. The peritoneum is assigned to the mesothelia and like the epithelia its function is the homeostatic regulation of peritoneal fluid and plasma composition. This regulation is achieved by its capability of transporting water, electrolytes and nonelectrolytes across two parallel pathways of transepithelial as well as transperitoneal movement. Besides active transport systems several types of ion channels have been demonstrated in the epithelial cells (for review see Refs. 1 and 2). Much less is known about the mechanism of ion transport through the peritoneal cells. Here we report on observations of single K^+-selective channels in the apical membrane of peritoneal cells from *Xenopus laevis* and *Rana esculenta* using the patch-clamp technique [3]. The experiments were carried out on pieces of frog mesentery at room temperature. Before the experiment the frog was decapitated and the spinal cord was destroyed. Then the abdomen was opened surgically and the intestinal mesentery was isolated. The tissue was folded to allow better observation of its apical membrane of mesothelial cells. The surface of the cell membrane was clean enough to form giga-ohm seals (20–50 GΩ) with glass pipettes without any additional enzyme treatment.

After seal formation current fluctuations of large amplitude corresponding to a conductance of 200–250 pS were observed from membrane patches in 'cell-free' and in 'cell-attached' configurations, Fig. 1A. Traces B and C in Fig. 1 represent two sections of the record shown in A at more expanded time scales. This presentation shows more clearly that the current fluctuations are superimpositions of several discrete steps (here up to 11). They have approximately the same amplitude as the smallest events seen at the beginning of traces A and B (compare also amplitude histogram in Fig. 1D). Though this record is atypical, it was selected to show the multiplicity of the

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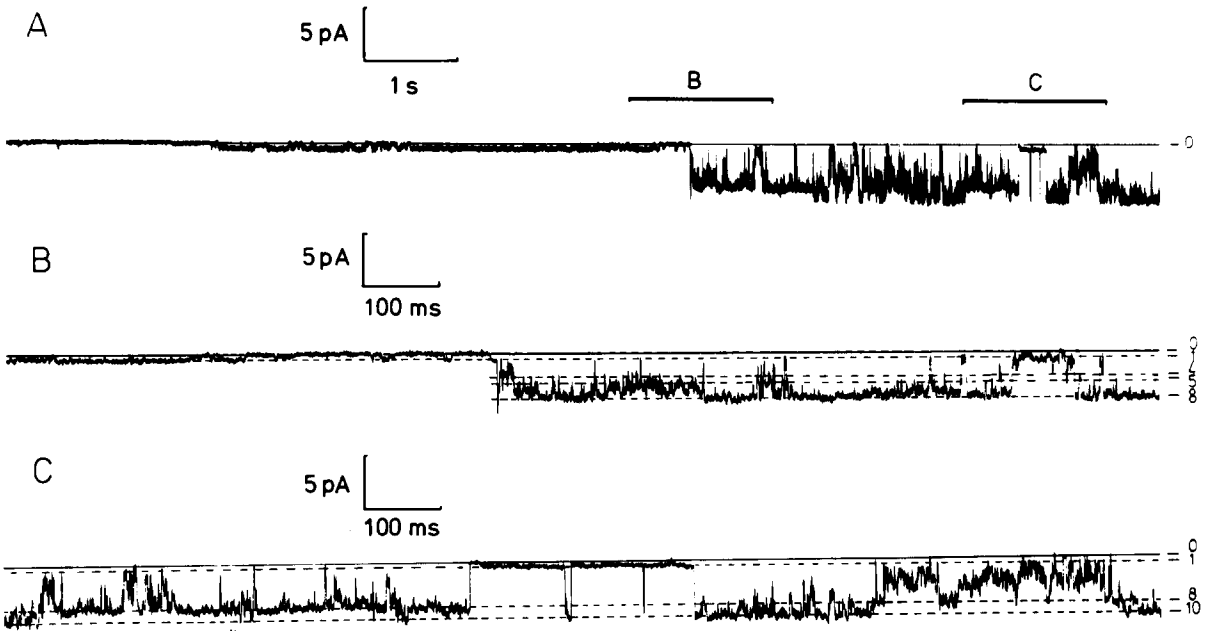

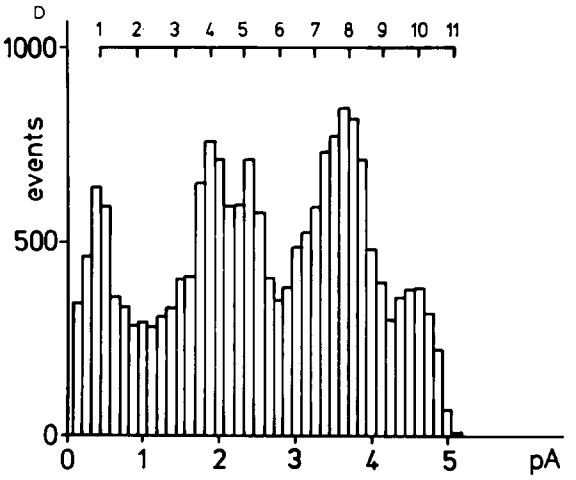

Fig. 1. (A–C) Current fluctuations recorded at −20 mV (voltage of the bath solution on the cytoplasmic surface of the membrane with respect to the pipette solution) from an excised, inside-out patch of apical membrane of peritoneum (*Rana esculenta*). The bath and pipette contained the same solution soln. 2 (60 mmol/l KCl, 60 mmol/l NaCl, 1 mmol/l $MgCl_2$, 10 μmol/l $CaCl_2$, 10 mmol/l Mops as a buffer, pH 7.6). Downward deflections represent currents flowing from the pipette to the bath. Current was recorded on an FM tape recorder and subsequently played back for plotting by brush recorder (Gould). Two sections from the top trace A are shown in B and C in more expanded time scale. Numbers on the right of each record and the broken lines shows integer multiples of the lowest level seen. Solid line show the baseline current level (closed channel). (D) Amplitude histogram of current fluctuations shown in (A–C). To construct the histograms, records were digitized at sampling rate 2 kHz, transferred to the floppy diskette and analysed by means of an LSI 11/23 computer. The upper abscissa has the units that are approximately the largest, common multiplicity of the differences between all five peaks seen in the current amplitude histogram.

conductance levels, and the smallest sublevel is clearly visible. At the beginning of an experiment transitions between fully open and fully closed states or fluctuations between higher conducting sublevels dominate (see Figs. 2 and 4). Appearance of the intermediate and low conductance sublevels such as those seen in Fig. 1 usually happens only at the end of an experiment, 20–40 min after seal formation and probably results from splitting of the high conducting sublevel(s). Fig. 2 demonstrates that channel activity depends on membrane potential and increases as the holding potential becomes more negative. With 120 mmol/l KCl on both sides of the membrane the current-voltage (*I-V*) relation of the elementary current step is linear with a slope conductance of about 22 pS, Fig. 3, curve 1. Under asymmetrical conditions with $120K^+$ in pipette (soln. 1) and

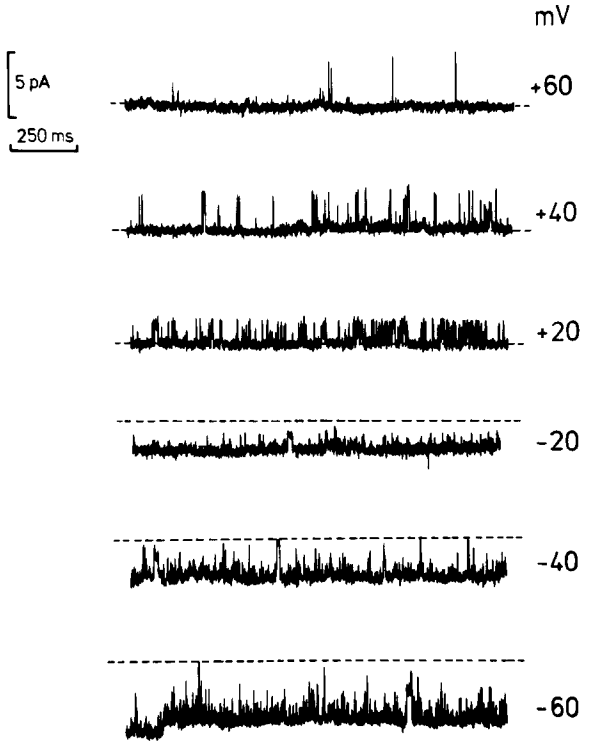

Fig. 2. Voltage dependence of channel activity in membrane patch (cell-free, inside-out) from apical side of peritoneum (*Xenopus laevis*). Channel current was recorded at the holding potentials indicated on the right of each trace. Upward deflections represent currents flowing from bath to the pipette and downward deflections currents flowing in opposite direction. Broken lines show baseline current level. The bath and pipette contained the same solution soln. 1 (120 mmol/l KCl, 1 mmol/l $MgCl_2$, 80 μmol/l EGTA, 10 mmol/l Mops (pH 7.6)).

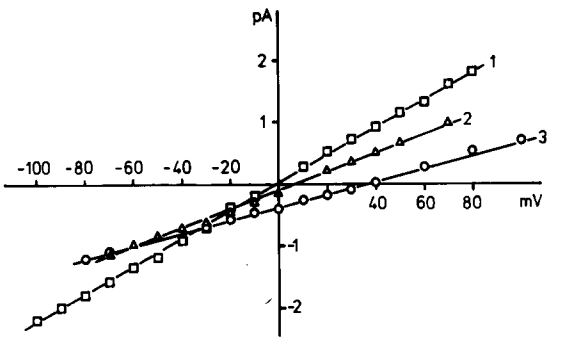

Fig. 3. Current-voltage relations of the studied channel for three different bath solutions. For the curve 1 pipette and bath contained the same solution soln. 1 ($120K^+$) as described in Fig. 2. For curve 2 bath solution was replaced by soln. 2 ($60K^+/60Na^+$) and for curve 3 by Barth's solution (90 mmol/l NaCl, 1 mmol/l KCl, 0.74 mmol/l $CaCl_2$, 10 mmol/l Hepes (pH 7.6)). Each point is an average from 2–5 experiments and represents the size of an 'elementary' current step at a given membrane potential. The slopes of the curves 1–3 correspond to conductances of 22, 15 and 11 pS, respectively.

$60K^+/60Na^+$ (soln. 2) or $1K^+/90Na^+$ (Barth's solution) in bath (for detailed solutions composition see figure legends), the *I-V* curve shifts to positive potentials as expected for K^+-selective channels (curves 2 and 3 in Fig. 3). To plot such relations amplitude histograms were usually constructed to find the size of an 'elementary' current step at a given membrane potential. From the dependence of reversal potential of the *I-V* curves on the Na^+-K^+ composition of the solutions, using Goldman's equation, a selectivity ratio P_K/P_{Na} was estimated to about 3.5. K^+ channels of such low selectivity were recently reported by Labarca and Miller [4] in sarcoplasmic reticulum and by Berger et al. [5] in smooth muscle. Under asymmetrical conditions with high K^+ externally and high Na^+ internally the *I-V* curves remain linear and do not show rectification as for example the low selective K^+ channel described in Ref. 5. Application of Ca^{2+}-free solution to both sides of the membrane patch does not influence channel activity as seen in Fig. 4A, indicating that this channel is not activated by Ca^{2+}. Intracellular application of 20 mmol/l tetraethylammonium (TEA), a typical K^+-channel blocker, reversibly blocks inward and outward currents (e.g. Fig. 4B). After wash out of the TEA the activity did not completely recover as was observed also in other preparations e.g. the potential-dependent, Ca^{2+}-insensitive K^+ channel in smooth muscle [6]. In most membrane patches studied here we observed channel activity which nearly exclusively was due to the channel described above. The interesting feature of the channel studied is that it shows: (1) several equally spaced conductance levels, as if in the membrane patch at the same time several identical ionic pores were present and organized in a cluster; (2) high percentage of rapid transitions between closed and fully open states indicating cooperative gating of the elementary channels; (3) irreversible splitting, during the time of an experiment, of dominating conducting level(s) into several lower conductance levels, which may result from disturbance of the synchronism in operation

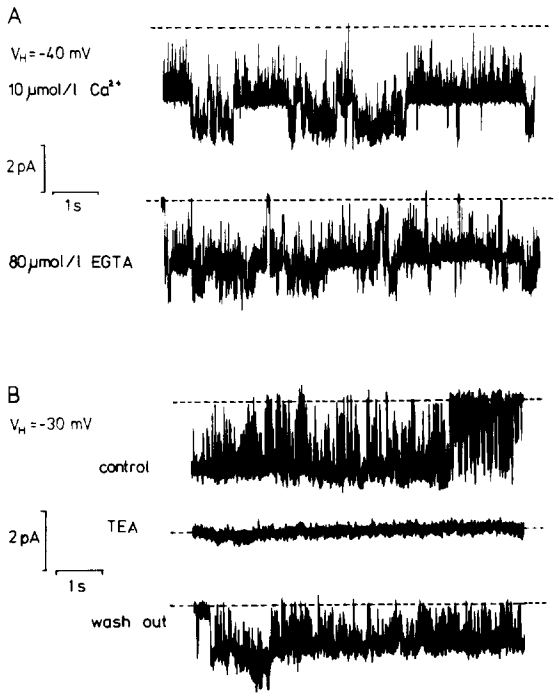

Fig. 4. Effect of Ca^{2+} and tetraethylammonium (TEA) on channel current. The pipette and bath contained the same solution as described in Fig. 2 but in A for upper trace EGTA in bath was replaced by 10 µmol/l $CaCl_2$ and for middle trace in B a 20 mmol/l TEA was added to the bath. A broken line represents a baseline current level. Membrane patch the same as in Fig. 2.

of the elementary channels. K^+ and Cl^- channels having such characteristics were recently recognized in molluscan neurons [7,8], as well as anion-selective channels in pulmonary epithelial cells [9]. The former authors also report observation of similar K^+ channels in molluscan glial and cardiac cells and in rat neurones. Multiplicity of conducting levels have also been shown for the Cl^- channel from *Torpedo californica* incorporated into planar lipid bilayers [10]. Those observations were interpreted as evidence for channel clusters formed by several co-channels opening and closing independently; in addition a shared gating mechanism can synchronously render all of them non-conducting [9]. Channel aggregates can irreversibly degrade with time of experiment, splitting into smaller pieces down to an elementary subchannel [8], a phenomenon consistent also with our observations. Thus, in our view, results reported here are consistent with such interpretation and give further evidence that clusters of synchronously operated co-channels might be common in a wide variety of biological membranes.

The voltage dependence of channel activity favours channel operation at negative membrane potentials. In two cases, by disruption of the patched membrane in cell-attached configuration we found a resting potential of the studied cells of −28 and −38 mV with Barth's solution in the bath and 120K^+ (soln. 1) in the pipette. Calculation of the reversal potential of K^+ currents, E_K, under those conditions with a selectivity ratio $P_K/P_{Na} = 3.5$, gives the value of −38 mV, matching the cell resting potential mentioned above. Thus our results indicate that the apical membrane of peritoneum shows high K^+ permeability which is governed by the voltage-dependent K^+ channels organized in clusters. Systematic investigation of K^+ permeability in peritoneal cells has not yet been reported. The high occurrence of the K^+ channel in patches from the apical membrane of peritoneum and its high conductance suggest an important role of this channel in K^+ permeation through peritoneal cells. Similar significant K^+ conductance was found in apical membranes of other preparations: rabbit gallblader, frog skin and rabbit colon, which may serve as the exit step for active K^+ secretion [1].

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