Potassium channels in primary cultures of seawater fish gill cells. I. Stretch-activated $K^+$ channels

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Previous studies using the patch-clamp technique demonstrated the presence of a small conductance $Cl^-$ channel in the apical membrane of respiratory gill cells in primary culture originating from sea bass $Dicentrarchus labrax$. We used the same technique here to characterize potassium channels in this model. A $K^+$ channel of 123 ± 3 pS was identified in the cell-attached configuration with 140 mM KCl in the bath and in the pipette. The activity of the channel declined rapidly with time and could be restored by the application of a negative pressure to the pipette (suction) or by substitution of the bath solution with a hypotonic solution (cell swelling). In the excised patch inside-out configuration, ionic substitution demonstrated a high selectivity of this channel for $K^+$ over Na$^+$ and Ca$^{2+}$. The mechanosensitivity of this channel to membrane stretching via suction was also observed in this configuration. Pharmacological studies demonstrated that this channel was inhibited by barium (5 mM), quinidine (500 $\mu$M), and gadolinium (500 $\mu$M). Channel activity decreased when cytoplasmic pH was decreased from 7.7 to 6.8. The effect of membrane distension by suction and exposure to hypotonic solutions on $K^+$ channel activity is consistent with the hypothesis that stretch-activated $K^+$ channels could mediate an increase in $K^+$ conductance during cell swelling.

mechanosensitive $K^+$ channels; patch clamp; gill epithelium

IT WAS PREVIOUSLY DEMONSTRATED that primary cultures of respiratory cells of the sea bass ($Dicentrarchus labrax$) gill serve as a good model for studying $Cl^-$ secreting epithelia (2, 10). This model could also help to clarify the mechanism of ion balance regulation in marine teleosts. Taken together, the results of previous studies (2, 10) have permitted functional characteristics of this cell model to be more precisely determined. In this way, the Na$^+$/K$^+$-ATPase pump, by maintaining low intracellular Na$^+$ and $Cl^-$ levels, provides the driving force for $Cl^-$ entry into the cell through the basolateral membrane via Na$^+$/K$^+$-2$Cl^-$ cotransport and the $Cl^-$/$HCO_3^-$ exchange mechanism. Intracellular $Cl^-$ accumulation above its equilibrium leads to $Cl^-$ transport across the apical membrane via small conductance $Cl^-$ channels sensitive to cAMP. It is now well established that transepithelial $Cl^-$ secretion is also dependent on $K^+$ channel activity. These channels are required for the control of membrane potential and also to allow $K^+$ ion recycling across the basolateral membrane. Moreover, $K^+$ channels could also be involved in cell volume regulation. It was therefore postulated in the present study that $K^+$ channels in gill cells could be of significant physiological importance in the control ofionic homeostasis in fish. For these reasons we have undertaken the study of $K^+$ channels in primary cultures of sea bass gill cells in an attempt to elucidate mechanisms of transepithelial ion transport.

MATERIALS AND METHODS

Animals

Sea bass ($Dicentrarchus labrax$) weighing 50.5 ± 7.9 g ($n = 14$ for patch-clamp experiments) were obtained from a local fish farm (Cannes Aquaculture, Cannes, France). The fish were kept in 1-m$^3$ tanks containing seawater from the Mediterranean sea (36 g/l salinity) in a semi-open circuit configuration (water completely renewed every 6 h). The fish were kept in water maintained at ambient temperature (16–17°C over the course of the year) and were exposed to a natural photoperiod.

Primary Culture of Gill Cells

Conditions for the primary culture of gill cells were described previously by Avella et al. (1). Briefly, the following steps were performed at 18°C in an air-conditioned room. Before cell culture preparation, fish were held 2 h in a 10-liter tank of aerated seawater containing the antibiotics and fungicides furaltadone (0.02%; Sigma) and temerol (0.02%; France). Fish were killed by a blow to the head, then decapitated, and the gills were excised and weighed.

The following procedures were performed in sterile conditions under a laminar flow hood. The gill arches (cartilaginous part) were removed, and the remaining filaments (primary lamellae) were dipped in the washing medium L15 (see Solutions and Chemicals). The filaments were washed under gentle automatic shaking (5 × 10 min, 100 agitations/min).

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and then teased into small tissue fragments (explants). Each explant was inoculated in a plastic culture dish (Nunc; 35 mm diameter, multiwell plates). The culture growth medium L15 was then added gradually to the explants. Explants were then maintained in a precision-controlled low-temperature incubator (Jouan) at 17°C in a humidified air atmosphere (atmospheric PCO₂). The growth medium was changed every second day.

**Patch-Clamp Experiments**

Prior to the patch-clamp experiments, explant culture cells were briefly treated (15–30 s) with a solution of trypsin (0.02%) plus EGTA (0.05%). This slight treatment was performed to isolate electrically each cell from the other without modifying the monolayer epithelium structure. Single-channel currents were recorded in 7- to 13-day-old cultured cells. Patch pipettes made from borosilicate glass (1.5 mm OD, 1.1 mm ID; Clay Adams) were pulled in two steps using a vertical puller (PP-83 Narishige). Pipettes, with resistances from 2 to 5 MΩ were connected via an Ag-AgCl wire to the headstage of an RK 300 patch-clamp amplifier (Biologic). Seals were achieved spontaneously or by applying slight suction to the patch pipette.

**Single-Channel Experiments**

Single-channel currents were stored on digital audiotapes (48 kHz) using a DTR 1200 recorder (Biologic) and later displayed on a digital oscilloscope for analysis (Gould Electronics, Instrument Systems). In the cell-attached configuration, the potential difference across the patch is equal to the cell membrane potential (V_m) minus the pipette clamp potential (V_p). Because V_m is not known exactly, values of V_p are used and are expressed as −V_p, so that the data for potential changes refer to the extracellular side of the membrane. In excised inside-out patch recordings, −V_p (mV) indicates the potential on the cytoplasmic face of the patch membrane relative to the pipette. In some experiments, the pipette perfusion technique described by Tauc et al. (33) was used. The perfusion catheter was made of polyethylene tubing (PE-10, Clay Adams) and tapered at one end (internal diameter 30–50 μm). The perfusion catheter was inserted through the filled patch pipette and connected to the outlet of a syringe containing the test solution. After a control period, test solutions were perfused through the pipette by applying pressure (+10–20 mmHg) to the syringe. The catheter was positioned as close as possible to the pipette and electrode tips to replace quickly (5 s) all the pipette solution and to avoid excessive background noise.

All experiments were performed at room temperature (22 ± 2°C).

**Data Analysis**

Channel current amplitudes were measured from audio-taped data replayed on the digital oscilloscope. Current-voltage (I-V) relationships of channel activity were established from the average amplitude of well-defined transitions between closed and open current levels at each potential (−V_p). For the analysis of channel kinetics, data were transferred to an ERN computer at a sampling frequency of 1 kHz. Channel open probability (P_o) was estimated from current amplitude histograms created and analyzed using Biopatch software (Biologic). The ratio of the areas of the Gaussian-like distributed current-amplitude histograms corresponding to the closed and open states of the channel was taken as an estimate of the P_o. When more than one channel was present in the patch, the level of K⁺ channels activity was quantitated by defining the mean number of open channels (N P_o) as

\[ N P_o = \sum_{n=1}^{N} n Pn \]

where Pn is the probability that n identical channels are open simultaneously and N is the apparent number of channels in the patch.
Reversal potentials ($E_{rev}$) were estimated by interpolation after a polynomial function of the I-V curve was fitted using Sigma Plot and Sigma Stat software (Jandel Scientific).

The $K^+$-$Na^+$ permeability ratio was calculated from $E_{rev}$ in excised patches according to the modified equation of Goldman-Hodgkin-Katz:

$$\frac{P_K}{P_{Na}} = \frac{[Na^+]_{bath} \exp[-E_{rev}/(RT/F)]}{[K^+]_{bath} \exp[-E_{rev}/(RT/F)]}$$

where $P_K$ and $P_{Na}$ are $K^+$ and $Na^+$ permeabilities, respectively, and $R$ is the gas constant, $T$ is the absolute temperature, and $F$ is Faraday's constant. The use of this equation to evaluate selectivity requires the tacit assumption that the channel being studied has a negligible selectivity for anions.

**Solutions and Chemicals**

**Primary cultures.** All solutions were prepared using tissue-culture quality chemicals.

**WASHING MEDIUM.** Leibovitz L15 medium (GIBCO-BRL) was supplemented with 20 mM NaCl, 0.1 mg/ml fungizone, 200 U/ml penicillin, 200 mg/ml streptomycin, and 400 mg/ml gentamicin. All antibiotics were supplied by Sigma. The final pH was adjusted to 7.8 with 1N NaOH.

**CULTURE GROWTH MEDIUM.** Leibovitz L15 medium was supplemented with 10% fetal bovine serum (Multiser, Cytosystems), 20 mM NaCl, 100 U/ml penicillin, 100 mg/ml streptomycin, and 200 mg/ml gentamicin. The final pH was adjusted to 7.8 with 1N NaOH.

**Solutions for electrophysiological studies.** The standard bath and pipette solutions contained (in mM) 140 KCl, 5 HEPES, and 0.1 CaCl$_2$ (pH 7.8 adjusted with 1M KOH) for control conditions. In some experiments, cells were also bathed in a NaCl solution containing (in mM) 140 NaCl, 5 HEPES, and 0.1 CaCl$_2$ (pH 7.8 adjusted with 1N NaOH). For determination of the channel ionic selectivity, solutions containing (in mM) 60 KCl, 80 NaCl, 5 HEPES, and 0.1 CaCl$_2$ or with 100 mM CaCl$_2$ and 5 HEPES were also used.

For pH studies, the standard 140 mM KCl bath solution described above was used; pH was adjusted to 6.8 or 8.2 with stock 1M KOH solution.

For calcium studies, the bath solution contained (in mM) 140 KCl, 5 HEPES, and 0.5 EGTA. The Ca$^{2+}$-free concentration was lowered to $10^{-9}$ M by buffering calcium ions with EGTA.

Barium and gadolinium (Gd$^{3+}$) solutions were prepared from a 1 M BaCl$_2$ or GdCl$_3$ stock solution (Sigma) and added directly to the 140 mM KCl solution. Tetraethylammonium (TEA) and quinidine were each obtained as chloride and sulfate salts (Sigma) and were added to the 140 mM KCl solution at final concentrations of 20 mM for TEA and 50–500 $\mu$M for quinidine.

**RESULTS**

Gigaseals were obtained in 20.5% of the cells tested (in all, 521 cells from 56 monolayers). A total of 107 patches exhibited channel activity. However, this activity depended on the age of the culture in that single-channel activity was never recorded from cells aged <7 days, whereas the maximum activity was found in cells from monolayers aged 10 ± 3 days.

Patch-clamp studies performed with 140 KCl in the pipette and bath solutions on the same cell preparation showed a Cl$^-$ channel with same biophysical characteristics (small conductance <10 pS, potential depen-
was 123 ± 3 pS (n = 24). The zero-current potential (1.6 ± 0.5 mV, n = 24) was close to the expected $E_{\text{rev}}$ for K⁺ (assuming an intracellular K⁺ concentration ranging between 120 and 140 mM). This indicates that the observed current was carried by K⁺. The analysis of four independent seals presenting only one open state revealed that the probability of the channel being in the open-state was independent of the membrane potential ($P_o$ at $-40$ mV = 0.24 ± 0.03; $P_o$ at +40 mV = 0.25 ± 0.01; n = 4). The kinetic analysis of recordings made at $-40$ mV revealed that the channel showed one open state with a time constant of 27 ± 5 ms (n = 4).

Cell-attached recordings were also performed with the NaCl solution in the pipette and the standard KCl solution in the bath (Fig. 1B). Under these conditions, positive currents were observed from $-60$ to $+40$ mV. The corresponding I-V curve for these experiments is shown in Fig. 1C. The relationship was nonlinear over the range $-60$ to $+40$ mV, whereas the extrapolated $E_{\text{rev}}$ (-78 mV) was close to the expected $E_{\text{rev}}$ for K⁺.

The Goldman-Hodgkin-Katz constant field equation was applied to the data on the assumption that the channel was selective for K⁺. As shown in Fig. 1C, the experimental data are described accurately by the theoretical curve. Assuming the cell-attached configuration, internal potassium concentration is 140 mM and the calculated mean permeability is $2.24 \times 10^{-7}$ (n = 3). The maximal channel conductance, calculated as the slope of the curve between 0 and $+40$ mV, was 96 ± 12 pS (n = 3). The I-V curve in Fig. 1C also illustrates the effect of Rb⁺ when the pipette was filled with an RbCl solution. The substitution of KCl with RbCl did not modify the slope conductance (114 ± 7 pS, n = 4) nor the $E_{\text{rev}}$ (1.3 ± 5.4 mV, n = 4), indicating that the channel was equally permeable to K⁺ and Rb⁺ ions.

Channel activity rapidly declined with time in 100% of the cell-attached recordings, irrespective of the ionic composition of the bath and pipette solutions. This spontaneous run-down phenomenon is illustrated in the recording shown in Fig. 2A. In this experiment, the pipette contained the standard KCl solution and the $V_h$ was maintained at $-40$ mV. One minute after seal formation, the channel activity was so low that it was often very difficult to obtain a reliable estimate of the mean $NP_o$. In fact, the time necessary to obtain complete inactivation of channel activity varied from 1 to 3 min. The curve in Fig. 2B shows average results for six cell-attached patches in which inactivation occurred within 1 min.

The mean $NP_o$ decreased with time. After channel activity had been lost, it was possible to restore it by application of a negative pressure to the patch pipette. A typical experiment is shown in Fig. 3A. Increasing the negative pressure >20 mmH₂O produced a rapid and reversible increase in $NP_o$ (Fig. 3C). This stretch activation of the channel was effective only up to a negative pressure of 40 mmH₂O, after which patches usually ruptured. The I-V curve of the reactivated channel (with a negative pressure ranging between 30 and 40 mmH₂O) was linear, with a slope conductance of $132 \pm 6$ pS (n = 7) and a $E_{\text{rev}}$ unchanged from 0 mV (Fig. 3B). Finally, the main characteristics of the channel before and after stretch activation were very similar.

To investigate whether these stretch-activated channels could also be activated by osmotic pressure, the effect of cell swelling, induced by exposure of cells to a hypotonic solution, was then studied. The perfusion of six different cell-attached patches with a 200 mosmol/kgH₂O solution brought about an increase in channel activity. A typical recording obtained at a $V_h$ of $-20$ mV and with the KCl solution in the pipette, is given in Fig. 4A. The mean $NP_o$ increased from 0.05 ± 0.01 to 0.53 ± 0.06, n = 6 (Fig. 4B) within 25 s of the onset of perfusion of the hypotonic solution. This effect was reversible after washout with an isotonic solution. The

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**Fig. 3.** A: negative pressure dependence of K⁺ channel activity for cell-attached configuration. Up to 3 channels are activated simultaneously by increasing negative hydrostatic pressure (applied as suction to the patch pipette) as indicated on left. The pipette potential was held at $-V_p = -20$ mV. Pipette and bath contained 140 mM KCl solution. B: I-V relationship of K⁺ channel in cell-attached configuration during negative pressure stimulation of between 30 and 40 mmH₂O. C: mean $NP_o$ as a function of pipette pressure (suction).
single-channel currents recorded when the pipette solution contained 140 mM KCl and the bath medium 140 mM NaCl. At a $V_h$ of 0 mV, channel openings showed downward deflections with an average unitary current amplitude of $-2.3 \pm 0.1$ pA ($n = 8$). By definition, this had to correspond to the passage of K$^+$ ions, because Na$^+$ ions would have produced upward deflections and Cl$^-$ is at electrochemical equilibrium. The mean I-V relationship of eight experiments performed under these conditions is shown in Fig. 5E. From the current $E_{rev}$ estimated by extrapolation (+74 ± 6 mV), a K$^+$-to-Na$^+$ permeability ratio ($P_K/P_{Na}$) equal to 19 was calculated. The maximal slope conductance was 84 ± 6 pS.

Further experiments were performed by replacing the 140 mM NaCl bathing solution with a solution containing 60 mM KCl and 80 mM NaCl. Figure 5C illustrates single-channel currents recorded under these conditions. The corresponding I-V relationship reported in Fig. 5E displayed an inward rectification over the applied potential range from −50 to +40 mV. In these experimental conditions, the K$^+$ Nernst $E_{rev}$ was calculated at +21.3 mV. The experimental $E_{rev}$ was determined at +19.8 ± 1.2 mV ($n = 7$), leading to a $P_K/P_{Na} = 20.8$. The average conductance of the channel was 76 ± 4 pS ($n = 7$).

To examine the permeability of the channel to Ca$^{2+}$ ions, experiments were performed with 100 mM CaCl$_2$ solution in the pipette and 140 mM standard KCl solution in the bath, respectively. Figure 5D illustrates the kinetic properties of the channel, while the I-V curve in Fig. 5E shows that the currents were always positive for $V_h$ ranging from +20 to −40 mV. The $E_{rev}$ tended toward that of K$^+$; however, it was not possible to record channel activity at potentials more negative than −40 mV because larger hyperpolarizations ruptured the patch seal. Nevertheless, the recorded current was mainly due to K$^+$, indicating an absence of Ca$^{2+}$ permeation through the channel. Under these conditions, the maximal slope conductance of the channel was 104 ± 11 pS ($n = 7$).

As observed in cell-attached experiments, channel activity decreased within 1 to 3 min after the patch was excised. In 17 of the 28 inside-out excised patches, channel activity was completely lost. The application of negative pressure to the patch pipette produced an increase in channel activity that was rapid and reversible on removal of the suction. Such stretch-activation was observed in 11 of 15 patches studied. Figure 6A illustrates this phenomenon for a $V_h$ of −40 mV, when the bath and the pipette solutions contained standard 140 mM KCl. In this typical recording, the application of suction (46 mmH$_2$O) to the pipette induced the opening of three channels. As illustrated in Fig. 6B, this stretch activation did not depend on the Ca$^{2+}$ concentration at the inside surface of the membrane. In fact, lowering Ca$^{2+}$ to $10^{-9}$ M in the bath (see MATERIALS AND METHODS) did not suppress the stretch activation of K$^+$ channels in these cultured cells.

Channel characteristics were then studied in excised patch experiments. Such a study was complicated by the inactivation properties of the channel observed in the cell-attached configuration. For this reason, channel activity was first tested in the cell-attached mode at a $V_h$ of −40 mV. The patch was then excised, and the pulse protocol was rapidly applied between $V_h$ of −40 mV and +40 mV. Figure 5A illustrates the single-channel currents recorded in an excised inside-out patch when the pipette and bath solutions both contained 140 mM standard KCl. In 13 patches, upward and downward currents were observed at positive and negative potentials, respectively. The I-V relationship (Fig. 5E) was linear, with a zero-current potential at 0.47 ± 0.46 mV and a channel conductance of 121 ± 4 pS ($n = 13$).

Under these experimental conditions, the ion selectivity of this channel could not be accurately determined because K$^+$ or Cl$^-$ was able to induce the same current. To verify the K$^+$ selectivity of the channel, inside-out excised patches were perfused on the cytoplasmic side with a K$^+$-free solution or a solution containing only 60 mM K$^+$. Figure 5B illustrates the

$NP_o$ returned to control levels with a similar delay ($NP_o = 0.08 \pm 0.03$, $n = 6$).

Single Channel K$^+$ Current Recorded in Excised Inside-Out Configuration

Figure 4. A: activation of K$^+$ channel in cell-attached patch during change of external hypertonic saline solution to hypotonic solution. Fifty seconds before time zero, the external hypertonic bath medium (350 osmol/kgH$_2$O NaCl) was replaced by a hypotonic medium (200 osmol/kgH$_2$O NaCl). Note the increase in channel activity during the "hypotonic" period. B: mean $NP_o$ during hypotonic shock ($NP_o$ was calculated by computer analysis of periods of 5-s duration) ($n = 6$).
Pharmacological Properties

To identify the nature of the channels under investigation here in cultured seawater fish gill cells, the effect of 5 mM Ba\(^{2+}\) was studied on five excised patches, with standard KCl solution in the pipette and in the bath. As shown in Fig. 7A, the application of Ba\(^{2+}\) to the cytoplasmic side of the channel considerably modified the current recordings. Three channels were opened simultaneously under control conditions at a \(V_h\) of +40 mV. The addition of Ba\(^{2+}\) to the bath reduced the channel openings and the current amplitude. The mean \(N_P\) was estimated from the amplitude histograms of Fig. 7B, the surface of the Gaussian curves corresponding to the \(N_P\) of the three channels measured under control conditions (\(N_P = 0.70\)). In the presence of Ba\(^{2+}\), two of the three open states completely disappeared (\(N_P = 0.14\)).

Gd\(^{3+}\) is a blocker of stretch-activated channels in a variety of tissues, acting principally at the extracellular surface of the channels. To investigate whether K\(^+\) channel activity could be blocked by Gd\(^{3+}\), the perfusing pipette technique of Tauc et al. (33) was used. Figure 7C illustrates the effect of Gd\(^{3+}\) on K\(^+\) channel activity recorded from an inside-out patch. Under control conditions, two channels were open simultaneously. The perfusion of 500 \(\mu\)M Gd\(^{3+}\) through the patch pipette strongly blocked channel openings, thus reducing the mean \(N_P\) from 0.75 ± 0.20 to 0.04 ± 0.02 (\(n = 5\), Fig. 7D). The unitary conductance of the channel, however, remained unchanged.
The present study focused on the identification of potassium channels present on gill cells in primary culture. Patch-clamp experiments were performed on the apical membrane of these cells. This single-channel analysis revealed the existence of a channel that was selective to potassium over sodium ions. In the cell-attached mode and under control conditions (KCl standard solution), the channel was spontaneously active at the resting membrane potential and could permit K\(^+\) efflux from the cell. The unitary conductance of the K\(^+\) channel under these conditions ranged between 100 (physiological conditions) and 120 pS (symmetrical K\(^+\) solutions).

The kinetic properties of the channel in excised configuration were not distinguishable from those seen in cell-attached patches. For both recording configurations and in the presence of identical K\(^+\) concentrations on both sides of the patch, the inward and outward K\(^+\) currents were similar. The rectification observed in the presence of asymmetrical solutions was the consequence of a Goldman-type rectification. Moreover, the open state probability of the channel did not exhibit voltage-dependence characteristics over the range of imposed potentials.

The channel was blocked by Ba\(^{2+}\) ions, with the kinetics of this inhibition being characteristic of the action of slow blocking agents. Thus Ba\(^{2+}\) ions reduced the length of the burst duration and induced long periods of inactivity between any two bursts. Moreover, Ba\(^{2+}\) reduced not only the open probability of the channel, but also the amplitude of the current flowing

Dependence on pH

To examine the pH dependence of K\(^+\) channel activity, the pH of solutions was varied at the cytoplasmic surface of inside-out patches. In this way, pipettes were filled with the standard KCl solution, whereas the bath contained the same solution at one of three different pH values. Figure 9A shows single-channel current trace for one patch held at -20 mV and in the presence of bath solutions at pH 7.7, 6.8, and 8.2. It can be clearly seen that a change in pH from 7.7 to 6.8 reduced channel activity, corresponding to a large decrease in the mean NP\(_o\) from 0.71 ± 0.21 to 0.03 ± 0.01 (n = 7; Fig. 9B). The activity of the patch was partially recovered when the pH was increased secondarily to 8.2. To test the reversibility of the pH effect, we performed additional experiments with repetitive decrease and increase of pH over the time in the same recording. In that case, we observe a total reversible effect of acidification (Fig. 9C). The correlated mean number of open channels followed the same inhibition and stimulation profile (Fig. 9D).

**K\(^+\) CHANNELS IN FISH GILL CELLS**
through the channel. Quinidine and gadolinium were also potent inhibitors of the channel, whereas TEA did not influence its activity.

Cytosolic acidification has been shown strongly to reduce open-time probability in many \( K_1 \) channels (8, 12). The maximal channel activity observed in the present study was with an intracellular pH ranging from 7.5 to 7.8. It is possible that a culture medium maintained at pH 7.8 represents physiological pH values in seawater fish gill cells (considering that this pH value is close to that of sea bass plasma). In the present study, the effect of acidification was partially or totally reversible. Repetitive decrease and increase of pH over the time in the same recording induced a reversible effect of acidification showing that the inhibition of the activity of the channel was a real effect and not a run-down phenomenon.

Some interesting findings made here concerned the fact that channel activity exhibited a spontaneous run-down phenomenon that could be reactivated by the application of a negative pressure via the patch pipette. The reactivated channel exhibited kinetic and conductance characteristics very similar to those determined before the inactivation process taking place. This suggests that the pipette suction probably reactivated the same channel and that the \( K_1 \) channel in the present study was a stretch-activated channel. Ion channels activated by membrane stretch have been detected in several epithelial cells (7, 35, 36). Many of these channels are nonselective stretch-activated channels with single conductances ranging between 25 and 35 pS. In primary cultures of gill cells, it appears that the channel was at least 19 times more permeable to \( K^+ \) than to \( Na^+ \) and relatively impermeable to \( Ca^{2+} \).
Stretch-activated K⁺ channels with small conductances and insensitive to calcium have been identified in amphibian proximal tubules (15, 16, 27–29). However, none of these channels exhibit characteristics such as those identified here in gill cell K⁺ channels. Maxi K⁺ channels have also been shown to exhibit mechanosensitive properties in different cell preparations. For example, Pacha et al. (23) described a high-conductance K⁺ channel in the apical membrane of intercalated cells of the rabbit collecting duct that shares some of its properties with the K⁺ channel examined in the present study. In particular, the conductance was close to 100 pS and the channel could be activated by pipette suction in the cell-attached as well as inside-out patch-clamp configurations. Moreover, this mechanical activation was not dependent on cytosolic calcium. Some important differences between the two channel types, however, also exist. Notably, the activity of K⁺ channels in gill cells was not gated by voltage and was not sensitive to TEA. Therefore, the mechanical activation could definitely not be attributed to an increase in cytosolic Ca²⁺. This observation led to the conclusion that the channel was activated directly by membrane stretching. It is therefore evident that the channel is considerably different from the large conductance K⁺ channel (maxi K⁺ channels) typically found in other epithelial tissues (4, 22, 24). Finally, because very little information exists concerning K⁺ channels in fish gill cells, it is not possible to directly compare the K⁺ channel in the present investigation with K⁺ channels described in other reports. The most relevant studies concerning our findings are those of Gögelein et al. (12) and Greger et al. (13) on the rectal gland of the dogfish and of Chang and
solution. The pipette potential was held constant at
the bath solution from 7.7 to 6.8 and a partial reactivation by
inside-out patch. Note the inhibition of K1
channel. Therefore, to our knowledge, the present work
and K1
channel with a unitary conductance,
channel in cultured gill cells. The
former work revealed that the basolateral membrane
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To understand the physiological role of the K+ chan-
nels in the apical membrane of gill cells, it is necessary
to know whether this channel is active at the resting
membrane potential. This question is important be-
cause the channel inactivated rapidly after seal forma-
tion in the cell-attached and inside-out patch-clamp
configurations. The inactivation process could be inter-
preted in light of two different hypotheses. 1) The
channel was inactive at rest before seal formation and
the application of suction to seal the membrane-in-
duced stretching that activated the channel. Subse-
sequently, when the application of negative pressure was
terminated, the patch returned to its inactive state. 2) The
channel was active at rest, and its spontaneous
activity was, therefore, representative of the cell con-
ductance under normal conditions. After the seal for-
mation, the channel run down observed could have been due to mechanical constraints on the cell mem-
brane induced by the patch pipette.

The importance of the membrane geometry on mech-
anosensitive K+ channels was recently studied by Pa-
tel et al. (25). They identified a cloned mammalian two
P-domain mechanogated S-like K+ channel and demon-
strated that the opening of TREK 1 (tandem of P
domains in a weak inward rectifying related K+ chan-
nel) was highly sensitive to the curvature of the mem-
brane. We favor this second hypothesis over the first
because, in whole cell experiments (see companion
paper, Ref. 9a), a spontaneous decrease of the ionic
conductance was never observed. According to the data
of Patel et al. (25), it is reasonable to postulate that the
cell membrane exhibits a crenated form at rest, which is
compatible with channel activity. Soon after the
formation of a gigaseal, this notched configuration of
the membrane progressively disappears because the
membrane beneath the patch becomes more rigid. The
crenated form is then replaced by a cup form (which is
not conductive to channel activity). Afterward, the
application of a negative pressure reinforces the crenated
form and channel activity is restored. The fact that a
positive pressure was unable to induce channel activity
would tend to favor this explanation.

Considering that the K+ channel could be active in
resting cells, it may be involved in K+ fluxes across the
apical membrane of gill cells. We previously demon-
strated that primary cultures of respiratory cells of the
sea bass gill represent a good model for studying Cl-
secreting epithelia (2, 10). In accordance with classical
models for electrogenic chloride secretion, the secretion
of K+ takes place across the basolateral membrane and
serves to recycle K+ ions accumulated in the cytosol by
the Na+-K+-ATPase and the Na+-K+-2Cl- cotrans-
porter mechanisms (13). However, K+ channel activity
has been recorded in the apical membrane of some
chloride-secreting epithelia, including lacrimal acinar
cells (31) and vas deferens epithelial cells (30). More-
over, K+ fluxes have been also measured across the
isolated skin of the marine teleost Gillichthys mirabilis
(20) and seawater opercular epithelia (21).

Using an equivalent circuit model, Cook and Young
(9) examined the effect of an apical K+ conductance on

Fig. 9. A: effect of pH variations on K+ channel activity in an excised
inside-out patch. Note the inhibition of K+ channels by acidification
of the bath solution from 7.7 to 6.8 and a partial reactivation by
increasing pH to 8.2. Pipette and bath contained 140 mM KCl
solution. The pipette potential was held constant at −Vp = −20 mV.
B: representation of the mean NPo for 3 different values of pH: 7.7,
6.8, and 8.2. Values are means ± SE for 7 experiments.
C: repetitive decrease (from 7.7 to 6.8) and increase (from 6.8 to 7.7) of pH over the
time showing a total reversible effect of acidification.
D: correlated mean NPo after the same inhibition and stimulation profile.
the secretion of fluid by an epithelium in which secretion was driven by the secondary active transport of Cl\(^-\). They concluded that a K\(^+\) conductance in the apical membrane could enhance secretion with a maximal effect when the proportion is \(\sim 10-20\%\) of the total K\(^+\) conductance. In the marine teleost, this effect could be of importance, because the gill cell must always excrete high quantities of Cl\(^-\) against a strong electrochemical gradient. Therefore, an apical K\(^+\) conductance could improve Cl\(^-\) secretion by maintaining a high potential difference across the apical membrane.

Many studies have demonstrated that stretch-activated channels are also activated by hypotonic shock and could be implicated in the cell volume regulation (see Ref. 14 for review). Different types of K\(^+\) channels could be involved in such regulation. First, osmotic shock could modulate Ca\(^{2+}\) influx through stretch-activated cation channels, whereas a local increase in cytosolic Ca\(^{2+}\) could activate Ca\(^{2+}\)-sensitive K\(^+\) channels. This hypothesis was first proposed by Christensen (7), whereas a similar mechanism had already been postulated to explain regulatory volume decrease in different epithelial cell types (17, 19, 26, 32, 34, 35). Second, mechanical stress on the cell membrane induced by the osmotic shock could open two different types of truly stretch-activated K\(^+\) channels. These K\(^+\) channels are either Ca\(^{2+}\) sensitive (18, 23) or Ca\(^{2+}\) insensitive (11, 28). The K\(^+\) channel described in the present study probably belongs to the second category. Two types of Ca\(^{2+}\)-insensitive stretch-activated K\(^+\) channels have been demonstrated in the basolateral membrane of *Necturus* proximal tubule. These channels exhibit a lower conductance than that of the channel described here, but interestingly they are activated by pipette suction and by osmotic swelling (11, 16).

In the cell-attached experiments described here, exposure of cells to a hypotonic solution induced an increase in channel activity. The kinetics of this form of channel activation were similar to that obtained when negative pressure was applied to the patch pipette. Thus, in cultured gill cells, the stretch-activated K\(^+\) channel is regulated by both mechanical pressure and osmotic change, suggesting that channel activation could be involved in the control of cell volume following hypotonic shock.

**Perspectives**

The sea bass is considered euryhaline and capable of surviving in an estuarine environment where seawater salinity is drastically reduced (3). Therefore, the fish could be naturally subjected to significant changes in osmotic gradients that would initially affect the gill epithelia. It is thus reasonable to postulate that the gill cells have developed efficient mechanisms of cell volume regulation. The large conductance stretch-activated K\(^+\) channel described here could form part of these mechanisms.

In a companion paper (9a), we investigate potassium conductances and fluxes in both apical and basolateral membranes and their behavior in response to exposure of the membranes to a hypotonic medium. For this purpose, whole cell patch-clamp and \(^{86}\)Rb\(^-\) efflux experiments on primary cultures of gill cell were employed.

**REFERENCES**


