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Invertebrate epithelial Na^+ channels: amiloride-induced current-noise in crab gill

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Epithelial sheets (including cuticle) from posterior gills of the freshwater-adapted euryhaline crab *Eriocheir sinensis* were obtained according to the method of Schwarz and Graszynski ((1989) Comp. Biochem. Physiol. 92A, 601–604; (1989) Verh. Dtsch. Zool. Ges. 82, 211 and (1989) Arch. Int. Physiol. Biochim. 97, C45). With external NaCl-saline, the outward-directed short-circuit current (I_{sc}) could hardly be influenced by external amiloride up to 100 μmol/l but was, on the contrary, strictly dependent on apical Cl^- (Onken, Graszynski and Zeiske (1991) J. Comp. Physiol. B 161, 293–301). In absence of external chloride an inward-directed, amiloride-inhibitable I_{sc} was observed which depended on external Na^+ (thus, $I_{sc} \approx I_{Na}$) in a two-step, saturating mode. The I_{sc}-block by amiloride obeyed saturation kinetics (half-maximal at $\leqslant 1$ μmol/l, suggesting apical Na^+-channels). Only for Na^+ concentrations below 100 mmol/l we found an indication for a competitive interaction between Na^+ and amiloride at the channel. Current fluctuation analysis revealed the presence of an amiloride-induced relaxation (Lorentzian) component in the I_{sc}-noise (so-called 'blocker-noise'). The Lorentzian parameter-shifts with increasing amiloride concentration indicate first-order kinetics of the blocker with its apical receptor. Using a 'two-state' blocking model we calculated, for amiloride concentrations between 2 and 5 μmol/l, a mean single-channel current of 0.46 pA and a mean channel density of $250 \cdot 10^6$ cm^{-2}.

Introduction

NaCl absorption by crustacean posterior gill has been shown to be composed of two parallel, active and electrogenic transport processes. The underlying membrane transport mechanisms are, however, far from being clear. Thus, it has been found (for review, see Ref. 5) that, in perfused gill preparations under open-circuit conditions, a transepithelial, inside-negative potential difference develops which depends on the presence of both, external Na^+ as well as Cl^-. In *Uca* or *Carcinus* gills, some interdependence of Cl^-- and Na^+-transport appears to exist as the fluxes of these ions and the electrical parameters may be influenced by the same manoeuvers, for instance, blockage of the basolateral Na^+/K^+-ATPase by ouabain. On the other hand, for the gill of the Chinese crab, *Eriocheir sinensis*, treatment with ouabain affects only the Na^+-related part of the PD in perfused gills [6], or of the respective short-circuit current in split-lamellae preparations [7], while the Cl^--related portion of the PD [6] or the respective I_{sc} [4] remain unaffected. Application of amiloride to the external bath of isolated gills reduced net sodium uptake in all preparations, and the PD, when measured, often attained even more negative values. Many PD data would be compatible with the assumption of the involvement of electrically responsive apical Na^+ channels, like postulated for *Uca* [8]. On the other hand, the reported comparably moderate inhibitory effect of amiloride on Na^+ fluxes and PD [9,10], led to the assumption that a Na^+/H^+ exchanger might be responsible for the apical sodium transfer.

A new preparation method allows to mount half-platelets of crab gill lamellae in an Ussing-type chamber under voltage-clamp conditions [3]. It was found for the gills of freshwater-adapted *Eriocheir* and in the absence of ambient chloride, that a Na^+-dependent, inward-directed short-circuit current (I_{sc}) could be completely eliminated with internal ouabain or external amiloride. The observed low apparent amiloride

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dissociation constant of less than 1 μmol/l suggested the participation of apical Na^+ channels [7,11].

In the present study, we describe, for the absence and presence of amiloride, the kinetics of this electrogenic sodium transport across the voltage-clamped Chinese crab 'split-gill' preparation. We further investigate the amiloride-induced fluctuations in the short-circuit current which is carried by Na^+ ions. Our data strongly suggest that apical amiloride-blockable sodium pores, or channels, rather than exchange mechanisms, are the principal mediators of the transepithelial electrogenic Na^+ uptake across crab gill.

A preliminary communication of part of our findings has been presented at the 1991 Spring Meeting of the German Physiological Society [12].

Materials and Methods

A. The preparation

Chinese crabs (*Eriocheir sinensis*) were obtained from commercial fishermen at the coast of the North Sea. The animals were kept in running tap water at about 15°C for at least one month before use. For the experiments, posterior gills were removed and split-lamellae were prepared as described by Schwarz and Graszynski [1,2]. The so-obtained half-platelet of a gill lamella (area about 2×4 mm^2) consists of a single epithelial layer with many finger-like apical membrane protrusions [7], which is lined by an apical cuticle and rests on a basal laminar structure. The epithelium was, by means of a small forceps and under microscopic control, mounted cautiously and avoiding edge-damage (Glisseal sealing grease) in a modified Ussing-chamber with an epithelial area of only 0.0078 cm^2 exposed to the saline-containing 1 ml-chamber compartments [1,4,7].

The chamber perfusion with aerated saline was continuous (gravity-driven) at a rate of about 10 ml/min. Thus, a sufficiently fast solution exchange could be matched with the requirement of noise analysis to avoid mechanical disturbance [13], but also to prevent contamination with Cl^- ions from the agar bridges (see below) which was especially important when chloride-free saline had to be used.

B. Solutions and chemicals

The standard, hemolymph-like saline which was always used as basolateral bathing solution, was composed of (in mmol/l): 300 NaCl, 8 KCl, 2 $NaHCO_3$, 5 Hepes, 8 Ca-gluconate, 2 glucose; pH 7.6 (Tris, gluconolactone). In chloride-free external salines the chlorides were substituted by gluconates while NaCl-free salines were prepared with Tris-gluconate as a substitute. Amiloride was a gift from Merck, Sharp and Dohme; theophylline was purchased from Serva. The latter agent is known to stimulate, by preventing cAMP-breakdown, Na^+ uptake in a variety of tight vertebrate epithelia [14], and it also stimulates Na^+ current across the crab gill epithelium [15]. Theophylline was employed in preparations where the spontaneous rate of sodium transport was small.

C. Measurement of short-circuit current and current fluctuations

PD and I_{sc} were recorded as described before [4]. For the current fluctuation ('noise') analysis experiments, we used a specially constructed low-noise voltage-clamp apparatus designed and modified after the original version by Van Driessche and Lindemann [16].

Fluctuations of the short-circuit current were recorded digitally after initially passing the clamp current through a set of (anti-aliasing) high- and low-pass filters, and after appropriate amplification at each step. Details may be found elsewhere, e.g. in Refs. 13, 17 and 18. Fast Fourier analysis of the I_{sc}-noise yields the I_{sc}-variance/frequency distribution, the so-called power density spectrum (PDS; see Fig. 4A).

When a Lorentzian (see below) noise component was contained in the PDS (cf. Fig. 4A), the data points were fitted [19] by the sum $S(f)$ of a Lorentzian noise component (S_L; see Eqn. 1) and a presumably linear background noise, $S_B = K/f^{\alpha}$, where the fitting parameter K is the noise power at the frequency of 1 Hz and α the negative slope of the low-frequency background noise in the PDS (see inset Fig. 4A). The Lorentzian (or relaxation-) component (S_L) in the PDS of the I_{sc}-fluctuations (Fig. 4A) is described by

$$S_L = S_0/[1 + (f/f_c)^2] \tag{1}$$

with the fitting parameters S_0 and f_c: S_0 is the low-frequency plateau, f_c is the ('corner') frequency where $S_L = S_0/2$.

If amiloride blocks Na^+ channels with pseudo-first-order kinetics ([14,20–23]; see also Fig. 4 in the present paper), the following relations are valid:

$$2\pi f_c = k_{01} c_{AMI} + k_{10} \tag{2}$$

$$I_{Na(A)} = i \cdot M \cdot P_0 \tag{3}$$

$$P_0 = k_{10}/2\pi f_c \tag{4}$$

$$S_0 = 4 \cdot I_{Na(A)} \cdot i \cdot (1 - P_0)/2\pi f_c \tag{5}$$

We define: association (k_{01}) and dissociation (k_{10}) rate constant for the amiloride–Na^+ channel interaction; amiloride concentration c_{AMI}; apparent amiloride inhibition constant $K_A = k_{10}/k_{01}$; sodium current in presence of amiloride $I_{Na(A)}$; single-channel Na^+ current i; channel area density M; channel open probability P_0. With the macroscopic current $I_{Na(A)}$ and the Lorentzian

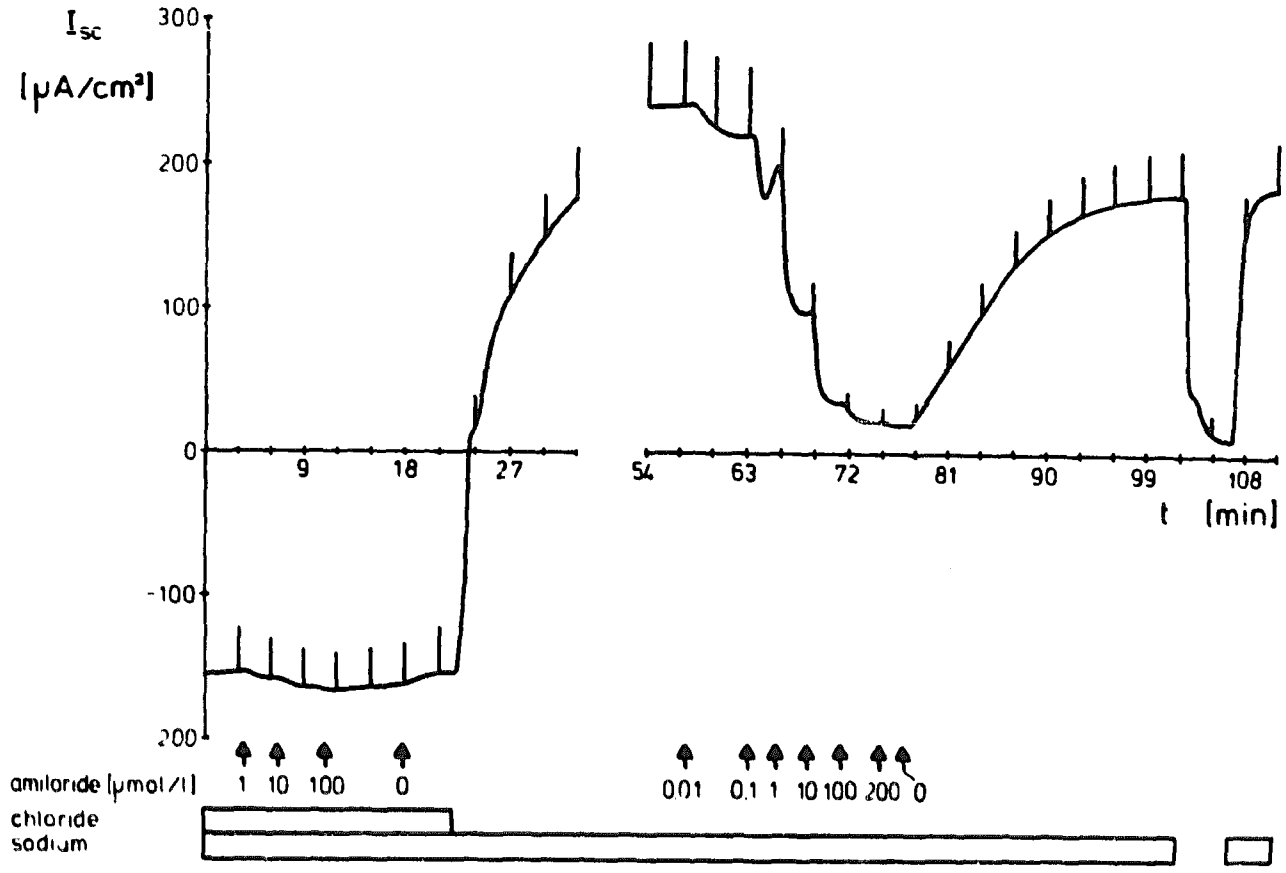

Fig. 1. Dependence of the short-circuit current (I_{sc}) on external Na^+, Cl^- and amiloride. Internal standard NaCl-saline without theophylline. Conductance: Vertical current deflections induced by a 10 mV voltage-clamp pulse. Cl^- substitute: gluconate. Na^+ substitute: Tris.

fitting parameters S_o and f_c for a given amiloride concentration, combination of the above equations enables to calculate i and M for a given Na^+ concentration c_{Na}.

Results

A. Currents

In absence of external sodium but presence of chloride, the gill epithelium of *Eriocheir sinensis* shows an outward-directed short-circuit current, I_{sc}, which reflects the electrogenic inward movement of Cl^- ions [4]. When the tissue is bathed with NaCl-rich standard saline on both sides (Fig. 1) an analogously oriented I_{sc} is obtained. The addition of amiloride (up to 100 μmol/l) to the external solution leads to only a very small change in I_{sc} whereas the complete removal of external chloride quickly abolishes the current. Moreover, a large, inward-directed current develops within a few minutes after Cl^--omission. Subsequent addition of amiloride results in a fast current drop which seems to be complete at 200 μmol/l of the drug. After washout of the diuretic, the positive I_{sc} is recognized to be a sodium current (inward; I_{Na}) as it practically disappears when apical Tris is substituted for Na^+. I_{Na} is reduced by about 50% with 1 μmol/l of the diuretic, and the transepithelial conductance (proportional to the length of the vertical bars) follows closely the course of I_{sc}. The current reduction by amiloride is reversible and the changes of the electrical parameters seem to occur as fast as the solution changes can be achieved, which may be taken as indication of an apical location of the I_{Na}-blocking site. The findings in Fig. 1 are similar to previously published data [7,11].

Fig. 2 illustrates in a semilogarithmic plot for three tissues that the percent inhibition of I_{Na} by gradually rising the amiloride concentration follows saturation

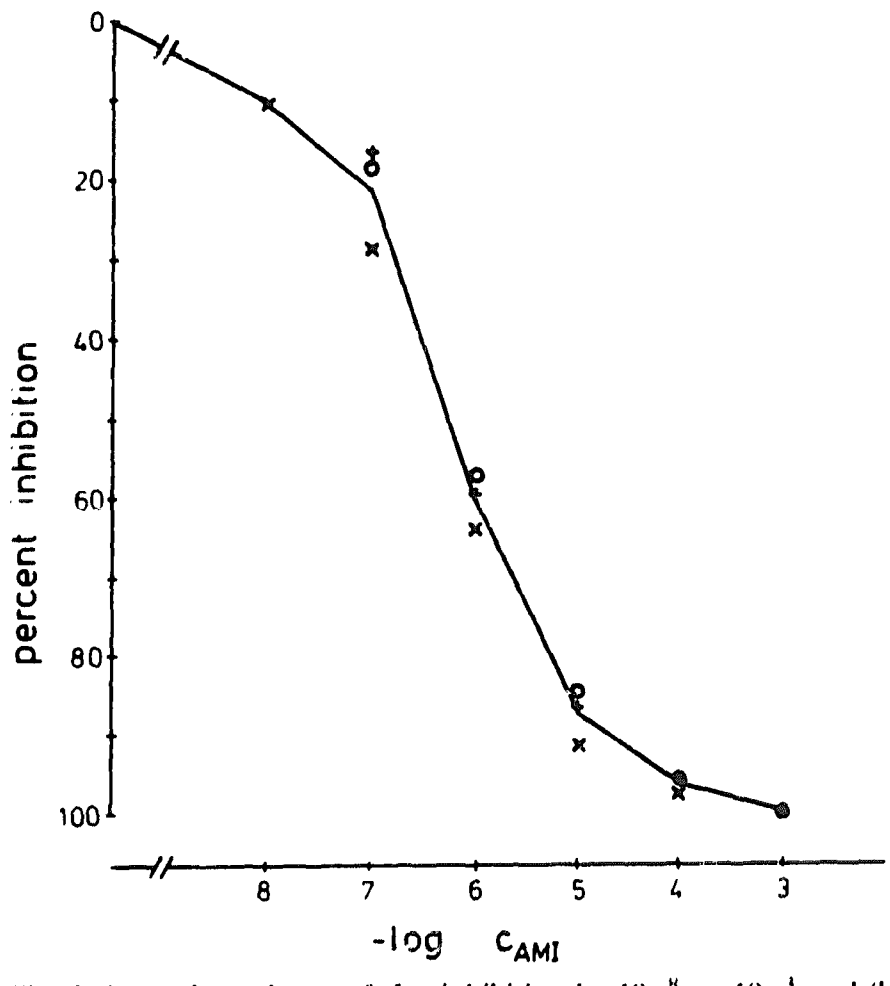

Fig. 2. Dose-dependence of I_{Na}-inhibition by 10^{-8} to 10^{-3} mol/l amiloride (three tissues not stimulated with theophylline; symbols +, ○, ×). I_{Na} is defined as I_{sc} corrected for shunt currents in presence of $2 \cdot 10^{-4}$ to 10^{-3} mol/l amiloride in the external Na^+ gluconate saline.

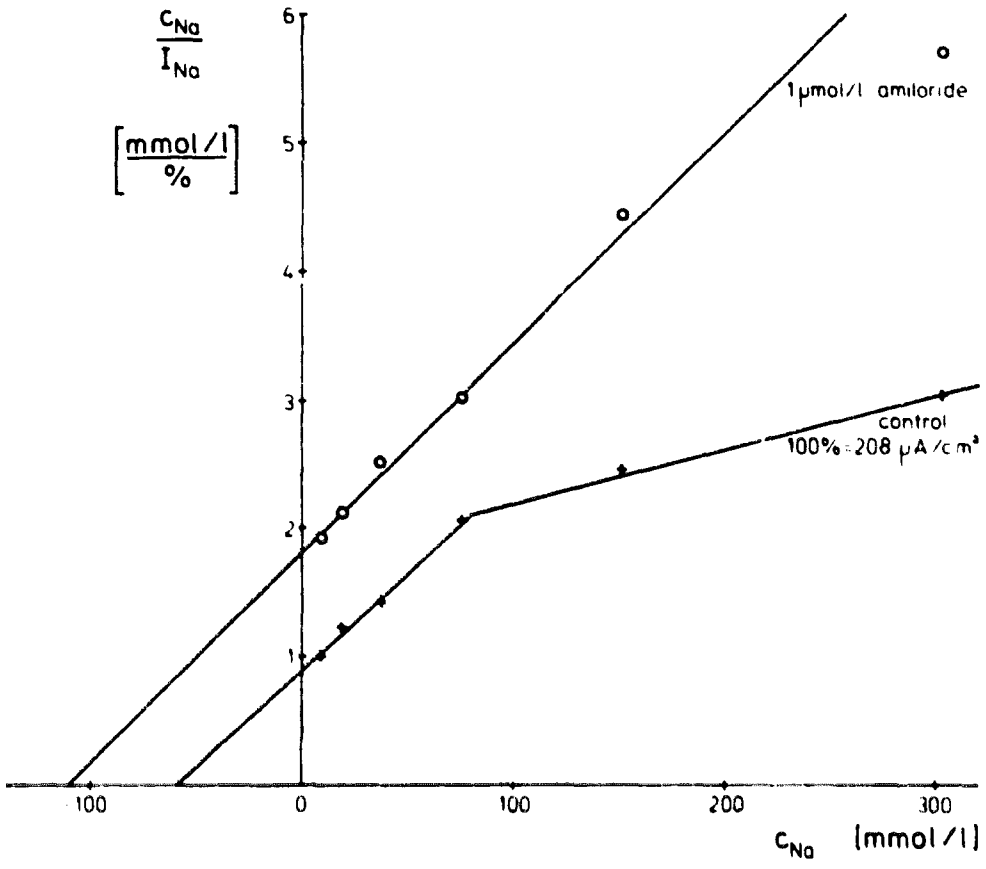

Fig. 3. Hanes plot (c_{Na}/I_{Na} vs. c_{Na}) for a typical saturating sodium current with increasing external c_{Na} in the absence (control) or presence of 1 μmol/l external amiloride. Sodium current was defined as the difference between the short-circuit current without, and in presence of, 200 μmol/l external amiloride at each c_{Na}. Furthermore, I_{Na} was normalized to the Na^+-current obtained with 302 mmol/l Na^+. For the control case, the I_{Na}-saturation level (determined from the inverse slope at high c_{Na}) is 500 μA cm^{-2}. 1 μmol/l amiloride shifts the line left from the break (control) upwards; the abscissa intercept increases from 60 to 110 mmol/l.

kinetics. Half-maximal blockade occurs near 0.6 μmol/l which is consistent with earlier findings by Schwarz and Graszynski [11], who postulated that amiloride acts on an apical Na^+ channel in the gill epithelium of *Eriocheir*.

In order to establish the nature of I_{Na} inhibition by amiloride, we studied the I_{Na}-dependence on the concentration of external Na^+ and the influence of amiloride on the so-obtained current kinetics. When c_{Na} is gradually increased (substitution of Tris), I_{Na} increases in a relatively flat, but subproportional manner (not shown). The corresponding Hanes diagram of this Na^+-induced I_{sc}-increase (termed I_{Na}) is shown for a representative experiment in Fig. 3: This analysis reveals a 'two-step' saturation behavior (control) of the sodium current (here normalized to the value recorded with 302 mmol/l Na^+) since we observe a well-defined 'break' in the otherwise linear relationship.

Complete linearity would be a hint for Michaelis-Menten kinetics, yielding the Michaelis constant from the negative abscissa intercept and the maximal I_{Na} from the inverse slope. However, linearity is only observed after addition of amiloride: With 1 μmol/l of the drug the break disappears and, for almost the entire range of c_{Na}, we now observe a straight line relationship in the Hanes plot. This line appears shifted upwards and parallel, when compared to the c_{Na} range left from the break in control. Such a shift would, indeed, be expected if Na^+ and amiloride were competitors, at least in that c_{Na} range.

For three analogous experiments we found as mean values (± S.E.) for the negative abscissa intercepts 60 ± 6 (CTR) and 112 ± 6 mmol/l (AMI). The inverse low-c_{Na} slopes were 133 ± 11 (CTR) and 96 ± 16 (AMI) μA/cm^2. The inverse high-c_{Na} slope was 500 ± 100 μA/cm^2 (only control).

B. Current fluctuations

A power density spectrum (PDS) of the I_{sc}-noise is depicted in Fig. 4A. When the apical, Na^+-rich but Cl^--free, saline contains no amiloride an all over quite flat (slope about −1) frequency distribution of the noise power can be seen. After addition of 2.5 μmol/l amiloride, a relaxation, or Lorentzian, noise component (S_L) arises in the power spectrum. The data fit (cf. Methods) yields as Lorentzian parameters a plateau value of $9.1 \cdot 10^{-20}$ A^2 s cm^{-2} and a corner frequency of 37 s^{-1}.

Increasing the blocker concentration to 50 μmol/l shifts the still well observable relaxation noise component right-downward. The shift is displayed, in the diagram of Fig. 4B, for the same tissue but for a wide range of amiloride concentrations: With increasing dose of the diuretic, the plateau value first reaches a sharp maximum near K_A followed by a steep decay, whereas the corner frequency shows a perfectly linear increase up to 50 μmol/l amiloride. This behavior is theoretically exactly predicted for a first-order rate process between blocker and ionic channel ([19,20], see also Methods). For this (theophylline-treated) epithelium,

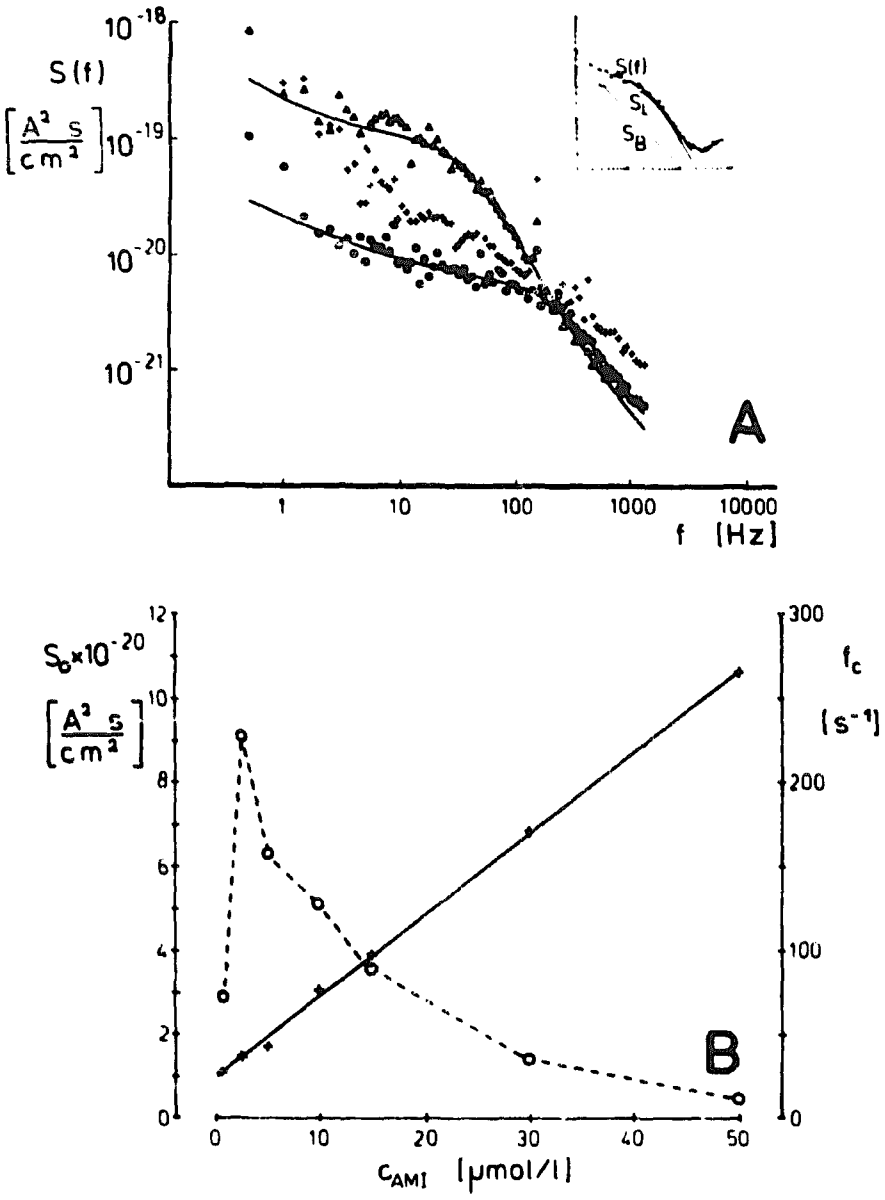

Fig. 4. (A) Power density spectra (PDS) of the I_{sc}-fluctuations in absence (crosses; slope roughly -1) and presence of 2.5 (triangles) and 50 μmol/l (dots) external amiloride. The spectral data are fitted (smooth lines) by the sum $S(f)$ of a Lorentzian component (S_L; high-frequency slope -2 and low-frequency plateau) and a linear background component (S_B); see inset and Methods. Tissue with 5 mmol/l theophylline. More details in text. (B) Dependence of Lorentzian plateau value, S_0 (open circles), and corner frequency, f_c (crosses), for the same tissue on increasing amiloride concentrations.

we find, according to Eqn. 2, as slope of the $2\pi f_c$-c_{AMI} relationship 31 μmol^{-1} s^{-1} l and as ordinate intercept 157 s^{-1} (see also Fig. 4B). For K_{AMI} we then obtain 5 μmol/l which is higher than estimated from the half-maximal current suppression (see, e.g. Fig. 2). Fig. 5 illustrates, for a data pool of $n = 3$ to 5, the average $2\pi f_c$-[AMI] relationship. From a linear regression over the entire range of amiloride concentrations ($r = 0.99$, smooth line) we determine the slope $k_{01} = 32.9$ μmol^{-1} s^{-1} l and the ordinate intercept $k_{10} = 105.8$ s^{-1} which are similar to the data obtained from the above single experiment (Fig. 4B). At the highest amiloride concentration, a correct determination of Na^+ current and the Lorentzian fit becomes much more sensitive to errors. Therefore, we fitted this relation also with the exclusion of the data for 50 μmol/l amiloride (dashed line). We now obtain $k_{01} = 42$ μmol^{-1} s^{-1} l and $k_{10} = 31$ s^{-1}, giving a K_A very close to the one obtained from Fig. 2. Both rate constants are well in the range as determined for other tight epithelia [24].

From the set of the equations given in Methods, we may calculate i and M for a particular c_{AMI} at the given high c_{Na}. To this purpose, the blocker concentration has to be chosen such that a large Lorentzian response is obtained (see Fig. 4B). This will not only reduce the possibility of an erroneous determination of the Lorentzian parameters with respect to the background noise, but at the same time I_{Na} is still large enough to be accurately determined (here by correcting the I_{sc} for the presumably non-specific current in presence of 200 μmol/l amiloride, a concentration which is likely to maximally block I_{Na}; cf. Figs. 1 and 2). These conditions are met with amiloride concentrations between 1 and 5 μmol/l.

Table I lists macroscopic and microscopic (i.e. noise) parameters from six preparations. We may learn from this evaluation that the single-channel current is in a range which may be taken as evidence [25] that the amiloride-blockable molecular entity must be a pore structure with inherent high ion-turnover rate. Obviously, i is of the same order of magnitude as for Na^+ channels of other tight epithelia [14]. Since no consistent and systematic variation with c_{AMI} was seen, we may calculate a mean ($\pm$S.E.) single-channel current of 0.46 ± 0.05 pA and a mean channel density of $(250 \pm 60) \cdot 10^6$ cm^{-2}.

TABLE I

Macroscopic and noise parameters obtained from six crab gill epithelia

Displayed are: sodium-specific current I_{Na} (μA cm^{-2}) in absence of amiloride; amiloride concentration c_{AMI} (μmol/l); channel open-probability P_0 (i.e. ratio sodium current in presence/sodium current in absence of blocker); Lorentzian plateau value S_0 (A^2 s cm^{-2}); chemical rate $2\pi f_c$ (s^{-1}); single-channel current i (pA); channel area-density M (cm^{-2}).

I_{Na}	c_{AMI}	P_0	$2\pi f_c$	S_0 ($\times 10^{20}$)	i	M ($\times 10^{-6}$)
98.7	2.5	0.39	232	9	0.40	208
53.4	2.5	0.37	132	16	0.41	59
184.8	3.1	0.32	371	7	0.43	484
196.8	3.1	0.25	170	26	0.47	163
349.0	3.1	0.27	232	60	0.67	302
296.2	5.0	0.15	270	18	0.36	280

Discussion

1. Methodical aspects

A. Basic experimental conditions

Among vertebrates, e.g., frogs or freshwater fish experience much the same osmotic problem as do freshwater (-adapted) crustacea. Active Na^+ uptake across the frog skin [26,27], the fish gill [28,29] and several crustacean gills (for review, see Ref. 5) are well documented for numerous experimental conditions and with many different methodical approaches.

The experiments dealt with in the present paper are designed to register electrogenic Na^+ pathways in the apical crab gill cell membrane. To this purpose, we tried to keep close-to-natural internal conditions with a hemolymph-like NaCl-saline. When the 'spontaneous' Na^+ current was low after mounting the epithelium in the Ussing chamber, we enhanced the Na^+ current by adding 2 to 5 mmol/l theophylline, which is a well-known inhibitor of the cAMP-decomposing phosphodiesterase [30], and a potent stimulator of Na^+ transport in a number of epithelia [14] including Chinese crab gill [15]. The external saline is not close-to-natural as it is of the same osmolarity and ionic strength as the internal bathing saline, but it lacks chloride since this ion seems to impair the Na^+ transport (see Fig. 1) which is unusual for other epithelia containing Na^+ channels.

Defining I_{Na} (see Figs. 2 and 3) as amiloride-sensitive I_{sc} eliminates the need to discuss the so-generated, if small (Fig. 1; cf. Refs. 4, 5 and 10), shunt currents.

Strictly speaking, we here demonstrate the existence of electrically active Na^+ channels (by means of noise analysis) only for the conditions described here, but the experimental conditions used by different workers vary greatly. Thus, transmembrane and saturating Na^+-influxes via non-channel pathways, as postulated by others [9,10], may well exist, and different types of sodium transporters (e.g., Na^+/H^+ antiport vs. Na^+ channel) could simply be active under different experimental conditions. It is beyond doubt that Na^+ channels mediate sodium absorption not only in the present preparation but also at close-to-natural conditions in the frog skin [31] or the fish gill [29]. However, for situations other than described here, channels as Na^+-supplying pathways in the crab gill still remain to be established.

B. Na^+ conduction-noise induced by amiloride: Localization of the reaction site and applicability of the two-state model

The changes in current seem to occur immediately following the switch between different salines, and rapid onset and full reversibility of the effects (amiloride, Na^+- and Cl^--substitution) suggest that we observe reactions of a rate-determining barrier at the external face of the gill epithelium.

The gill cuticles of *Carcinus* and *Eriocheir* have been shown to possess a certain selectivity among small monovalent ions as well as a sensitivity towards amiloride [32] but the contribution of the cuticle to rate-limiting transport phenomena should be small: This is indicated by its low resistance [1] and a many-fold higher permeability to small cations than observed with the whole gill [5]. The low basolateral fractional resistance (below 0.2) observed with microelectrodes for Cl^--transporting- [4] as well as for the present conditions (Onken, H., unpublished data) would exclude the observability of basolateral noise [33].

Thus, we may extend to the Chinese crab gill epithelium the current paradigm that amiloride blocks apical epithelial Na^+ channels [34] and so induces a

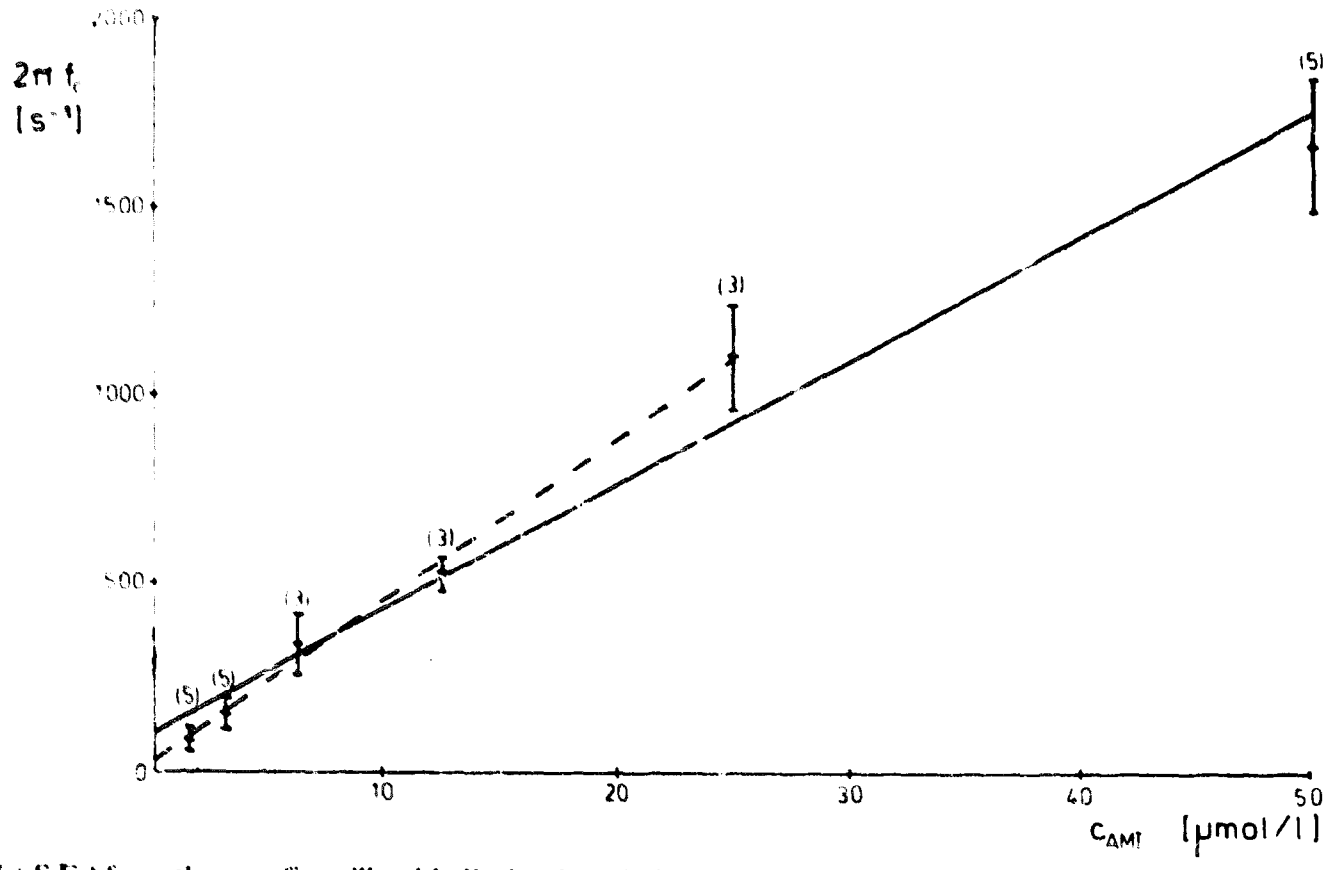

Fig. 5. Pooled data (± S.E.) from three to five gill epithelia for the relationship $2\pi f_{c_2}$ vs. [AMI]. All tissues were theophylline-stimulated (2.5 to 5 mmol/l). Two linear regressions were carried out (smooth and dashed lines; see text). From the slope (k_{01}) and the ordinate intercept (k_{10}) the microscopic K_{AMI} can be calculated (see Methods).

Lorentzian component in Na^+-current fluctuations [14].

Lorentzians are usually recorded only for amiloride concentrations above 1 μmol/l ([14]; see Fig. 4 this paper). Then, with reference to Fig. 3, we analyse single-channel parameters pertinent to the kinetic situation described by the upper straight line. Thus, irrespective of the non-uniform control situation ('break' in I_{Na}-kinetics) we apparently deal with a single sodium channel population displaying simple saturation kinetics in the range of c_{AMI} used for noise analysis.

In many preparations the Lorentzian noise was visible with a prominent clarity and a distinguished plateau down to very low frequencies. The Lorentzian parameters, S_0 and f_c, shifted in exactly the manner as expected for a simple first-order reaction of amiloride with its apical receptor site at/in the channel: S_0 reached a maximum at c_{AMI} near K_{AMI} while f_c increased linearly with c_{AMI} over the investigated frequency range (Figs. 4B and 5). Presumably due to the small chamber area, the normally visible high-frequency a.c.-noise from the amplifier did not overlap too much with the relaxation noise. Thus, the latter could be registered up to amiloride concentrations of 50 μmol/l which is about 5-times higher than reported for any other tight Na^+-transporting epithelium [14].

Competition between Na^+ and amiloride has been described for other tissues [34] and the analysis of our Fig. 3 also suggests competition, although clearly only for $c_{Na} \leq 100$ mmol/l. If competition would also hold (which we are yet unable to show) for higher c_{Na}, a Na^+-loaded channel would then be inaccessible for amiloride. Thus, the estimate of channel density would only yield the number of Na^+-free pores at a given Na^+- and amiloride concentration. On the other hand, it is just the high reaction rate ($2\pi f_c$) at high c_{AMI} which may enable us to record more than 90% [24] of the total density of Na^+ channels, provided that their interaction with Na^+ ions is much slower as compared to that of the blocker [14,21]. Epithelial patch-clamping [35] as well as, in rare cases, fluctuation analysis [24] have provided evidence that epithelial sodium channels are, as previously postulated [14,20], slowly fluctuating. In the present epithelium, the channel interaction with Na^+ seems to be quite weak (Fig. 3). Furthermore, a (possibly Na^+ -dependent) 'spontaneous' Lorentzian Na^+ channel noise in the gill could never be recorded in the investigated frequency range (which is mostly also true for other tight epithelia [20,24]). So, we may speculate that amiloride will seldom encounter a channel occupied by Na^+ ions. This is the reason why, in concert with many previous evaluations of microscopic data from amiloride-induced noise in I_{Na} [14,20-23], we propose for the crab gill a calculation of i and M according to the two-state model as outlined in Methods. The respective figures should, however, be regarded as tentative until later experiments will give conclusive evidence of the type of amiloride interaction with the sodium channel at high c_{Na}.

2. The apical Na^+-channel in the Chinese crab gill epithelium

With respect to Na^+ transport we do not yet know for sure whether an apical Na^+ channel is, like in frog skin [27] or in fish gill [29], responsible for the mass flux of sodium ions under natural conditions (low NaCl and osmolarity outside, open circuit); on the other hand, we established its role under 'Ussing' conditions. Unambiguous evidence comes from the amiloride noise experiments: The on-rate is slightly higher, and even more so is the off-rate, when compared to the vertebrate epithelial sodium channels [24]. Their ratio, the blocker dissociation constant K_A (see Methods) is low which is typical for Na^+ channels [14,24]. We found a high Na^+ ion turnover (single-channel current) of more than 10^6 per second and translocation site, thus characterizing a channel, or pore [25]. The range of single-channel currents as well as of channel densities from the present tissue compares favourably with the data obtained in a number of vertebrate, serosally non-depolarized epithelia [14,24]. The high 'macroscopic' amiloride affinity for its receptor which could be demonstrated independently in current kinetics ([11]; see also Fig. 2 this paper) strongly supports the above conclusions from noise data (Figs. 4 and 5). To our knowledge, this Na^+ channel in gill epithelium of *Eriocheir sinensis* has been the first one described for invertebrate tissues [7,11,12]; another one has most recently been communicated for the dorsal skin of the leech [36].

In order to establish the nature of the interaction of amiloride with the Na^+ channel we recorded the sodium current kinetics and found a two-step profile the origin of which is unclear at present. We might envisage the existence of more than one channel population with different affinities for amiloride as is suggested by the 'linearizing' influence of the blocker (Fig. 3). Two populations of Na^+ channels in toad colon have, indeed, been described [37] with K_A values of about 6 nmol/l and 0.8 μmol/l, respectively. Alternatively, one set of channels which depend in their kinetics (e.g., I_{max}) on the external c_{Na} and/or on the ensuing transport state of the cell, may generate the complex I_{Na} kinetics. Both possibilities seem compatible with more recent findings from our laboratory [15] which revealed a c_{Na}-related, two-step current increase in time within the first minute after a sudden change from Na^+-free to Na^+-containing saline. It seems easy to explain the discrepancy between the estimated c_{Na} for half-maximal current saturation and the small value (below 20 mmol/l) obtained from Na^+-influx measurements [38]: The fluxes were recorded at open-circuit in

presence of external Cl^-, a condition where Na^+ transport via channels is hardly detectable (Fig. 1, cf. also Refs. 4 and 7).

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