

# Anion-Cation Interactions in the Pore of Neuronal Background Chloride Channels

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**ABSTRACT** Background Cl channels in neurons and skeletal muscle are significantly permeable for alkali cations when tested with asymmetrical concentrations of the same salt. Both anion and cation permeation were proposed to require binding of an alkali cation with the pore (Franciolini, F., and W. Nonner. 1987. *Journal of General Physiology*. 90:453–478). We tested this hypothesis by bilaterally substituting large alkali cations for Na and found no significant changes of unitary conductance at 300 mM symmetrical concentrations. In addition, all organic cations examined were permeant in a salt gradient test (1,000 mM internal//300 mM external), including triethanolamine, benzyltrimethylamine, and bis-tris-propane (BTP, which is divalent at the tested pH of 6.2). Inward currents were detected following substitution of internal NaCl by the Na salts of the divalent anions of phosphoric, fumaric, and malic acid. Zero-current potentials in gradients of the Na and BTP salts of varied anions (propionate, F, Br, nitrate) that have different permeabilities under bi-ionic conditions, were approximately constant, as if the permeation of either cation were coupled to the permeation of the anion. These results rule out our earlier hypothesis of anion permeation dependent on a bound alkali cation, but they are consistent with the idea that the tested anions and cations form mixed complexes while traversing the Cl channel.

## INTRODUCTION

Chloride-permeable channels active at the resting potential are present in patches excised from the plasmalemma of vertebrate muscle fibers (Blatz and Magleby, 1985; Woll, Leibowitz, Neumcke, and Hille, 1987; Fahlke, Zachar, and Rüdell, 1992) and neurons (Franciolini and Nonner, 1987; Shukla and Pockett, 1990; Blatz, 1991; Strupp and Grafe, 1991). These chloride channels have the unusual feature of also being able to conduct cations under certain ionic conditions: alkali cations including Na have relative permeabilities of 0.1–0.35 with respect to Cl in asymmetrical concentrations of the same salt. The amphoteric character of these channels is interesting in at least two respects. First, if manifested under physiological ionic

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conditions, it would significantly interfere with Cl channels' presumed electrical function of stabilizing the resting potential. Second, it offers an experimental opportunity to elucidate factors that determine the charge selectivity in an ion channel.

This paper describes experiments in which neuronal Cl channels were probed with different species and combinations of anions and cations. We have previously demonstrated that substitution of Cl by sulfate, an impermeant anion, eliminated any currents attributable to cation, suggesting that the removal of permeant anion abolished not only anionic but also cationic conduction in the channel (Franciolini and Nonner, 1987). To explain this result, we hypothesized that the pore contains a relatively strong site that binds an alkali cation. In this proposed scheme, anion current would be mediated by anion association with the site-cation dipole, whereas cation current would result from exchanges of the bound cation itself. In the present experiments we tested this hypothesis by symmetrically substituting alkali by large organic cations and found, contrary to expectation, that at salt concentrations of 300 mM, this substitution had only small effects on unitary current magnitude. Measurements of zero-current potentials in gradients of the same salt revealed that large cations (up to 19 major atoms, including a divalent species) were permeant and that the apparent (alkali and organic) cation permeability tracked apparent anion permeability. The magnitudes of unitary currents at large salt concentrations depended on the nature of the bulk cation. These new results indicate that, whereas the channel does not require a bound cation for allowing anion permeation, both permeant anions and cations are present in the pore during cation permeation.

The accompanying paper (Franciolini and Nonner, 1994) presents additional support for a multi-ion permeation mechanism involving mixed Na-Cl complexes, which are required for Na permeation and which facilitate Cl permeation. In this model, cation/anion selectivity varies with absolute ion concentrations. Physiological ionic conditions and a negative membrane potential are predicted to render the channel virtually anion selective.

#### METHODS

The purpose of the experiments was to determine unitary current/voltage ( $i/V$ ) relationships of a neuronal "background" Cl channel under varied ionic conditions.

##### *Neuronal Culture*

Dissociated cell cultures of neurons were prepared from embryonic rats at day 15 of gestation. The hippocampal region was dissected, dissociated mechanically by repetitive pipetting in N5 culture medium (Kawamoto and Barrett, 1986) and plated onto a tissue culture plastic dish previously coated with poly-L-lysine. Cells were grown in N5 medium supplemented with 5% (1 mg/ml protein) of an acid-stable horse serum fraction (55 kD; Kaufman and Barrett, 1983). Half of the culture medium was replaced weekly. In the first week after plating, ~95% of the cultured cells were neuronal as assayed by immunochemical staining for neurofilament protein and ability to generate action potentials. Electrical recordings were done on neurons maintained in vitro for 1–3 wk.

### *Solutions*

Test solutions bathing both sides of the membrane patches contained, in addition to the specified concentration of bulk salt, 2 mM of the pH buffer MOPS (3-[*N*-morpholino]propane-sulfonic acid) and 1 mM of the Ca buffer EGTA (ethylene glycol-bis( $\beta$ -aminoethyl) ether) *N,N,N',N'*-tetraacetic acid). Unless otherwise specified, solutions were titrated to a pH of 7.2 with the bulk base or acid. Controls performed in a previous study (Franciolini and Nonner, 1987) showed that these buffers did not affect Cl channel unitary currents. No divalent cations were added, except when they constituted part of the bulk salt, and no neutral compounds were included to balance osmolality. Where necessary, solutions of organic salts were made fresh before each experiment. All solutions were sterile filtered.

The osmolality of solutions was assessed by vapor-pressure analysis (No. 5500XR, Wescor Inc., Logan, UT) and, where applicable, Cl<sup>-</sup> and/or Na<sup>+</sup> activities were determined with ion-specific electrodes (Orion 94-17B for Cl<sup>-</sup>, Orion 84-11BN for Na<sup>+</sup>). Activity ratios determined by both electrodes were in good agreement with published mean activity coefficients (Robinson and Stokes, 1965). However, when we used the Cl-specific electrode to compare Cl activities in solutions of organic bulk cations, the electrode exhibited drift and a significantly smaller slope than with inorganic cations (probably due to complexation between amino groups and Ag<sup>+</sup> in this Ag/AgCl-based electrode). In testing salt gradients involving organic cations, we therefore assume that mean ionic activity ratios are similar to those of analogous salines made with alkali or earth alkali cations.

### *Recording Techniques*

For recordings under voltage clamp, patches of soma membrane were excised in the inside-out configuration (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981) and transferred into a microchamber that was continuously perfused with one of the test solutions. Patch pipettes were pulled from borosilicate glass tubing and had resistances of 3–10 M $\Omega$  when filled with 300 mM saline. Pipette bath capacitance was minimized by the design of the microchamber (Franciolini and Nonner, 1987); no resin coating was necessary for low-noise recordings. Patch membrane currents were recorded using a home-made patch clamp instrument based on an integrating headstage (2 pF feedback capacitor). The temperature of the solution perfusing the microchamber was controlled by a Peltier heat exchanger; several temperatures between 10 and 25°C were used.

An on-line computer operated a programmable generator of command voltage and control signals and recorded the digitized current signal (12-bit resolution) onto digital ½ inch magnetic tape. A typical recording sequence consisted of 10–20 repetitions of a staircase voltage pattern that visited voltage levels from -80 to +80 mV in 10-mV steps, maintaining each level for 0.5 to 2 s. The holding potential between staircases was 0 mV. Before currents were recorded on tape, the computer executed one test staircase at low current amplification to determine the optimal gain for the current preamplifier. The headstage capacitor was shunted under computer control during each change of voltage, and sampling of the current signal began at least 50 ms after the voltage change. The current signal was low-pass filtered at 1–2 kHz (-3 dB, 4-pole Bessel type) and sampled at 3–6 kHz.

An experiment involved several changes of bath saline, while the pipette saline remained the same. Typically, measurements with symmetrical salines alternated with measurements in asymmetrical salines. Recordings began  $\geq 1$  min after a bath saline exchange was complete; solution changes were monitored by recording the holding current on an inkwriter.

The ionic conditions and the (zero) holding potential in our experiments minimized activity of non-Cl channels. Background Cl channels were identified by their conductance, voltage-

dependent activity pattern, and anion/cation selectivities, which were assessed in each experiment using a salt gradient.

### *Analysis*

Unitary current amplitudes were determined from the stored records using an interactive computer program. Recorded traces corresponding to a particular voltage step in the staircase pattern were inspected, and segments during which the channel remained closed were identified. A current baseline was computed by averaging these segments. This baseline was fitted with a polynomial, which was subtracted from subsequently-analyzed samples to compensate for leak current and slow relaxations after each voltage change. Voltages were corrected for junction potential changes of the bath electrode measured against a double-junction reference electrode.

Baseline-corrected samples (e.g., traces in Fig. 1) were redisplayed and corresponding segments of each staircase were analyzed. The amplitude histogram computed for unitary currents at each particular voltage was displayed using a square-root ordinate scale. Occasionally the histogram was recomputed after additional low-pass filtering of the samples as needed to resolve currents smaller than 1 pA; the smallest identifiable currents were  $\sim 0.25$  pA. The histogram was evaluated by specifying peaks with a vertical cursor and averaging the bins about each peak that exceeded a size selected by a horizontal cursor. If a patch contained several active channels, up to three peaks were used, and the unitary current was determined from the average offset between these peaks. The current levels corresponding to the histogram peaks were indicated in a display of the original current record as a control. Unitary current estimates thus obtained were entered into a cumulative current-voltage array (Fig. 1, *symbols*).

Every  $i/V$  relationship was fitted by a third-order polynomial, which was plotted together with the points (Fig. 1, *solid lines*). Because these fits were satisfactory throughout,  $i/V$  relationships were routinely represented by their polynomials for comparisons between repeated experiments or varied ionic conditions. Reversal potentials were determined from the abscissa intersections of the polynomials. Reversal potentials measured with symmetrical salines were used to control for electrode offset (the  $i/V$  curves in Fig. 1 were plotted after these corrections). Altogether, 247  $i/V$  relationships were analyzed for 77 different ionic conditions.

## RESULTS

### *Cation Dependence of Conductance*

Franciolini and Nonner (1987) hypothesized that the neuronal background Cl channel might require an adsorbed alkali cation for conducting anion current. This hypothesis was based on the observations that alkali cations are permeant and that substitution of internal NaCl by CsCl increases unitary currents of both polarities. We tested this hypothesis by substituting large organic cations for Na. Fig. 1A shows unitary currents and the  $i/V$  relationship recorded from a patch bathed in symmetrical 300 mM triethanolamine (TEoA) chloride. Solid lines in Fig. 2A plot  $i/V$  relationships obtained from five membrane patches bathed in 300 mM symmetrical glucosamine (GA) chloride solutions. These  $i/V$  relationships were not significantly different from the average  $i/V$  relationship determined from 28 patches bathed in symmetrical 300 mM NaCl salines (*dotted line*). Analogous results obtained with other organic cations are summarized in Fig. 3: the chloride salts of TRIS, glucosamine, and triethanolamine yielded closely similar chord conductances. Among the organic

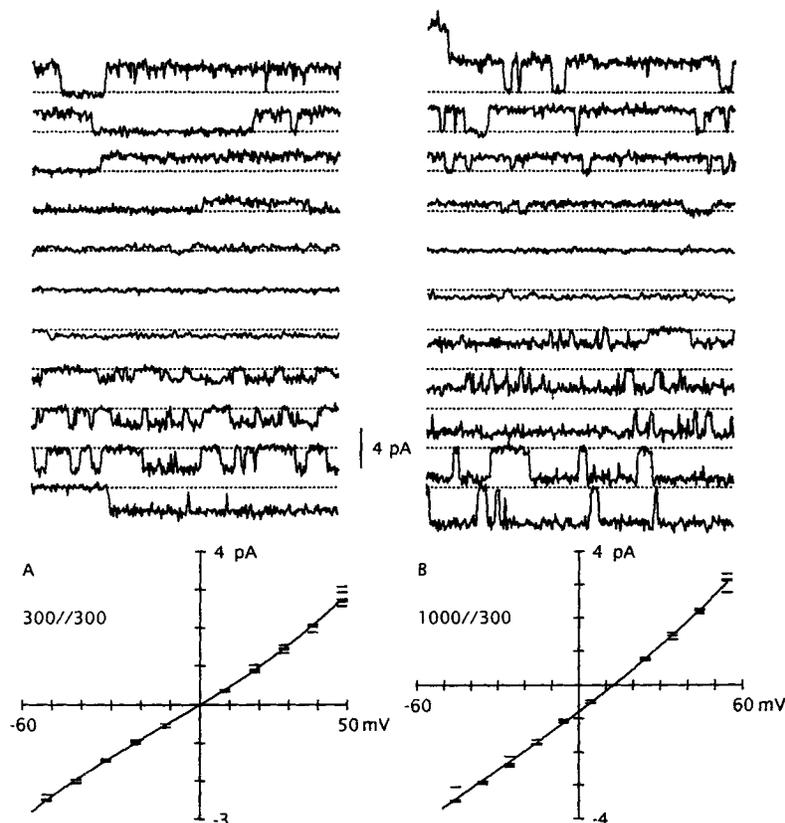


FIGURE 1. Unitary currents (*top*) and unitary  $i/V$  relationships (*bottom*) in triethanolamine chloride solutions (pH 6.2). (A) 300//300 mM (int//ext); (B) 1000//300 mM. The current traces of 300-ms duration were recorded at 10°C during a voltage staircase and correspond to the voltages for which  $i/V$  points were plotted below (*top trace*: most positive voltage). The records were corrected for time-dependent baseline currents as described in Methods; zero-current levels are indicated by dotted lines. The currents were low-pass filtered at 2 kHz; additional Gaussian filtering at 500 Hz was applied to recordings near the zero-current potentials. The individual  $i/V$  points (*symbols*) were determined via histogram analysis from individual record segments like those shown. Each  $i/V$  relationship is least-squares fitted by a polynomial (*solid lines*). Because such polynomials represented the individual  $i/V$  points accurately, the polynomials rather than the original points are used for comparing  $i/V$  relationships in the following figures.

cations tested, only benzyltrimethylammonium (BTMA) reduced the unitary conductance; this cation differs from the others by the absence of hydroxyl groups and, by analogy with tetraethylammonium (TEA) ion (Sanchez and Blatz, 1992), may hydrophobically bind to and block the pore. Channel currents were also measured with the chloride salt of bis-tris-propane (BTP, which is mostly divalent at the tested pH 6.2) (Fig. 3), and in 100 mM symmetrical solutions of  $MgCl_2$  (not shown).

Fig. 2 B shows a converse experiment, in which symmetrical 300 mM solutions of a

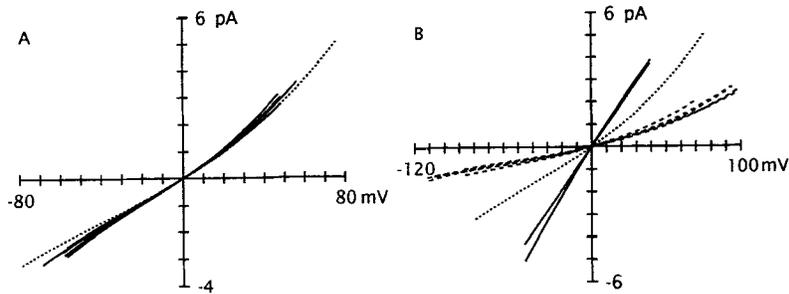


FIGURE 2.  $i/V$  relationships in symmetrical 300-mM salines. (A) Glucosamine Cl at pH 6.2 (solid lines, from individual patches) yields currents similar to those in NaCl (dotted line, average of 28  $i/V$  relationships). Temperature, 10°C. (B) Bilateral substitution of Cl anion (dotted line, average) by nitrate (solid lines) increases unitary currents, whereas substitution by propionate (dashed lines) reduces currents (cation: Na). Temperature was 10 (Cl, propionate) or 25°C ( $\text{NO}_3$ ). All lines represent third-order polynomials that were fitted to the unitary currents determined from amplitude histograms (see Methods).

fixed cation (Na) and a varied anion (propionate, Cl, nitrate) were tested. Here each anion produced a significantly different unitary conductance. Fig. 3 shows that with Na salts there was a continuous increase in unitary conductance from propionate to nitrate, a lyotropic order that parallels that of the zero-current potentials measured under bi-anionic conditions (Franciolini and Nonner, 1987). The fluoride, chloride, and nitrate salts of BTP yielded analogous variations of unitary conductance, but conductances in the BTP salines (which contained nearly twice the anion concentration of the Na salines) were not increased in proportion to anion concentration (Fig. 3). This could indicate saturation of anion flux or a mild block by BTP.

These results with 300-mM salines are consistent with a primarily anion-specific channel and suggest that permeant alkali cations are not required for conduction. It remains possible that a high-affinity binding of alkali cation might be sustained by impurity levels of alkali cation in the organic test salts. The block by BTMA (and

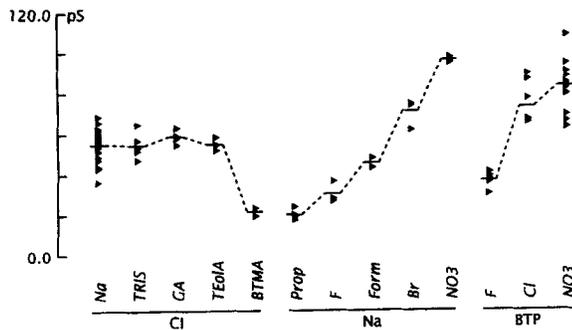


FIGURE 3. Unitary conductances in symmetrical 300-mM salines. The ordinate plots chord conductances determined at +40 mV. Abbreviations: tris[hydroxymethyl]aminomethane (TRIS), D-glucosamine (GA), triethanolamine (TEoLA), benzyltrimethylammonium (BTMA), 1,3-bis[tris(hydroxymethyl)methylamino]propane (BTP), propionate (Prop), formate (Form).

The pH was 6.0 in solutions containing BTP, 6.2 in those containing GA or TEoLA, and 7.2 in the others. Temperatures (from left to right): 10, 25, 10, 10, 10, 10, 25, 10, 25, 25, 10, 10, and 10°C. Each symbol represents measurements in one membrane patch. Averages are indicated by the lines.

possibly BTP) indicates an interaction of the organic cation with the channel, but does not require that the organic cation compete for a cation site deep within the pore. A stronger argument against high-affinity binding of alkali cation could be made if the organic substitute were permeant and hence could be proved to access the entire length of the pore.

#### *Permeability to Organic Cations*

We assessed organic cation permeabilities by measuring zero-current potentials in a salt gradient. Fig. 4 *A* shows the results of a control experiment in which the external solution contained 300 mM NaCl and the internal solution contained 300 (*dashed line*) or 1,200 mM (*solid lines*) NaCl. The zero-current potentials in the salt gradient were substantially less depolarized than the equilibrium potential for Cl ion (32 mV), which demonstrates that Na ion is permeant in the Cl channel, in agreement with

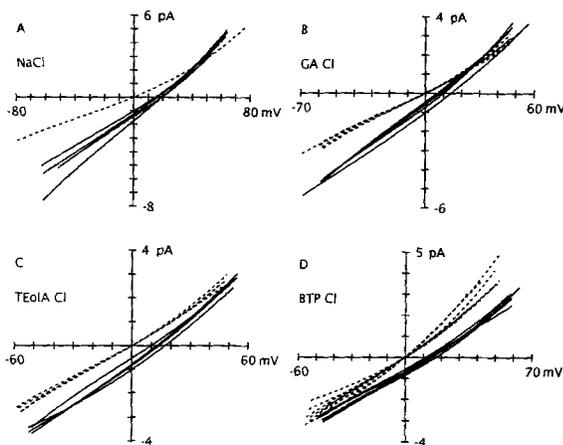


FIGURE 4.  $i/V$  relationships resulting from salt gradient tests for cation permeability. The dashed lines represent measurements in symmetrical 300-mM concentrations of various Cl salts (Na in *A*, GA in *B*, TEoIA in *C*, and BTP in *D*). (*Solid lines*) Measurements made while the intracellular concentration was elevated to 1,000 mM (1,200 mM for NaCl). The dashed line in *A* is identical to the average  $i/V$  curve in Fig. 2 *A*; other dashed lines represent individual  $i/V$  curves.

The zero-current potentials in the salt gradient are less depolarized than the Cl Nernst potential, indicating that all cations tested were permeant. pH was 7.2 in *A*, 6.2 in *B* and *C*, and 6.0 in *D*. Temperature, 10°C.

published observations (Franciolini and Nonner, 1987; Blatz, 1991). Fig. 1 *B* shows unitary currents and the  $i/V$  relationship obtained from a membrane patch bathed in 1,000/300 mM triethanolamine chloride. Fig. 4, *B–D* plot the  $i/V$  relationships of several analogous experiments using the chloride salts of three organic cations. Fig. 5 (*top*) summarizes the zero-current potentials determined with Cl salts of these and other organic cations. In all cases, including the divalent cation, BTP, and the blocking cation, BTMA, the zero-current potentials were similar to that observed with Na as the cation.

The similarity of the zero-current potentials measured in gradients of the various tested cations indicates that the channel distinguishes poorly between the small alkali cation, Na, and a large tertiary or quaternary amine ion. This might be expected if the width of the pore allows cations to pass without significant dehydration or interaction with the pore lining. The permeability of the divalent cation, BTP, in this

mainly anion-specific channel, however, suggests that permeating cations interact significantly with other mobile ions while in the pore. One could envisage that  $\text{BTP}^{++}$  cation combines with an anion to form a monovalent cationic complex,  $(\text{BTP Cl})^+$ . The association would have to occur during the passage through the pore, because measurements of osmolalities (see Methods) of BTP salines did not reveal a substantial degree of ion-pair formation in the bulk solutions.

To test for possible anion-cation interactions within the pore, we determined zero-current potentials for salts that contained a fixed cation and a variable anion.

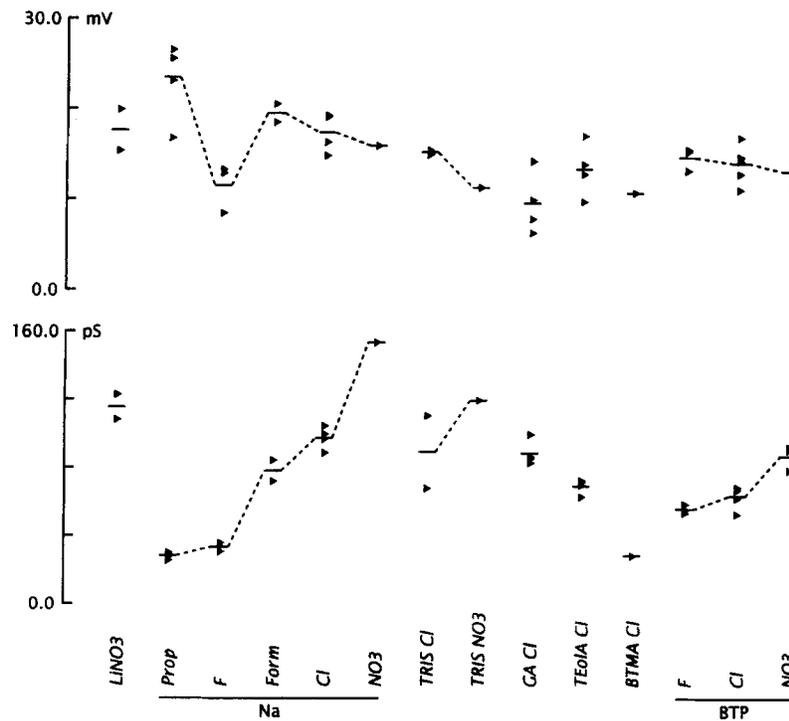


FIGURE 5. Summary of zero-current potentials (*top*) and unitary conductances (*bottom*) in gradients of various salts. Measured from  $i/V$  relationships recorded in 1,000//300 mM gradients (except NaCl, 1,200//300, and NaF, 800//300; the maximal concentration of NaF was 800 mM because of low solubility). The (chord) conductances were determined for a potential 40 mV positive to the respective zero-current potential. Symbols give values for individual membrane patches, lines represent the averages. For pH values see Fig. 3.

The anions chosen reveal different permeabilities when compared to Cl under bi-ionic conditions. This was shown by Franciolini and Nonner (1987) for Na salts, and is demonstrated in Fig. 6A for BTP fluoride and nitrate. Fig. 6B plots  $i/V$  relationships recorded in gradients of Na propionate and NaBr. Again, the zero-current potentials are similar to that measured with Cl as the anion, albeit the potential for propionate is slightly more depolarized than that for Br. If Na and these anions permeated independently of each other, one would expect cation permeation to cause larger deviations of zero-current potentials from the anionic Nernst

potential when the anion has a low permeability. Hence, with Br the zero-current potential should be closer to the anion potential than that measured with the less permeant propionate. However the small observed difference was in a direction opposite to that predicted. The zero-current potentials for Na salts summarized in Fig. 5 (*top*) generally became slightly less depolarized as anion permeability and unitary conductance (Fig. 5, *bottom*) increased. With divalent BTP as the cation, fluoride and nitrate have different permeabilities when compared under bi-ionic

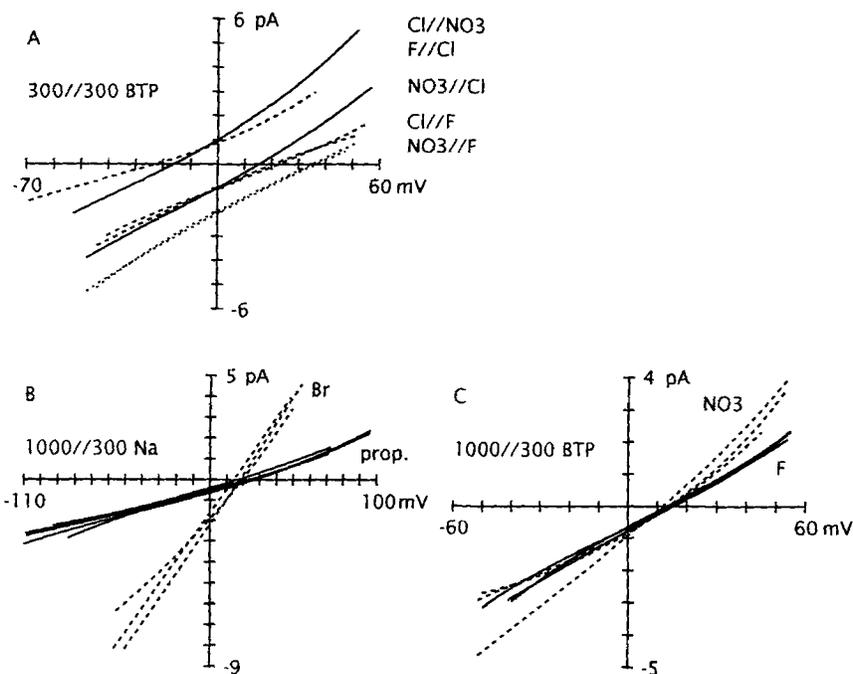


FIGURE 6. (A)  $i/V$  relationships obtained under bi-anionic conditions with 300 mM BTP salts (pH 6.0). In the order of zero-current potentials (left to right): F//Cl, Cl//nitrate, nitrate//Cl, Cl//F, and nitrate//F (int//ext). (B, C) Salt gradient tests of cation permeability in the presence of different anions. All  $i/V$  relationships were measured with 1,000//300 mM (int//ext) concentrations of the salt. (B) Na propionate (*solid lines*) compared to NaBr (*dashed lines*). (C) BTP fluoride (pH 6.0, *solid lines*) compared to BTP nitrate (pH 6.0, *dashed lines*). When interpreted in terms of Goldman-Hodgkin-Katz permeabilities, the zero-current potentials in B and C would indicate similar cation/anion permeability ratios for a given cation and a varied anion, whereas the zero-current potentials in A indicate significantly different permeabilities of the varied anions.

conditions (Fig. 6A), but similar zero-current potentials when tested in the salt gradient (Fig. 6C). The variations of unitary conductance are smaller for BTP than for Na salts (Fig. 5), indicating that BTP reduces current more effectively in the presence of nitrate (the most permeant anion) than in the presence of fluoride.

These results indicate an interdependence of anion and cation fluxes in the Cl channel pore. The anion-cation interaction could be essential for permeation of the

divalent cation, BTP, but must occur to a significant extent also during permeation of the alkali cation, Na.

Also relevant to the issue of anion-cation interactions are the  $i/V$  relationships shown in Fig. 4. Whereas increasing the internal salt concentration significantly increased the slope of the  $i/V$  relationship for the Na salt (compare *solid* with *dashed lines* in Fig. 4 *A*), smaller increases were found with GA (Fig. 4 *B*) and TEoA (Fig. 4 *C*), and even a decrease with BTP (Fig. 4 *D*). At high concentrations of the Cl and nitrate, but not F, salts, unitary conductances were smaller with BTP than with Na ion (Fig. 5, *bottom*), although the anion concentrations were significantly larger (because BTP is divalent). This apparent blocking effect of high [BTP] indicates that at elevated concentrations organic cations interact with the pore, effecting a reduction of anion flux.

#### Currents Associated with Divalent Anions

Fig. 7 (*solid lines*) shows  $i/V$  relationships measured under bi-ionic conditions with two divalent anions, phosphate (pH 8, Fig. 7 *A*) and malate (*B*). The external anion was

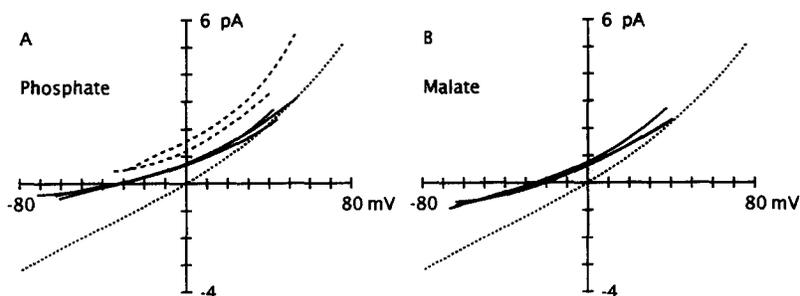


FIGURE 7. Some divalent anions are permeant.  $i/V$  relationships measured in 300-mM salines under bi-anionic conditions (external saline: NaCl; *dotted lines* plot the average  $i/V$  relationship for symmetrical 300 mM NaCl). (*A*) The divalent  $\text{HPO}_4$  anion dominates at pH 8 (*solid line*), whereas the monovalent  $\text{H}_2\text{PO}_4$  anion dominates at pH 6 (*dashed lines*). (*B*) Di-sodium salt of DL-malate ( $\text{NaO}_2\text{CCH}_2\text{CH}(\text{OH})\text{CO}_2\text{Na}$ , pH 7.2) (*solid lines*). A reversal of current was observed in the presence of both divalent anions, but no inward current was detected with monovalent phosphate anion.

chloride, and the cation, Na. With both internal divalent anions, inward currents were detected; experiments with fumarate (not shown) gave a similar result.

The observed inward current is consistent with an efflux of the divalent test anion, but could also be carried by Na ion. Two observations argue against an anion-independent Na influx as the major carrier of inward current. First, no inward current was detected in earlier tests with internal sulfate (Franciolini and Nonner, 1987). Second, no inward current was found with monovalent phosphate (pH 6) as the internal anion (Fig. 7 *A*, *dashed curves*). Here only outward currents were detectable (no currents could be resolved between  $-40$  and  $-80$  mV), and at each voltage the resolved currents were more positive than those found with divalent phosphate. A test with a Na-specific electrode (Methods) showed that, despite a

twofold ratio of Na concentrations, Na ion activities were virtually identical in the monovalent and divalent phosphate salines and close to that determined in 300 mM NaCl (which is consistent with mean activity coefficients listed in Robinson and Stokes, 1965). Hence, if different Na fluxes underlay the differences between the  $i/V$  relationships in Fig. 7 A, these fluxes could not be independent of the anions present. Specifically, the intracellular divalent phosphate would permit a significant influx of Na ion, whereas the monovalent phosphate would not; this interaction, which involves ions from opposite sides of the membrane, would most likely occur in the pore itself. The alternate, and simpler, explanation involves different anion fluxes: internal divalent phosphate produces a detectable anion efflux, whereas monovalent phosphate does not. In this case, the channel would have the remarkable property of passing not only monovalent, but also divalent, ions of both polarities.

#### DISCUSSION

Our results rule out the possibility that a bound alkali cation is essential for anion conduction in the background Cl channel (Figs. 1–3), but do demonstrate that at elevated salt concentrations alkali and organic cations interact with the pore. First, the unitary conductance obtained with a given anion depended on the species of cation (Figs. 4 and 6), as if cations could partially block the pore with very fast kinetics. Second, all organic cations tested were permeant (Figs. 4 and 6). Together, these observations are consistent with the idea that cations can compete with anions over the entire length of the pore and, in case of a longer dwell time, can reduce the total charge flux below that sustainable by anions alone. Whereas high concentrations of large organic cations slow ion permeation rates in the pore, 300 mM internal Cs ion enhances unitary currents of either polarity with respect to that observed with Na (Franciolini and Nonner, 1987).

The range of permeant cations suggests a pore structure analogous to that previously determined for anions (Franciolini and Nonner, 1987). For example, the minimal effective pore diameter required for the largest tested cation (BTP) is  $\sim 0.7$  nm, similar to that necessary for glutamate permeability. The substantial permeability to large organic cations suggests that the pore has a lyotropic character, which also appears to underlie the anionic selectivity. The marked blocking effect of the permeant cation, BTMA, indicates a favorable hydrophobic interaction, which resembles characteristics observed for the permeant aromatic anion, benzoate. Organic cations that block to lesser extents are surface lined by hydroxyl groups, rendering them similar to a hydrated alkali cation.

These features suggest a wide aqueous pore whose lining contains no strongly electrostatic groups and allows for hydrophobic interaction. Nevertheless, the observed preference for anions over cations likely involves some electrostatic interaction between ions and pore walls, because anions appear to be permeant per se whereas cation permeation appears to require a cooperation of permeant anions. This dependency is manifested in the absence of observable Na current with impermeant sulfate as the anion (Franciolini and Nonner, 1987) and in the observations of Figs. 5 and 6: the apparent permeabilities of BTP, but also those of Na, are in an approximately constant ratio to the permeabilities attributable to the anion of the tested salts. It appears likely that when both anions and cations can access this pore,

they tend to aggregate more readily than in bulk water because their interactions are weakened to a lesser extent by interactions with the environment. The free energy changes associated with the formation of anion-cation complexes are expected to be small because the tested salt concentrations are not very different from the limits of aqueous solubility. For instance, NaF was tested at 800 mM, and the solubility limit of NaF is slightly larger than 1 M. The transient formation of cation-anion complexes might be particularly important for the permeability of the divalent cation, BTP (Fig. 4 D), and for the potential permeabilities of several divalent anions (Fig. 7).

Observations on other kinds of Cl channel consistently reveal a lyotropic sequence of anion selectivities (reviewed by Hille, 1992; Franciolini and Petris, 1992; Franciolini and Adams, 1994), but the observed cation/anion permeability ratios vary. For instance, Bormann, Hamill, and Sakmann (1987) determined  $P_K:P_{Cl} < 0.05$  in GABA<sub>A</sub>-receptor and glycine-receptor channels and Schoppa, Shorofsky, Jow, and Nelson, (1989) reported a  $P_{Na}:P_{Cl}$  ratio of  $\leq 0.09$  in CFTR channels, whereas Blatz and Magleby (1985) determined values of 0.15–0.2 for  $P_K:P_{Cl}$  in background channels of myotubes. The lower ratios were obtained in experiments in which the test salt (or permeant anion) concentration was unilaterally lowered below isotonic levels. The higher ratios were determined in salt gradient experiments in which the test salt concentration was unilaterally increased above isotonic levels. If cation permeation in Cl channels depends on a transient anion-cation complex, cation:anion permeability ratios will increase with absolute salt concentration, as appears to be the case for the ratios reported in the literature.

Effects commonly associated with multi-ion mechanisms were observed in Cl channels. Anomalous mole fraction effects on unitary conductance were found with NaCl/NaSCN mixtures in GABA<sub>A</sub>-receptor and glycine-receptor channels (Bormann et al., 1987) and the CFTR channel (Tabcharani, Rommens, Hou, Chang, Tsul, Riordan, and Hanrahan, 1993). These effects have been interpreted in terms of an interaction between two anions dwelling in the pore. The inter-anionic "repulsion factors" (Hille and Schwarz, 1978) needed to explain the effect (equivalent to 1.6 kT for Cl, or 3.2 kT for SCN) were substantially smaller than that determined, e.g., for Na–Na repulsion in Na channels (7 kT; Naranjo and Latorre, 1993). It seems possible that the small repulsion factor reflects ion interactions in a mixed (e.g., anion-cation-anion) complex rather than those between two anions alone.

The accompanying paper (Franciolini and Nonner, 1994) presents further observations on Na and Cl ion permeation, which support the hypothesis of mixed anion-cation complexes in the neuronal background Cl channel. This paper also elaborates a molecular interpretation based on quantitative kinetic modeling.

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