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The Ca-Activated Chloride Channel of *Ascaris suum* Conducts Volatile Fatty Acids Produced by Anaerobic Respiration: A Patch-Clamp Study

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Abstract. Plasma membrane vesicles prepared from the bag region of the somatic muscle cell of the parasite *Ascaris suum* contain a large conductance, voltage-sensitive, calcium-activated chloride channel. The ability of this channel to conduct a variety of carboxylic acids, a number of which are products of anaerobic respiration, was investigated using the patch-clamp technique and isolated inside-out patches of muscle membrane. The channel has a conductance of 140 pS in symmetrical 140 mM chloride. Replacement of internal chloride with various carboxylic acids (140 mM) caused large hyperpolarizing shifts in the reversal potential. Permeability ratios, relative to chloride, were calculated for each acid. The relationship between permeability ratio and ionic size is inverse and linear predicting a pore diameter of 6.55 Å. This simple relationship was not observed between ionic size and conductance. Calculation of the transition state energy required to transfer a single methyl group from aqueous phase to the binding site afforded a value that was low but favorable, indicating a cationic binding site of low field strength. As the channel is able to open fully at the resting membrane potential of *Ascaris* and is permeable to fatty acids produced by anaerobic respiration, the possible role of this channel in the removal of metabolic products across the muscle membrane is discussed.

Key words: *Ascaris suum* — Chloride channels — Permeability — Fatty acids — Anaerobic respiration

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Introduction

In this study we have examined further the calcium-activated chloride channel currents in the muscle membrane of the porcine nematode parasite *Ascaris suum.* This channel is located on the body muscle membrane of *Ascaris* and is selective for anions, cations are not detectably permeant (Thorn & Martin, 1987; Dixon, Valkanov & Martin, 1993). Because of biophysical properties so far documented (Thorn & Martin, 1987; Dixon et al., 1993), it is difficult to assign this channel to any of the four categories described by Franciolini and Petris (1987). The maxi chloride channel, as defined by Franciolini and Petris, imposes a minimum conductance of 200 pS in symmetrical 150 mM Cl; the Ca-activated chloride current of *Ascaris* only reaches such a conductance in symmetrical 175 mM Cl. The *Ascaris* chloride current remains active at holding potentials in excess of −100 mV, whereas the maxi chloride currents tend to inactivate at potentials outside the range ±30 mV from zero. As the opening of the *Ascaris* chloride channels is dependent upon calcium, it is possible to put this channel in the ligand-activated category of chloride currents. However, the large conductance of the calcium-activated channel does not fall in line with the small conductances (<50 pS in 145 mM KCl in mouse cultured spinal neurons) normally displayed by the GABA and glycine receptor gated channels (Bormann, Hamill & Sakmann, 1987). The further category of background chloride currents includes channels that are likely to be open at the resting membrane potential of the cell. The resting membrane potential of *Ascaris* is low at −35 mV and the Ca-activated chloride channel is indeed active at this potential. Unfortunately, the other criteria of this category are a mild voltage dependence and a conductance of between 10–100 pS in symmetrical 150 mM Cl. Clearly, the calcium-acti-

vated chloride channel fails to fulfill either criterion (Thorn & Martin, 1987) as it is greatly affected by membrane potential and its conductance is double the upper limit of this category. Further investigations into the properties of this channel are therefore required.

In this paper we have studied the ability of the channel to conduct several aliphatic organic acids including acetate and propionate, both of which are formed in the mitochondria as products of carbohydrate metabolism (Fig. 1*A* and 1*B*). In turn, acetate and propionate are condensed to α-methyl butyrate and two units of propionate condense to form α-methyl valerate. These two branch chained, volatile, fatty acids are the major fermentation products of carbohydrate metabolism in *A. suum* (Saz & Weil, 1962). In addition to being found in muscle tissue, α-methyl butyrate and α-methyl valerate have also been isolated from perienteric fluid (Fig. 1*C*), where they constitute two thirds of all extracellular anions (Saz & Bueding, 1966). A mechanism, therefore must be available to the cell for the movement of these fatty acids across the muscle membrane. In this paper we have attempted to determine whether the calcium-activated chloride channel forms part of such a mechanism.

Materials and Methods

Preparation of Muscle Vesicles

A. suum, obtained from the local slaughterhouse, were maintained at 34°C in Locke's solution that was changed daily. The first 5 cm anterior region of the worm was discarded and the next 2 cm section of worm was taken and cut along one lateral line. The gut was removed and the resulting flap pinned out, cuticle side down, onto Sylgard in an incubation chamber at 37°C. The preparation was washed (three times) with extracellular solution, composition (in mM): NaCl, 35; NaAcetate, 105; KCl, 2; $MgCl_2$, 2; HEPES, 10; glucose, 3; ascorbic acid, 2; EGTA, 1; pH adjusted to 7.2 with NaOH. The washed flap was then incubated for 10 min with enzyme solution, composition (in mM): NaCl, 35; NaAcetate, 105; KCl, 2; $MgCl_2$, 2; HEPES, 10; glucose, 3; ascorbic acid, 2; collagenase, 1 mg/ml; pH adjusted to 7.2 with NaOH. Following enzyme treatment, the preparation was washed (three times) with extracellular solution and incubated in extracellular solution at 37°C. Approximately 1 hr after enzyme treatment, vesicles could be seen "budding off" from the bag region of the muscle cell membrane. The vesicles were harvested using a Pasteur pipette and transferred to the recording chamber.

Recording Setup

Microelectrodes were pulled to a resistance of 1–3 MΩ (Garner Glass 7052) and coated with Sylgard to improve frequency responses. Single channel recordings were made from inside-out patches of muscle membrane with seal resistances >1 GΩ. Currents were monitored using a LIST EPC7 current-voltage converter, viewed on an oscilloscope, digitized and recorded on Betamax tape. Prior to analysis, records were filtered at 1 kHz (3 dB) by an 8-pole Bessel-type filter.

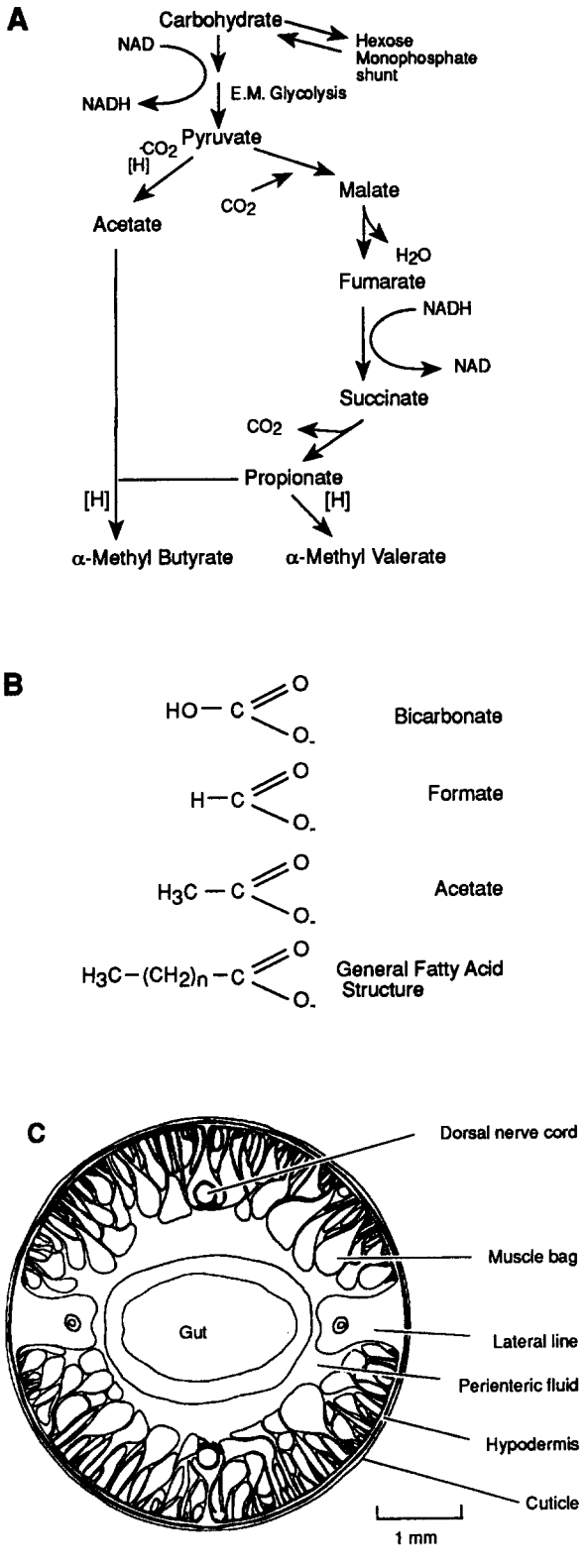


Fig. 1. (*A*) The anaerobic metabolism of carbohydrate in the muscle cells of *Ascaris suum.* (*B*) Diagramatic representation of the structures of the *n*-series carboxylic fatty acids. (*C*) Cross section through the body wall of *Ascaris,* illustrating the basic anatomy of the parasite.

Experimental Procedure

The experimental chamber was mounted on the stage of a Reichert-Jung Biostar inverted microscope. The vesicles were viewed at 200× magnification under phase contrast. All experiments were carried out at ambient temperature (15–22°C). The pipette solution remained constant throughout all experiments, composition (in mM): CsCl, 140; $Mg(Acetate)_2$, 2; $Ca(Acetate)_2$, 1; HEPES, 10; adjusted to pH 7.2 with CsOH. The bath solution was composed of (in mM): Test salt, 140; $Mg(Acetate)_2$, 2; $Ca(Acetate)_2$, 1; HEPES, 10; adjusted to pH 7.2 with CsOH, test ion being either chloride, bicarbonate, formate, acetate, propionate, butyrate, α-methyl butyrate (isovalerate), valerate (pentoate), caproate (hexoate), heptanoate. A high calcium concentration was used to ensure maximum activation of the channel currents (Thorn & Martin, 1987). All tested anions were supplied from Sigma: chloride and bicarbonate as cesium salts, the carboxylic acids were supplied as free acids.

For each patch, current records were obtained with chloride as bath test ion. The bath solution was then replaced with an alternative bath solution containing another test anion. In an additional series of experiments, records were obtained using each test ion in the initial bath solution, this solution was then exchanged for a bath solution containing chloride as test anion. This procedure ensured that it was the calcium-activated Cl channels that were present in the patch and that there was no contamination from other channels which may have been activated by the carboxylic acids.

The chamber was grounded using an agar bridge/AgCl electrode. In some experiments (with heptanoate) the pipette electrode was also encased in an agar jacket to ensure stability of junction potentials. Junction potentials were measured and taken into account in all calculations.

Analysis

Current records of at least 30 sec duration were made at fixed patch potentials between +100 and −100 mV and analyzed on an IBM PC2/70 computer using PAT, version 6.1 single channel analysis program (John Dempster, Strathclyde University). Current/voltage plots were constructed, and slope conductances and reversal potentials were determined from the region of the reversal potential to avoid errors incurred due to rectification of the current/voltage relationship. As an additional check on the stability of the current amplitudes, the potentials were revisited and the observations repeated. Each patch produced a very stable reversal potential but the slope conductance of the current/voltage plot sometimes reduced with time. If such a reduction occurred, the initial conductance value was recorded.

Determination of Permeability Ratios

In ion substitution experiments, the Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodkgin & Katz, 1949) was used to calculate the relative permeability (P_A/P_{Cl}) of anion A with respect to Cl.

$$Erev = \frac{RT}{F} \times \ln \left\{ \frac{[Cl_i] + (P_A/P_{Cl})\,[A_i]}{[Cl_o] + (P_A/P_{Cl})\,[A_o]} \right\} \quad (1)$$

Subscripts i and o denote internal and external ion species, respectively, and R, T and F have their usual meanings.

In all calculations, the activities of the anions were taken into account (Robinson & Stokes, 1959). All the organic acids were >99% completely ionized at pH 7.2. Results are expressed as mean ± standard error of the mean (SEM).

Results

Vesicles were used within four hours of harvesting since the quality of the preparation began to deteriorate after this time i.e., the frequency of seal formation begins to decline and the vesicles become fragile.

The current/voltage relationship, with chloride as internal anion, was linear with a slope conductance of 144.3 ± 1.4 pS and a reversal potential of 0.03 ± 0.47 mV. Substitution of bath chloride with bicarbonate, formate, acetate, propionate, butyrate, α-methyl butyrate (isovalerate), valerate, caproate (hexanoate), valerate, caproate (hexoate) and heptanoate showed that all these ions could produce inward current at sufficiently negative patch potentials and, therefore, that they could move outwardly through the channel (Fig. 2).

Figure 3 illustrates the effect of replacing internal chloride with butyrate on the conductance and reversal potential of the calcium-activated chloride channel. The conductance in butyrate solution decreased at hyperpolarized potentials; however, at depolarized potentials the conductance increased to a value corresponding to that expected when the channel is conducting predominantly chloride. This effect was seen with all other replacement anions. Consequently, slope conductances were determined using currents recorded at membrane potentials about 10 mV more negative than the reversal potential for each respective anion.

All anions tested produced hyperpolarizing shifts in the reversal potential when they were substituted for chloride in the internal solution. Substitution of bath chloride with bicarbonate, formate, acetate and propionate caused hyperpolarizing shifts in the current/voltage plot for each anion, thereby producing a negative reversal potential between −7.6 ± 0.6 and −20.4 ± 0.3 mV, respectively (Table). Other larger anions produced hyperpolarizing shifts in the reversal potential greater than 25 mV. These anions were: butyrate; α-methyl butyrate (isovalerate); valerate; caproate (hexoate) and heptanoate which had reversal potentials ranging from −26.4 ± 0.92 to −66.2 ± 1.3 mV (Table).

Permeability ratios for each anion relative to chloride (P_A/P_{Cl}) were calculated from the reversal potential of each anion using Eq. (1) and were subsequently related to ionic size (Fig. 4*A*). Ionic size can be estimated in a variety of ways, one of the most common being the Stokes diameter. This method applies Stokes Law for macroscopic particles to particles of ionic dimensions. The original law relates the movement of a sphere through an ideal hydrodynamic continuum to the frictional resistance in terms of the dimensions of the particle and the viscosity (η) of the medium. For a spherical particle this is given by:

$$v = F/(6\pi\eta r)$$

Table. Values of a variety of physical constants for the permeable anions and data obtained during single channel experiments

Anion	Stokes diameter (Å)	Limiting equivalent conductance λ^0 ($cm^2 Int\Omega^{-1} equiv^{-1}$)	Reversal potential (mV)	Conductance (pS)	Relative Permeability (P_A/P_{Cl})	Number of patches
Chloride	2.41	76.4	0.03 ± 0.47	144.3 ± 1.4	1.06 ± 0.02	5
Bicarbonate	4.13	44.5	−18.1 ± 3.03	65.6 ± 12.0	0.50 ± 0.06	4
Formate	3.37	54.5	−7.6 ± 0.62	86.7 ± 3.8	0.74 ± 0.02	4
Acetate	4.48	40.9	−12.8 ± 1.13	68.4 ± 4.7	0.59 ± 0.03	3
Propionate	5.13	35.8	−20.4 ± 0.24	58.9 ± 2.2	0.44 ± 0.01	4
Butyrate	5.37	34.5	−26.4 ± 0.92	68.3 ± 11.9	0.35 ± 0.01	3
Valerate	5.51	33.4	−43.0 ± 2.54	30.3 ± 3.7	0.18 ± 0.02	4
α-Methyl butyrate	5.62	32.6	−36.3 ± 1.50	58.7 ± 15.8	0.24 ± 0.01	3
Caproate (hexoate)	5.97	30.8	−59.0 ± 0.58	29.1 ± 7.9	0.10 ± 0.01	3
Heptanoate	6.30	29.2	−66.2 ± 1.30	63.0 ± 9.3	0.07 ± 0.01	3

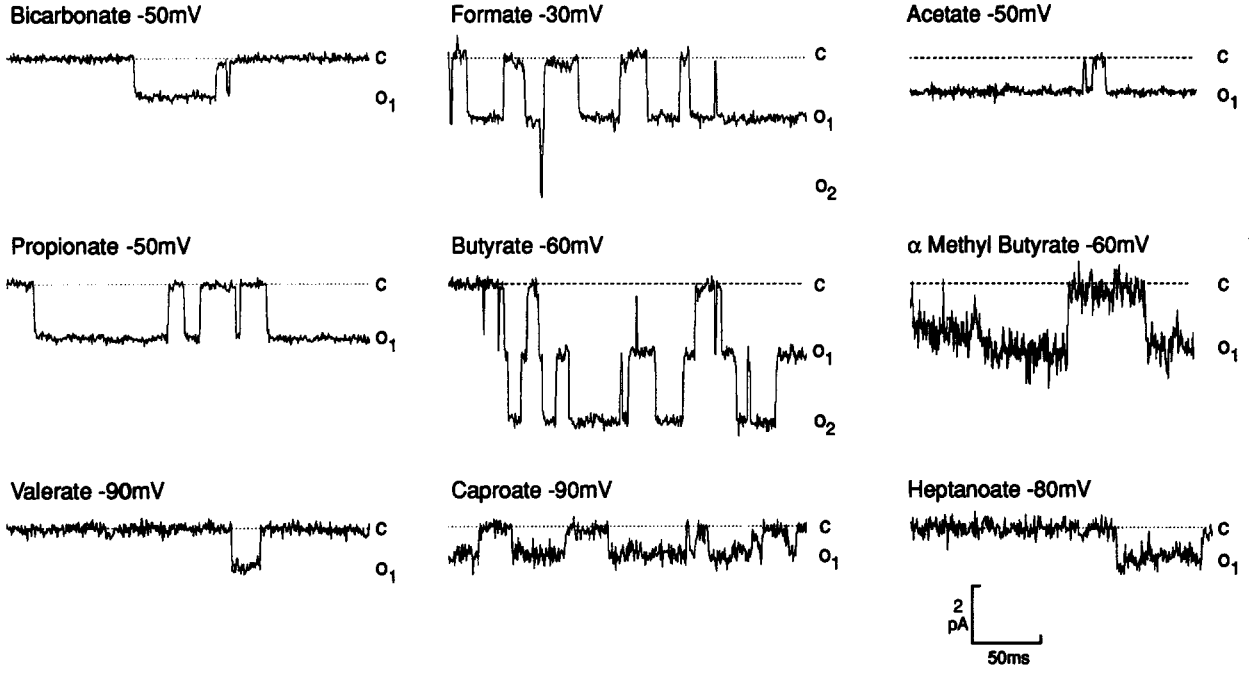


Fig. 2. Inward currents observed after replacement of the bath chloride with the test anion and isolated inside-out patches at the membrane potentials indicated. *C:* closed channel state. *O:* open channel state. Note that all these anions support inward currents at sufficiently negative patch potentials and that these anions may pass out through the channel.

where r = radius of sphere; v = velocity of sphere and F = Faraday constant. This can be applied to an ion whose radius is given by:

$$r = 1/(6\pi\eta u)$$

where u is the absolute mobility of the ion.

u can also be expressed in terms of the limiting equivalent conductivity:

$$r = |z|F^2/(6\pi N\eta^0\lambda^0)$$

where z = valency of the ion; λ^0 = limiting equivalent conductivity; η^0 = viscosity of the pure solvent and N = Avogadro number.

The limiting equivalent conductances are available for a wide variety of ions (Dippy, 1938; Robinson & Stokes, 1965), thereby enabling the calculation of r, the Stokes diameter of each ion (Table). The relationship between P_A/P_{Cl} and Stokes diameter was linear for all anions. It should be noted, however, that other workers use more complex analyses than described above (Bormann et al., 1987).

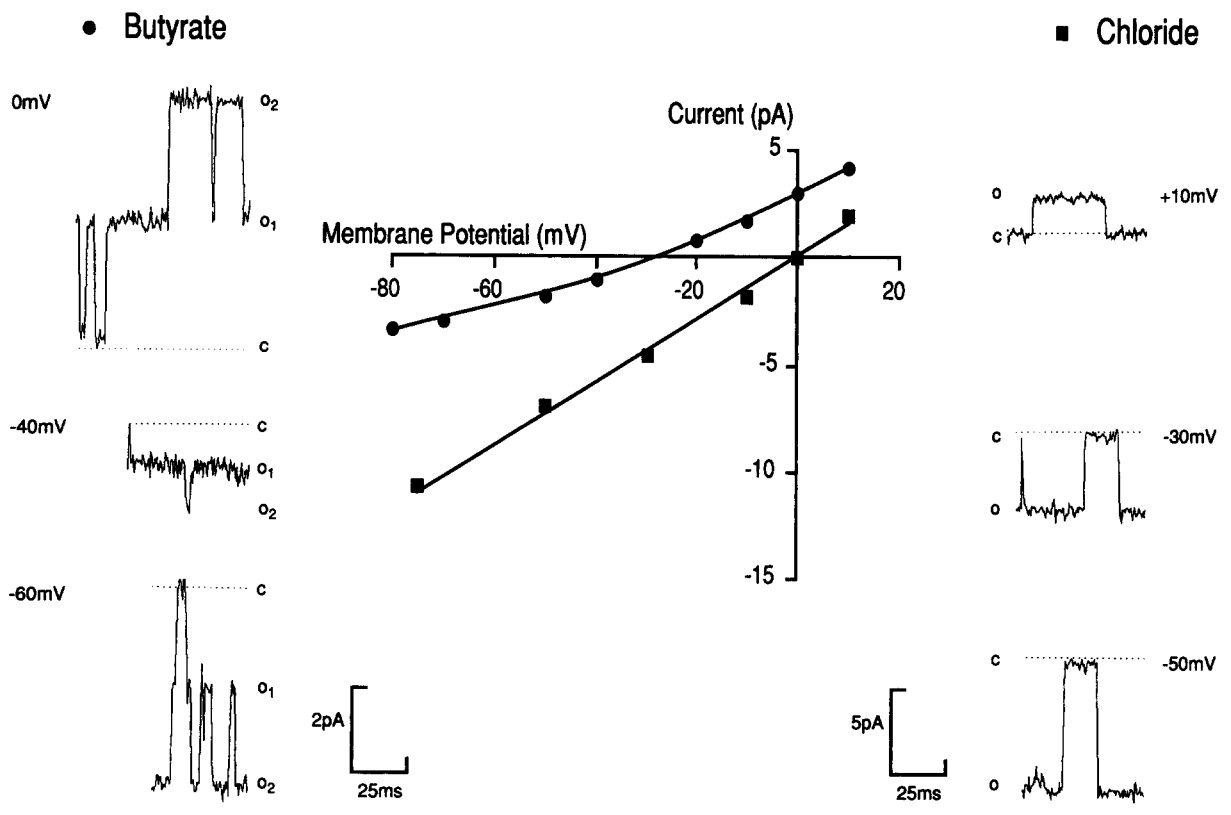


Fig. 3. Current/voltage relationship for symmetrical 140 mM chloride (squares) shown passing through the origin. The effect on the current/voltage relationship of replacing internal chloride with 140 mM butyrate is shown by the circles. The reversal potential shifts to −26 mV and the relationship is no longer linear, the conductance increases at potentials less negative than the reversal potential. Current records, obtained at the potentials indicated, are shown alongside the graph. *o* and *c* denote open and closed states, respectively. When butyrate was the internal anion, current records show the patch contained two channels denoted as o_1 and o_2. Currents are shown at a high time resolution to show the amplitude of the steps clearly.

Heptanoate, the seven carbon containing carboxylic acid, has a Stokes diameter of 6.30 Å and is measurably permeant. The diameter of the channel pore, therefore, must be greater than 6.30 Å. A simple linear regression fitted to the data intersects with the abscissa at 6.55 Å (Fig. 4*A*). This intersection provides an estimation of the effective diameter of the channel pore at its narrowest point. In addition to the Stokes diameter, an alternative measure of ionic size is the number of methyl groups within the organic acids. The relationship between P_A/P_{Cl} and the number of methyl groups in each acid is linear and shown in Fig. 4*B*. The intersect on the abscissa is 6.03, suggesting fatty acids with a total chain length greater than seven carbon atoms (six methyl groups) will not pass through the channel. Unfortunately, fatty acids longer than heptanoic are so lipophilic that the limit of solubility in aqueous solution is exceeded. Indeed, it is impossible to prepare aqueous solutions of sufficient concentration, of organic acids longer than nonoic acid.

In addition, the transition state energy (ΔG) between the binding site and the barrier peak (Andersen & Koeppell, 1992) can be expressed in terms of the permeability ratio of each acid relative to chloride

$$\Delta G = RT \ln (P_A/P_{Cl}) \quad (2)$$

The plot of ΔG against the number of methyl groups contained within each straight chained fatty acid (Fig. 5) can be described by two straight lines, whose slopes are a measure of the transition state energy change per methyl group for transfer from the aqueous phase to a binding site within the channel pore (Bormann et al., 1987; Murrell, Brown & Haydon, 1991). A least-squares regression through the points fitting the carboxylic acids, formate, acetate, propionate, butyrate and α-methyl butyrate, affords a slope of −0.609 kJmol^{-1}. The gradient of the second line describing the acids, valerate, caproate and heptanoate, is steeper at −1.157 kJmol^{-1}.

In addition to the hyperpolarizing shifts in the reversal potential, replacement of internal chloride with the respective anions also causes a reduction in slope

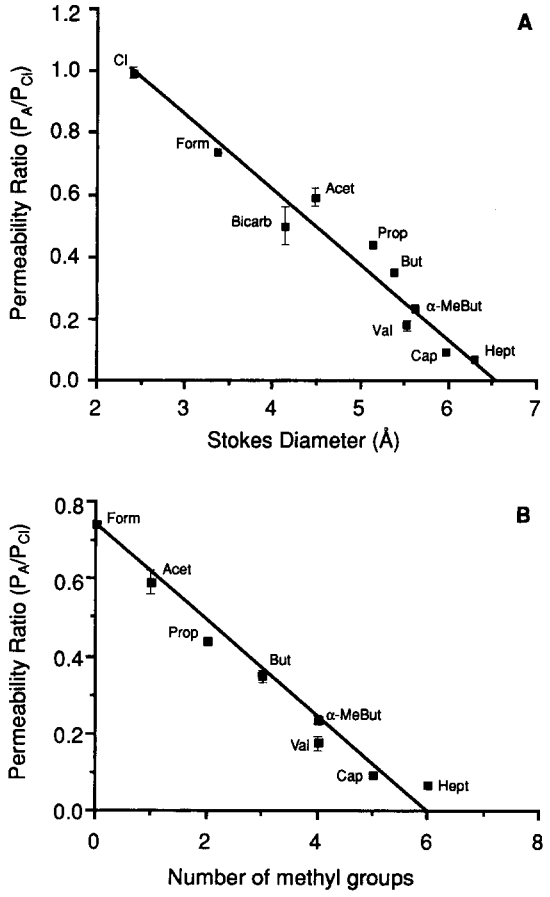


Fig. 4. (*A*) The relationship between permeability ratio and the Stokes diameter for each anion. The graph is fitted by a straight line described by the equation, $P_A/P_{Cl} = -0.25$ SD + 1.6, and cuts the abscissa at 6.55 Å. (*B*) The relationship within the aliphatic fatty acid chain. The graph is linear and crosses the abscissa at 6.03. Values are plotted as mean ± SEM (vertical bars). For the number of observations, *see* the Table.

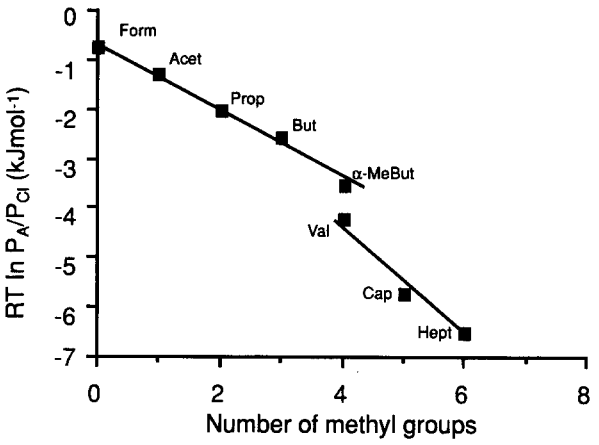


Fig. 5. Plot of ΔG, the energy required to move the fatty acid from an aqueous environment to the binding site within the channel pore ($RT \ln P_A/P_{Cl}$) against the number of methyl groups within the fatty acid chain. The slope of the individual lines affords an estimate of the transition state energy required to move a single methyl group from the bath solution to the binding site within the channel pore. The slope of the line fitting the acids, formate, acetate, propionate, butyrate and α-methyl butyrate, is -0.609 kJmol^{-1}. The gradient of the second line describing the acids, valerate, caproate and heptanoate, is steeper at -1.157 kJmol^{-1}.

conductance of the current/voltage relationships. Figure 6*A* shows the relationship between the slope conductance and the Stokes diameter for each anion. The relationship is linear with regard to chloride, bicarbonate, formate, acetate, propionate, valerate and caproate, which display conductances ranging from 144.3 ± 1.4 to 29.1 ± 7.9 pS (Table). Butyrate, α-methyl butyrate and heptanoate do not lie on the straight line graph; each has a conductance greater than expected for fatty acids of their ionic size, namely 68.3 ± 11.9; 58.7 ± 15.8 and 63.0 ± 9.3 pS, respectively. The relationship between conductance and the number of methyl groups within each acid (Fig. 6*B*) is also linear with the exception of butyrate, α-methyl butyrate and heptanoate. Again, all three anions display conductances greater than expected for their respective chain lengths.

Discussion

Size Limit for Permeant Ions

The relationship between permeability ratio and the Stokes diameter of each anion can be described by a straight line, the permeability ratio decreasing as the ionic diameter increases. Likewise, the relationship between P_A/P_{Cl} and the number of methyl groups in the fatty acid chain is also inverse and approximated by a straight line. This relationship is seen for a variety of other anionic and cationic channels (Bormann et al., 1987; McCleskey & Almers, 1985).

The pore size, as predicted from the graph of P_A/P_{Cl} *v*'s Stokes diameter, is 6.55 Å. The channel is therefore larger than the GABA and glycine receptor gated chloride channels of mouse cultured spinal neurons which have pore diameters of 5.6 and 5.2 Å, respectively (Bormann et al., 1987). However, a pore size of 6.55 Å is very much smaller than the lower limit of 20 Å predicted for the large conductance chloride channel found in rat cultured Schwann cells (Grey, Bevan & Ritchie, 1984).

Conductance

Butyrate, α-methyl butyrate (isovalerate) and heptanoate all show conductances that are greater than expected for anions of their size, whether their anionic size is de-

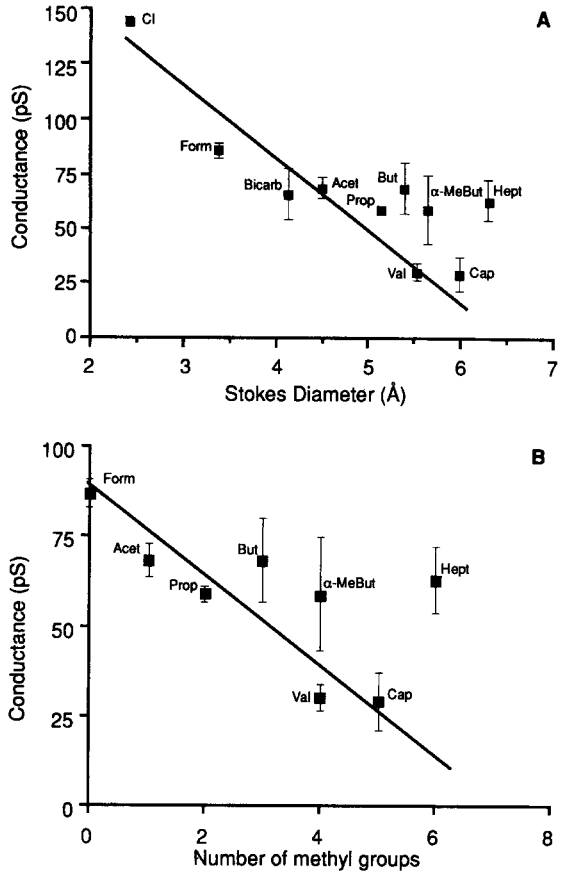
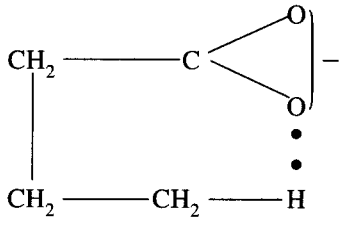


Fig. 6. (*A*) The relationship between conductance and the Stokes diameter for each anion. The graph can be described by a straight line with the exception of butyrate, α-methyl butyrate and heptoate. (*B*) The relationship between conductance and the number of methyl groups contained within the aliphatic fatty acid chain. The graph is linear, with the exception of butyrate, α-methyl butyrate and heptoate. Values are plotted as mean ± SEM (vertical bars). For the number of observations, *see* the Table.

scribed by Stokes diameter or the number of methyl groups in the fatty acid chain. In a paper to the chemical society in 1938, Dippy showed that butyric acid and α-methyl butyric acid have abnormally high dissociation constants as compared with other aliphatic fatty acids of the *n*-series up to nonoic acid. This observation was attributed to the ability of the terminal methyl group to associate with the oxygen of the carboxyl group thus:



This suggests that the secondary structures of butyrate and α-methyl butyrate are important in determining their conductance through the calcium-activated chloride channel. Heptanoic acid displays a normal dissociation constant, as measured by Dippy (1938), but in the light of the results obtained for butyrate and α-methyl butyrate, it is possible to assume that the secondary structure of a long chain fatty acid such as heptanoate could account for the abnormally high conductance of this fatty acid. Assuming all three fatty acids fold when in aqueous solution, then their ionic size, as perceived by the channel, may be smaller than that represented either by the Stokes diameter or the number of methyl groups within the chain.

Properties of the Channel-binding Site

The permeability sequence of the channel differs from the conductance sequence, a phenomenon usually ascribed to the presence of a binding site within the channel pore. Previous experiments investigating the permeability of the channel to other halides suggested between one and two cationic binding sites within the calcium-activated chloride channel (Dixon et al., 1993). The number of binding sites varied inversely with the size of the permeant anion. This same series of experiments also indicated that the internal environment of the calcium-activated chloride channel is relatively fluid compared to free water (Dixon et al., 1993). Unlike the relationship between the binding of *n* alcohols to a blockage site on the nicotinic acetylcholine gated channel of rat myocytes (Murrel et al., 1991), the graph of ΔG *vs.* the number of methyl groups cannot be described by a simple linear regression. The slope of the graph affords a transition state energy per methyl group for transfer from aqueous phase to the channel binding site. The observation that, in this case, the transition state energy for binding of the fatty acids is a curve indicates that ΔG for binding of individual methyl groups is dependent on the fatty acid presenting the methyl group to the binding site. It was possible, however, to fit two straight lines of good correlation (correlation coefficient >0.99) to the points on the graph, thereby affording two average values for the transition state energy per methyl group. The carboxylic acids, formate, acetate, propionate, butyrate and α-methyl butyrate, form the first straight line of slope -0.609 kJmol^{-1}, this value increases to -1.157 kJmol^{-1} for the line describing the relationship between valerate, caproate and heptanoate. It would appear, therefore, that the binding of the larger, more lipophilic acids to the channel wall is energetically more favorable than the binding of the smaller, less lipophilic acids. However, the larger energy change of -1.157 kJmol^{-1} is relatively low compared to the binding of *n*-alcohols to the blockage site on the nicotinic acetylcholine gated channel of rat

myotubes which afforded a standard transition state energy per methylene group of -3.3 kJmol^{-1}. This large energy change displayed by the *n*-alcohols is expected, as it is known that the environment of the binding site in the nicotinic acetylcholine channel is hydrophobic. The energy change involved in the move from an aqueous to a hydrophobic environment for increasingly lipophilic alcohols would therefore be large and favorable. If, in the case of the calcium-activated chloride channel, the difference between the aqueous environment surrounding the membrane and the environment within the channel is minimal, the energy change undergone by the fatty acid moving from aqueous phase to the binding site, although favorable, will be low. This being the case suggests that the environment within the channel is hydrophilic. The association, therefore, between permeant ions and the channel wall is not very strong.

Function of the Channel

The function of this channel, unlike its classification, is becoming easier to predict. The rectification displayed by the channel at depolarized potentials (Thorn & Martin, 1987) excludes an effective role in the repolarization of the cell that is attributed to other chloride currents (Owen, Segal & Barker, 1984; Geletyuk & Kazachenko, 1985). Other calcium-activated chloride currents have been found in rodent lacrymal glands (Findlay & Petersen, 1985; Marty, Tan & Trautmann, 1985; Evans & Marty, 1986) and in exocrine cells (Marty, Tan & Trautmann, 1985) where they appear to be involved in salt secretion. It is therefore of interest to note that the channel can conduct fatty acids involved in the anaerobic respiratory pathway of *A. suum*. Indeed, as stated above, the calcium-activated chloride channel is able to open at the resting membrane potential (-35 mV) of *Ascaris* muscle and is therefore able, under resting membrane conditions, to move intracellular anions across the muscle membrane to the perienteric fluid. At this potential, the chloride current flowing through a single channel is very low at -0.12 pA due to the ratio of internal to external chloride concentrations which in *Ascaris* muscle is 16.5:52.7 mM. Therefore, any anionic current flowing across the membrane at the resting membrane potential must be due to anions other than chloride. Unfortunately, it is experimentally very difficult to measure channel currents flowing across *Ascaris* muscle under normal physiological conditions, as the collagenase treatment required to obtain giga seals, results in the loss of membrane potential.

In addition, an increase in intracellular calcium concentration usually accompanies muscular activity, which in turn results in the formation of metabolic waste products that must be removed from the cell. Any increase in intracellular calcium would lead to an increase in the frequency of channel opening (Thorn & Martin, 1987), thereby facilitating the passage through the channel of the fatty acids that constitute metabolic waste. It is of interest to note that *Ascaris* eggs contain large amounts of the permeant acid α-methyl butyrate in the form of triglyceride (Tsang & Saz, 1973). This fatty acid plays an important role during development from egg to embryo. The eggs are unable to synthesize the fatty acid and must, therefore, absorb it from the perienteric fluid. In the light of the secretory role ascribed to other calcium-activated chloride channels, it is interesting to speculate on the possibility that this channel functions as a mechanism for the removal of metabolic substances across the muscle cell membrane. The internal pH of the intact muscle cells of *Ascaris*, as measured by hydrogen-selective microelectrodes, is 7.37 (Del Castillo et al., 1989). The low pKa values exhibited by the carboxylic acids ensure that the degree of ionization, at intracellular pH, is very high (>99%); consequently, a simple diffusion of the acids through the muscle membrane is unlikely. Indeed, the pH buffering power of *Ascaris* muscle appears to be high. Replacing the external sodium chloride in artificial perienteric fluid with sodium acetate only slightly reduced the internal pH to a new value of 6.93 (Del Castillo et al., 1989). If this channel does perform such an important physiological function, it will be necessary to establish whether muscle cells from other organisms also possess ion channels capable of conducting products of metabolism.

Once the volatile fatty acid metabolites have been excluded from the muscle, they accumulate in the perienteric space. Movement of the volatile fatty acids from the perienteric fluid through the cuticle of *Ascaris* to the outside has been observed by Sims et al. (1992). Although these authors suggested the possibility of an exchange process, the mechanism of the transport process across the cuticle was not identified. Our study raises the possibility that the transport process through the cuticle, like that through the muscle, may also involve a nonselective anion channel.

Considering the diversity of chloride channels, which are found in membranes as diverse as those of rat hippocampus (Franciolini & Nonner, 1987) and the bacterium *Escherichia coli* (Martinac et al., 1987), the description of a chloride current that does not fit comfortably in any contemporary classification is not altogether surprising. Our channel seems suited to transporting anions produced as a result of anaerobic respiration.

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