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A Cl Channel in *Ascaris suum* Selectivity Conducts Dicarboxylic Anion Products of Glucose Fermentation and Suggests a Role in Removal of Waste Organic Anions

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Abstract. The permeability of organic anions (produced from anaerobic fermentation of glucose) through a nonselective membrane Cl channel was examined. Single channel recording techniques were used to study the permeabilities of the anions: oxalate, succinate, oxaloacetate, malate, lactate and pyruvate in *Ascaris* muscle cell membranes. All of the anions, except malate, were found to be conducted through the channel. The relative permeability of most anions could be predicted from the component structure of the anions. The failure of the channel to conduct malate prevents an energy drain on the cell. These studies further the hypothesis that a Cl channel functions to transport waste organic anions across the cell membrane. This mechanism does not require specific exchange carriers for the anions. Channels with properties like the nonselective anion channels of *Ascaris,* are suitable for transport of carboxylic anions through cell membranes, down a concentration or potential gradient.

Key words: *Ascaris suum* — Cl channels — Permeability — Dicarboxylic acids — Anaerobic respiration

Introduction

In an anaerobic environment living cells produce energy by a process of fermentation in which molecules like glucose are oxidized. At the end of the process, one or more organic anions are excreted into the medium surrounding the cell as waste. The products that accumulate at the end of the process are often: lactate, acetate, propionate, butyrate and succinate. These anions, produced inside the cells, have to be removed through the cell membrane and so the question of how the anions cross the lipid barrier of the cell membrane is raised. An opportunity to examine aspects of this process is presented by a preparation of the parasitic nematode, *Ascaris suum.*

Adult *Ascaris* inhabit the small intestine of pigs in a low pO_2 environment and ferment glucose, Fig. 1, to produce energy and the carboxylic acids: acetic acid, propionic acid, succinic acid, butyric acid and α-methylbutyric acid (Bueding & Farrow, 1966; Saz & Bueding, 1966). The acids produced in the muscle cells, cross the cell membrane and accumulate in the surrounding perienteric fluid before further excretion across the cuticle of the parasite (Thompson et al., 1993). The flux of the organic anions across the muscle membrane is greater than the flux of sodium, the flux of potassium, or the flux of chloride, and appears to dominate the membrane potential (Ellory, 1967; Brading & Caldwell, 1971).

We have examined, using single channel recording techniques, properties of a high conductance Ca-activated Cl channel found in about 20% of patches made from muscle membranes of *Ascaris suum* (Thorn & Martin, 1987; Dixon, Valkanov & Martin, 1993; Valkanov, Martin & Dixon, 1994). The channel is a nonselective anion channel that can conduct simple monocarboxylic acid anions (acetate to heptanoate, including α-methylbutyric acid). We have suggested, as a result of the studies, that the Ca-activated Cl channel could function to transport organic anions across the cell membrane and that the membrane potential of the cell (−35 mV) would facilitate concentration of these anions outside. This mechanism does not require specific exchange carriers for the anions.

As a further test of this hypothesis, we now examine the permeability of the channel to the dicarboxylic anions: succinate, oxaloacetate and malate. Succinate is produced in mitochondria of muscle during glucose me-

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tabolism (Fig. 1) and is a major anion which is excreted from the muscle cell and accumulates in the perienteric fluid which surrounds the muscle cell. Malate and oxaloacetate are produced in muscle cytoplasm during glucose fermentation but they are not excreted and do not accumulate in the perienteric fluid. If malate or oxaloacetate leak out through this nonselective anion channel then there could be a substantial energy drain on the cell. In addition, we examine the permeability of the monocarboxylic acids lactate and pyruvate.

This paper, describes the results of experiments which show that the channel is able to conduct: succinate, lactate, pyruvate, oxaloacetate but not malate, and discusses the significance of the observations. We further suggest that channels with properties like the nonselective anion channels of *Ascaris,* are suitable for transport of carboxylic anions through cell membranes.

Materials and Methods

Preparation of Muscle Vesicles

Ascaris suum were obtained from the local slaughterhouse and maintained in Locke's solution at a temperature of 34°C. The Locke's solution was changed daily and the parasites discarded after 4–5 days. A 2-cm section of the worm, taken 5 cm from behind the head region, was cut along one of the lateral lines, the gut was removed and the body flap pinned, cuticle side down, onto Sylgard in a petri dish. The preparation was then washed with extracellular solution (mM): NaCl, 35; Na acetate, 105; KCl, 2; $MgCl_2$, 3; HEPES, 10; glucose, 3; ascorbic acid, 2; EGTA, 1; pH 7.2 with NaOH. The flap was then incubated in enzyme solution (collagenase, 1 mg ml^{-1} in EGTA-free extracellular solution) for ten min. The preparation was then washed well and incubated in extracellular solution at 37°C. After about 1 hr, vesicles could be seen budding off the bag region of the muscle cell. The vesicles were then collected with a Pasteur pipette and placed in the experimental chamber for recording.

Recording

The experimental chamber was mounted on an inverted microscope stage (Reichert-Jung Biostar) and the vesicles viewed at 200×. Experiments were carried out at room temperature (15–22°C). Except where stated in the results for the malate experiments, the pipette solution contained (mM): CsCl, 140; $Mg(Acetate)_2$, 2; Ca(Acetate), 1; HEPES, 1; adjusted to pH 7.2 with CsOH. The bath solution contained (mM): test salt, 140; $Mg(Acetate)_2$, 2; $Ca(Acetate)_2$, 1; HEPES, 1; adjusted to pH 7.2 with CsOH. A high concentration of Cs was used to block possible potassium currents. The test ion was: oxalate, succinate, oxaloacetate, malate, lactate and pyruvate. During some of the malate experiments, the bath and pipette solutions were reversed so that the malate was present in the pipette and Cl was the main anion in the bath. The bath solution with the test anion was initially exchanged for a solution with Cl to document the presence of active Ca-activated Cl channels; with the exception of malate, this control was unnecessary for all patches because the organic anion currents had easily recognized kinetics with long open-times. A high bath concentration of calcium was used to ensure activation of the Cl channels (Thorn & Martin, 1987). All the test anions were supplied by Sigma: Cl as a Cs salt and the carboxylic acids as free acids.

An agar Ag/Cl bath reference electrode was used. In the experiments with malate in the pipette, 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. Reversal potentials of the isolated patches are quoted conventionally with the outside membrane potential taken as zero and the inside membrane potential given relative to the outside.

Patch pipettes were made from capillary glass (Garner Glass 7092) and had resistances of 1–3MΩ and coated with Sylgard to improve frequency responses. Single-channel currents were recorded using the inside-out configuration and seal resistances were greater than 1GΩ. The currents were monitored with a List EPC-7 current-voltage amplifier and oscilloscope. The signal was filtered by an 8-pole Bessel filter (3dB, 1kHz) and recorded on DAT tape (converted Sony DTC 1000).

Analysis

Current records of at least 30-sec duration were made at fixed potentials between +100 and −100 mV and analyzed on an IBM PC2/70 computer using PAT 6.1 software (Dempster, Strathclyde University). Reversal potentials were determined, directly during recording, from the potential on the EPC-7 required to produce zero channel current — the potential at which the direction of the channel currents reversed. This method of determining the reversal potential permitted greater accuracy and precision and was better than interpolating between currents observed at more positive and more negative potentials. Current-voltage plots were drawn by eye but slope conductances were determined by least squares regression in the hyperpolarized region near the reversal potential to avoid errors due to rectification of the current-voltage plots. Each patch produced stable reversal potentials but the slope conductances sometimes reduced with time. If such a reduction occurred, the initial conductance was recorded.

Determination of Permeability Ratios of the Channel

The Ca-activated channel is only permeable to anions: the permeability of Cs in the presence of inorganic anions is negligible (Dixon et al., 1993) and in the presence of organic anions it is <0.07 (Valkanov et al., 1994). The Goldman-Hodgkin-Katz constant field equation (Goldman, 1943; Hodgkin & Katz, 1949) was used to calculate the relative permeability (P_A/P_{Cl}) of the monovalent or the divalent anions, A, from the single-channel current reversal potentials.

The general current equation for an anion given by constant field theory is:

$$I_A = \frac{F^2 Z_A{}^2 P_A V}{RT} \cdot \frac{[A]^o - [A]^i \exp(Z_A FV/RT)}{1 - \exp(Z_A FV/RT)} \quad (1)$$

where P_A is the permeability coefficient, Z_A is the valence, and $[A]^o$ and $[A]^i$ are the outside and inside activities, respectively of anion A.

At the reversal potential, E_{rev}, the net current in a perfectly selective anion permeable channel due to the flow of anions is zero; and when Cl is the only anion in the patch pipette and a monovalent anion A is the only permeant ion at the cytoplasmic surface of the membrane, the relative permeability P_A/P_{Cl} can be determined from:

$$P_A/P_{Cl} = \frac{[Cl]^o}{[A]^i} \cdot \exp(E_{rev} F/RT) \quad (2)$$

where R, T and F have their usual meaning and i denotes intracellular and o denotes extracellular concentrations.

When the anion A is divalent, the relative permeability was determined (Fatt & Ginsborg 1958) from:

$$P_A/P_{Cl} = \frac{[Cl]^o}{4[A]^i} \cdot \exp(E_{rev}F/RT) \cdot [\exp(E_{rev}F/RT) + 1] \quad (3)$$

In all calculations the activities of the anions were taken into account (Robinson & Stokes 1965). All the organic acids were at least 99% ionized at pH 7.2.

Results are expressed as mean ± SEM.

Determination of Anion Profiles and Mean Radii

The profile of each anion in this study was determined from space-filling models with the aid of ALCHEMY III software (TRIPOS Associates). Minimal energy conformations were obtained using TRIPOS' force field calculations and then side views and end on profiles obtained. The mean diameter of each anion was taken as the mean of the dimensions of a minimal rectangular box required to enclose the molecule (Hille, 1992).

Results

In a previous study (Valkanov et al., 1994) we had shown that monocarboxylic glucose fermentation products are permeant anions and pass through the Ca-activated Cl channel. Succinic acid, a dicarboxylic acid, is also produced in *Ascaris* muscle cells, Fig. 1, and accumulates outside the muscle cells in the surrounding perienteric fluid; it too might be expected to pass through the channel. We therefore decided to test the permeability of the channel to succinate and to a number of other appropriate dicarboxylic acids. Initially oxalate and then succinate, were selected for examination. Figure 2 shows the profiles of all the anions tested including oxalate and succinate.

Oxalate and Succinate

Figure 3 (oxalate and succinate) shows representative records of inward and outward currents through the channel, made using inside-out patches at positive and negative membrane potentials when the patch pipette contained 140 mM Cl and the bath anion was 140 mM oxalate or succinate. The channel supported inward and outward currents when either oxalate or succinate was present in the bath establishing that both of these anions are permeant and carry current.

The *I/V* plots of Fig. 4 (oxalate and succinate), show that the reversal potential for oxalate is near −3 mV and for succinate that it is near −15 mV. The slope conductance in the hyperpolarized region near the reversal potential of oxalate was 42pS and for succinate it was 41pS. The means ± SEM values for the reversal potentials and slope conductances are shown in the Table. Equation 3, was used to calculate the relative permeability of the anions. For oxalate P_A/P_{Cl} was 0.36 ± 0.01 (mean ± SEM, n = 3) for succinate it was 0.20 ± 0.01 (mean ± SEM, n = 4), Table. The profiles of these two anions, Fig. 2, show that succinate (mean diameter 5.5 Å) is larger than oxalate (mean diameter 4.5 Å,), and consistent with the lower permeability of succinate when compared to oxalate.

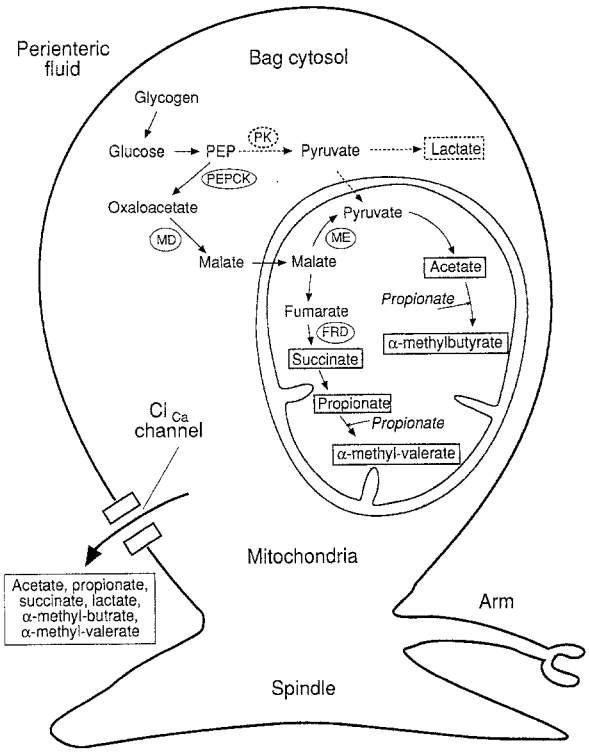

Fig. 1. Diagram of the glucose degradation pathway in *Ascaris* muscle, adapted from Saz, 1981, and Tielens and Van den Bergh, 1993. End products, which then accumulate in the perienteric fluid, are shown in boxes. PEP: phosho-enol pyruvate. PK: pyruvate kinase. PEPCK: phosho-enol pyruvate carboxy kinase. MD: malate dehydrogenase. ME: malic enzyme. FRD: fumarate reductase. The pathway outlined by the dashed arrows is absent or only present at a low level.

Oxaloacetate

We have seen that the simple dicarboxylic acids, oxalate and succinate, are permeant and support significant inward currents. The question of the permeability of other dicarboxylic acids like oxaloacetate, Fig. 1, that is part of the glucose fermentation pathway is then raised. If oxaloacetate is permeant and present in significant concentrations in muscle cytoplasm, then there could be an energy leak from the cell.

Figure 2 (oxaloacetate) shows the profile of oxaloacetate which has a mean diameter of 6.0 Å. It can be seen that the size of the end on profile is intermediate

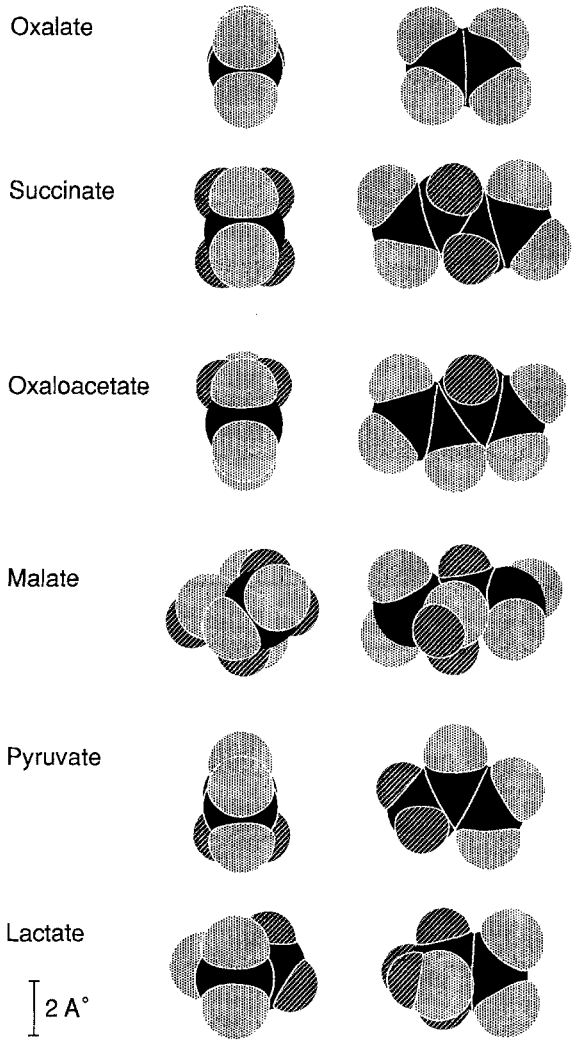

Fig. 2. Space filling models of oxalate, succinate, oxaloacetate, malate, pyruvate and lactate, end (left) and side (right) views.

between oxalate and succinate and if this profile determines relative permeability (Hille, 1992) then the oxaloacetate permeability would be expected to be between oxalate and succinate.

Figure 3 (oxalaoacetate) shows representative inward and outward current records and Fig. 4 (oxaloacetate) shows the *I/V* plot made from the same inside-out patch with 140 mM oxaloacetate present in the bath. The Table summarizes the mean ± SEM values for the reversal potential, slope conductance and P_A/P_{Cl}. The values for P_A/P_{Cl}, 0.32, together with the 64 pS slope conductance show that oxaloacetate is permeant and suggests that there could be a significant energy drain if oxaloacetate is available for diffusion from the cytoplasm. This point is considered further in the discussion.

The relative permeability of oxaloacetate, 0.32, is greater than succinate, 0.20, but less than oxalate, 0.36, and is consistent with the relative sizes of the end on profiles.

Malate

The mean diameter of malate is 6.5 Å. The end on profile of malate, Fig. 2, is greater than oxaloacetate and succinate, suggesting that malate would be less permeant than either of these other two anions. In our experiments (more than 50 patches) where malate replaced all the Cl in the bath, we consistently failed to detect the presence of inward channel currents. In an attempt to check if malate was directly gating the channel closed, experiments on four isolated patches, in symmetrical Cl and held at a potential of −20 mV, were carried out. In these patches, the addition of up to 120 mM malate in the bath failed to significantly reduce the probability of channel opening or change the amplitude of the channel currents. It appeared that the channel failed to conduct malate and conduct inward current.

We also reversed the location of the anions and carried out experiments on patches where the anion in the patch pipette was 140 mM malate and the bath anion was 140 mM Cl. In four patches at potentials ranging between +50 and −70 mV, only inward channel currents were recorded (Fig. 3, malate): the currents were only observed when the membrane was hyperpolarized and when the majority ion carrying the current was Cl. The *I/V* plot, Fig. 4 (malate) shows that the extrapolated reversal potential is near 0 mV. The Table shows mean ± SEM values. Interestingly, the calculated P_A/P_{Cl} for malate under these conditions is 0.5, but the channel fails to conduct an observable outward current. Malate appears to be able to enter the channel from the outside but does not conduct a current; this behavior may appear unusual but is explained by an ion binding to a site in the channel but having a low mobility in the channel (Gallacher, Maruyama, Petersen, 1984; Andersen & Koeppe, 1992). However malate may not enter the channel from the inside so easily since high concentrations of malate applied to the intracellular membrane surface did not block Cl channel currents.

Lactate and Pyruvate

In addition to the dicarboxylic acids above, the permeability of pyruvate and lactate were also examined. These anions might be produced during glucose fermentation, Fig. 1, and lactate has been reported in low concentrations in the perienteric fluid surrounding the muscle. Lactate has a larger mean diameter (5.5 Å) and end on profile than pyruvate (mean diameter, 5.2 Å), Fig. 2, and might be expected to be less permeant.

Figure 3 (pyruvate and lactate) shows representative

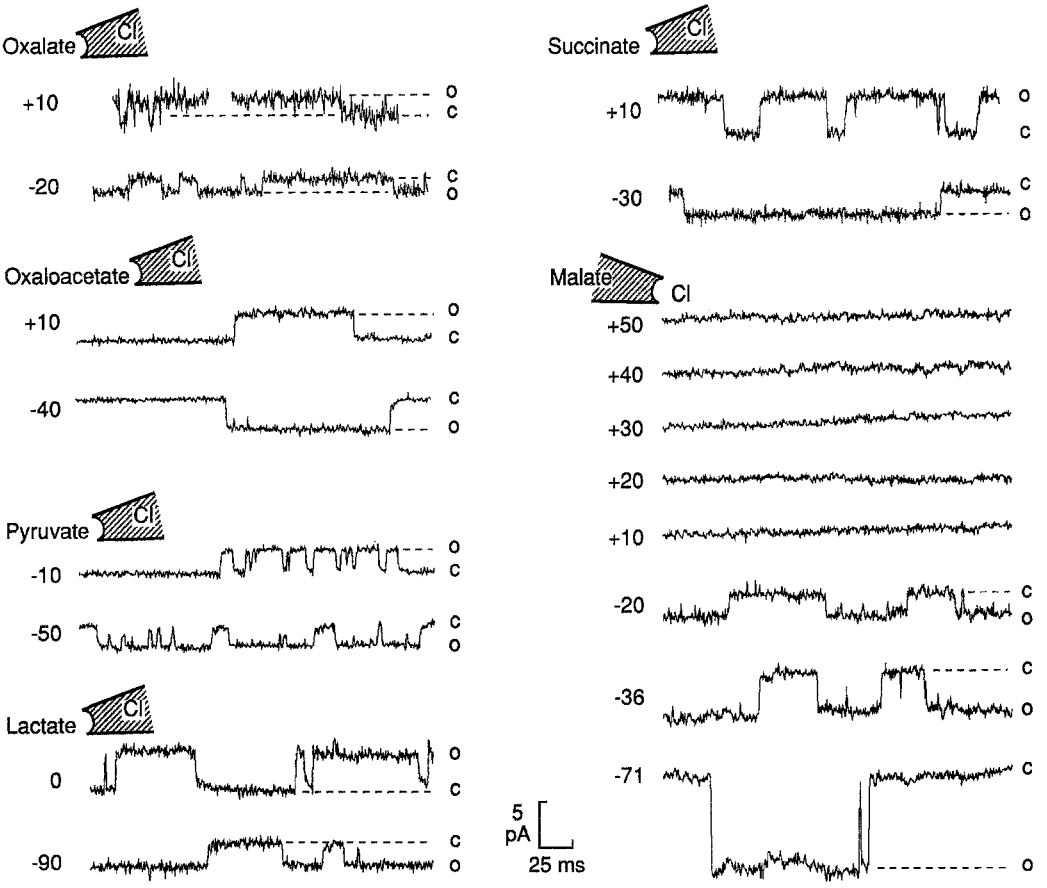

Fig. 3. Representative single channel currents from inside-out patch recordings. Inset: anion distribution. Trans-patch potentials are shown on the left of the current traces. *O:* open channel. *C:* closed channel; Oxalate: Pipette anion, 140 mM Cl; bath anion, 140 mM oxalate; Succinate: Pipette anion, 140 mM Cl; bath anion, 140 mM succinate; Oxaloacetate: Pipette anion, 140 mM Cl; bath anion, 140 mM oxaloacetate; Malate: Pipette anion, 140 mM malate; bath anion, 140 mM Cl; Pyruvate: Pipette anion, 140 mM Cl; bath anion, 140 mM pyruvate; Lactate: Pipette anion, 140 mM Cl; bath anion, 40 mM lactate.

inward and outward channel currents observed with pyruvate in the bath; the *I/V* plots for these patches is shown in Fig. 4. It can be seen that the reversal potential for pyruvate is near −30 mV and for lactate that it is more negative; the result demonstrates a lower relative permeability for lactate. The Table shows the mean ± SEM values of the reversal potentials, slope conductances and relative permeability of pyruvate and lactate.

Estimation of Transition Energies may be Made for Component Parts of the Organic Anions

In saturating barrier models of ion permeability with a one-ion pore, the relative permeability of an ion may be used to determine differences in transition energies between permeant anions transferring from the aqueous phase to a binding site in the channel (Bormann, Hamill & Sakmann, 1987; Andersen & Koeppell, 1992). Thus:

$$P_A/P_{Cl} = \exp[(G_A - G_{Cl})/RT], \quad (4)$$

where R and T have their usual meaning and $G_A - G_{Cl}$ is the difference in the transition energies for the anion A and Cl respectively. Even in multi-ion pores under 'constant offset energy' conditions the differences in transition energy barriers between two anions may be determined in the same way (Hille, 1975).

The difference $G_A - G_{Cl}$ between the energies for the anions observed in this study together with some of those reported by Valkanov et al., (1994) are plotted in Fig. 5: the graph shows $G_A - G_{Cl}$ against the number of methyl groups present and suggests that the component groups of the permeant anion behave additively to determine the transition energy of the whole anion. The line produced by the dicarboxylic acids oxalate and succinate is parallel to the plot formate to α-methylbutyrate. Both lines have least square slopes that afford a transition energy per methyl group from the aqueous phase to

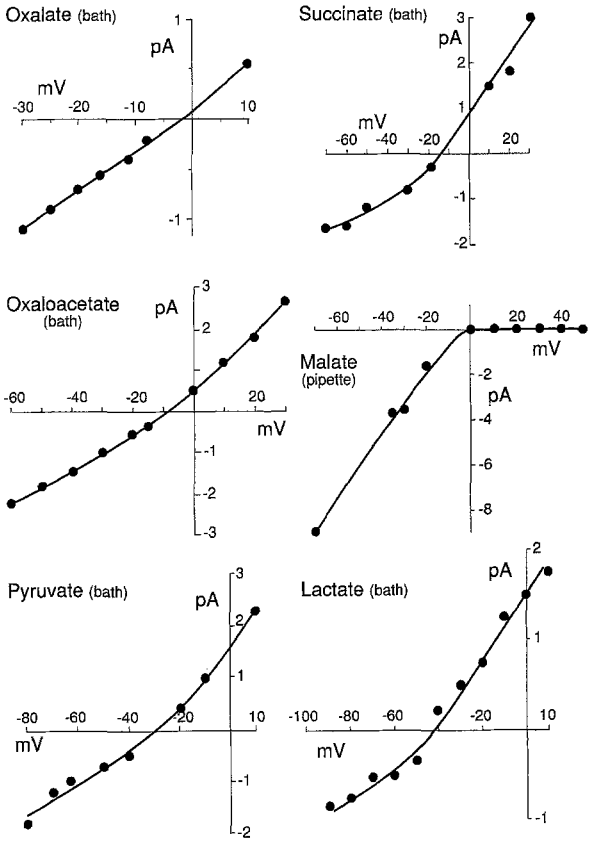

Fig. 4. Representative single channel *I/V* plots; Oxalate: E_{rev} – 3 mV; Slope conductance 44pS; Succinate: E_{rev} – 15 mV; Slope conductance 53pS; Oxaloacetate: E_{rev} – 8 mV; Slope conductance 55pS; Malate: Single channel *I/V* plot; E_{rev} 0.5 mV; note that malate was the anion in the pipette and that Cl was the anion in the bath. Pyruvate: E_{rev} – 30 mV; Slope conductance 50pS; Lactate: E_{rev} – 39 mV; Slope conductance 37pS.

the channel binding sites of –0.61 Kjmol^{-1}. The line oxalate-succinate is downwardly displaced from the formate to α-methylbutyrate plot by –1.9 Kjmol^{-1} suggesting that the extra carboxylic group increases the transition energy by that amount. The large transition energy of the $-COO^-$ group (compared to $-CH_2^-$ groups) can be explained by the greater dehydration energy (because of electrostatic interaction) that is required to permit translocation across the channel.

The downward displacement of pyruvate compared to acetate suggests that a –C=O group increases the transition energy by –1.5 Kjmol^{-1}. The downward displacement of lactate compared to acetate suggests that the addition of the –CHOH group increases the transition energy by –1.9 Kjmol^{-1}. The greater transition energy of lactate compared to pyruvate is not surprising because of the chiral carbon of lactate which makes the molecule of lactate larger than the molecule of pyruvate. It is pointed out however, that simple addition of the transition energies for the component groups of the anion does not predict accurately the observed relative permeability of malate or oxaloacetate; the failure may reflect the existence of selective ion binding sites within the channel.

Discussion

The Physiological Importance of the Permeability of the *Ascaris* Ca-Activated Cl Channel to Organic Anions

In a study of the membrane potential of *Ascaris* muscle based on the Goldman-Hodgkin-Katz voltage-equation, Brading and Caldwell (1971) showed that the relative permeability of the membrane to Cl is high (P_{Na}:P_K:P_{Cl} was 1:1:7); but the flux of organic anions is greater than the flux of sodium, the flux of potassium or the flux of chloride (Ellory, 1967), and appears to dominate the membrane potential (Brading & Caldwell, 1971).

In this paper and a previous paper (Valkanov et al., 1994), we have demonstrated the high permeability of the *Ascaris* Cl channel to carboxylic acids. This channel has a high density and is found in 20% of membrane patches in cluster of up to 15 channels per patch; these channels are active in recordings made from intact cells in a body flap preparation (Thorn & Martin, 1987). The high conductance (150 pS in symmetrical Cl) of the channel, the frequency with which it is observed and the fact it can be fully activated at the normal resting membrane potential, (–35 mV, Thorn & Martin, 1987) explains the permeability of the muscle membrane to both organic anions and Cl. The inward rectification shown by the channel in symmetrical Cl (Thorn & Martin, 1987) argues against a role in repolarization of the cell that is attributed to other Ca-activated Cl channels (Owen, et al. 1983; Geletyuk & Kazachenko, 1985).

Oxaloacetate

Oxaloacetate and malate are produced in *Ascaris* muscle cytoplasm during glucose metabolism (Saz, 1981; Tielens & Van den Bergh, 1993) but they do not accumulate in perienteric fluid. We found that malate was not normally conducted through the channel, but it was clear that oxaloacetate could be. With oxaloacetate having a conductance of 64pS and P_A/P_{Cl} of 0.32, the channel would permit extracellular accumulation of oxaloacetate. However this is not found to be the case in the whole animal.

The normal cytoplasmic concentration of oxaloacetate is relevant here: the cytoplasmic enzyme, malate dehydrogenase, rapidly reduces cytoplasmic oxaloacetate to malate (Saz, 1981). The cytoplasmic concentrations of oxaloacetate are therefore low. Another possible factor reducing oxaloacetate leak might be localization

Table. Mean + SEM values for the reversal potential, slope conductance and relative permeability of anions compared to Cl

Anion	W (Å)	H (Å)	L (Å)	D (Å)	E_{rev} (mV)	G (pS)	P_A/P_{Cl}	G_A/G_{Cl} (Kjmol-1)	Number of Patches
Oxalate	3.1	4.9	5.5	4.5	−5.6 ± 1.5	45.4 ± 2.0	0.36 ± 0.01	−2.50	3
Succinate	3.9	4.9	7.6	5.5	−17.2 ± 0.6	39.9 ± 12.4	0.20 ± 0.01	−3.94	4
Oxaloacetate	3.9	5.2	8.7	6.0	−7.4 ± 1.0	63.6 ± 13.1	0.32 ± 0.01	−2.79	5
Pyruvate	3.9	5.3	6.4	5.2	−29.3 ± 1.7	51.0 ± 10.9	0.32 ± 0.02	−2.79	5
Lactate	4.5	5.3	6.8	5.5	−33.5 ± 3.5	45.4 ± 12.6	0.27 ± 0.04	−3.21	4
Malate	4.8	6.3	8.5	6.5	0.1 ± 0.2	0	0.5 ± 0.01		5

Organic anion sizes (W: width; Height: height; L: length; D: mean dimensions). E_{rev}: reversal potential. *G:* slope conductance. P_A/P_{Cl}: relative permeability of the anion compared to Cl. G_A/G_{Cl}: transition energy. The correlation coefficient, *r*, between the relative permeability of all organic anions except malate and the slope conductance was 0.51.

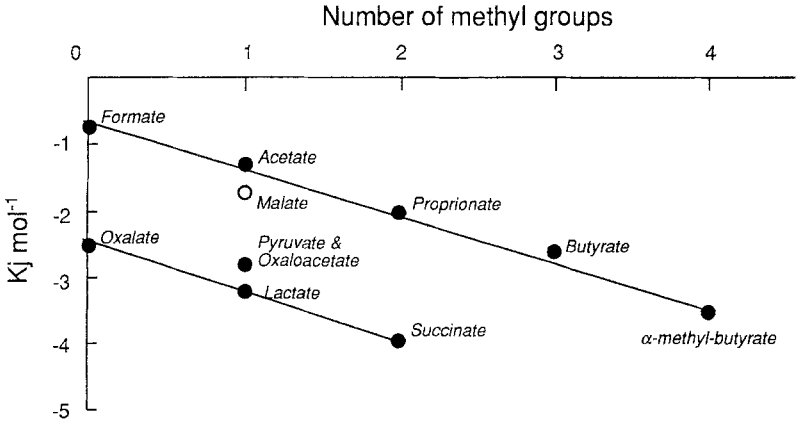

Fig. 5. Plot of the transition energies of anions determined from P_A/P_{Cl} values observed in this study and some from Valkanov et al. (1994) against number of methyl groups. The line fitted for the monovalent anions was obtained from a least squares fit of the points formate to α-methylbutyrate and the line for the divalent anions oxalate to succinate plotted parallel.

of metabolites as a result of the compartmentation of the glucose metabolic enzymes. However, a recent review on glucose metabolism in helminths, Tielens and Van den Berg (1993), suggests that compartmentation does not occur.

Malate

It is interesting that the mean diameter of malate, 6.5 Å, is near the limiting pore size of the Ca-activated channel, (6.6 Å Valkanov et al., 1994) suggesting that there is close contact between the malate anion and the walls of the channel. Malate did not enter and bind to the channel from the inside since experiments with malate in the bath failed to detect inward channel currents or to demonstrate evidence of blocking of Cl currents. When malate was placed in the patch pipette, the calculated P_A/P_{Cl} was 0.5 but the channel did not support current carried by malate. The observation is predicted if malate enters the channel from the outside and binds inside the channel (explaining the high relative permeability) but has low mobility in the channel (explaining the absence of malate current). In this respect, malate seems to behave in the same manner as rubidium in cation channels from mammalian exocrine acini (Gallacher et al., 1984). If binding to the channel site and mobility in the channel is not well related, it predicts and explains the poor correlation observed between the relative permeabilities and conductances of the other organic anions (Table, $r = 0.51$, and Valkanov et al., 1994). The ability of the Ca-activated chloride channel to prevent the movement of malate across the cell membrane would prevent an energy drain on the cell.

Lactate and Pyruvate

Very low levels of lactate have been detected in the perienteric fluid surrounding *Ascaris* muscle (Bueding & Yale, 1951) suggesting that a low level of excretion may occur through the channel. Pyruvate has not been reported in *Ascaris* perienteric fluid despite the fact that it is more permeant than lactate. *Ascaris* is not capable of forming significant quantities of cytoplasmic pyruvate, because the concentration of pyruvate kinase (which converts PEP to pyruvate) is very low (Saz, 1981). The level of cytoplasmic pyruvate appears to be very low and may only rise to 3.1 mM in mitochondria (Barrett & Beis, 1973). Thus, although the anion channel is permeable to lactate and pyruvate anions, significant loss may be prevented by low cytoplasmic levels.

The Physiological Function of Nonselective Anion Channels in a Number of Preparations may be the Membrane Transport of Carboxylic Acids Produced from Glucose Fermentation

The physiological functions of different types of Cl channels include: (i) GABA and glycine gated Cl channels which hyperpolarize and inhibit electrical activity in nerve and muscle cells (Bormann et al., 1987); (ii) Cl channels activated by osmotic stress and which may be involved in volume regulation (Worrell et al., 1989; Kubo & Okada, 1992); (iii) Cl channels which are spontaneously active and which contribute to the membrane potential and prevent hyperexcitability (Blatz & Magleby, 1983); (iv) Cl channels in parallel with ATP-dependent H^+ pumps involved in the maintenance of electroneutrality during acidification of intracellular organelles (Schmid, Burckhardt & Gogelein, 1989; (v) Cl channels involved in salt secretion (Findlay & Petersen, 1985; Marty, Tan & Trautmann, 1985). However, the function of a number of Cl channels remains unknown and in this group there is a group of nonselective anion channels with a relatively large (6.5 Å) pore. (Woodbury & Miles, 1973; Franciolini & Nonner, 1987).

The observations reported in this paper together with the report of Valkanov et al. (1994) demonstrate that the *Ascaris* Cl channel has a large pore (6.6 Å) and is permeable to both mono- and dicarboxylic acid products of glucose fermentation. The normal resting membrane potential of *Ascaris* muscle, around −35 mV (Martin, 1980), favors extracellular concentration of the organic anion products. Compared with ion channels known in animal cells and microorganisms (Hille, 1992), the Ca-activated Cl channel in *Ascaris* has the unusual property of conducting organic ions as a physiological substrates rather than an inorganic ion. Channels normally conducting organic anions are however, recognized in vacuolar membranes of plants (Hendrich & Marten, 1993).

It is usually assumed in vertebrate preparations that passage of most carboxylic acids across cell membranes is by simple diffusion of the uncharged protonated moiety. However, Woodbury and Miles (1973), who measured the resting membrane conductance of skeletal muscle using microelectodes, detected the presence of Cl channels with a pore cross section of 5.5 × 6.5 Å that was detectably permeable to butyrate and lactate. The existence of nonselective anion channels that have a large P_A/P_{Cl} for acetate (0.66) and propionate (0.5), has also been noted in rat hippocampal neuron membranes (Franciolini & Nonner, 1987) but the function was not identified. They too may conduct products of glucose fermentation.

In inner mitochondrial membrane preparations specific dicarboxylic acid and tricarboxylic acid carriers are recognized (Lehninger, 1965) but the presence of normally quiescent nonselective anion channels permeable to organic anions like acetate, propionate, and butyrate has been advocated (Galid & Baevis, 1986) and observed (Hayman, Spurway & Ashley, 1993). It seems possible that a function of large nonselective anion channels, could be to permit movement of carboxylic anions through lipid membranes.

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