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INTRACELLULAR CHLORIDE ACTIVITY IN FROG HEART

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SUMMARY

1. Chloride liquid ion exchanger micro-electrodes have been used to measure intracellular chloride activity (a^i_{Cl}) in isolated pieces of frog sinus venosus, atrium and ventricle.

2. a^i_{Cl} was found to be $35{\cdot}0 \pm 3{\cdot}2$, $17{\cdot}3 \pm 0{\cdot}8$ and $17{\cdot}6 \pm 0{\cdot}8$ mM in sinus venosus, atrium and ventricle, respectively.

3. In atrium and ventricle a^i_{Cl} is independent of whether the tissue is beating or non-beating.

4. In non-beating ventricle a^i_{Cl} changes rapidly and reversibly when a^o_{Cl} is reduced by replacing chloride with glucuronate.

5. E_{Cl} calculated from the measured a^i_{Cl} has been compared with E_{Ci} calculated from the constant field equation assuming either constant chloride conductance or constant chloride permeability.

6. It is suggested that chloride is actively transported into the cells in all three kinds of frog heart tissue.

INTRODUCTION

Intracellular chloride concentration in various kinds of heart muscle has been measured by means of total tissue analysis, which requires measuring extracellular space, and by washout of ^{36}Cl or ^{82}Br, assuming for the latter that bromide permeability and distribution are the same as those of chloride. Using the first of these methods the intracellular chloride concentration has been reported to be 32 m-mole/kg fibre water (Lamb, 1961), 37 m-equiv/l. intracellular water (DeMello, 1963) and 34 m-mole/l. intracellular water (Carmeliet & Janse, 1965) for rat atrium, rabbit atrium and cat papillary respectively. The chloride concentration as determined by radio-isotope washout is 21·3 m-mole/l. intracellular water in cat papillary (Carmeliet & Janse, 1965) and 19·5 m-mole/kg fibre water in rat atrium (Lamb, 1961).

Hutter & Noble (1961), studying the anion conductance of sheep Purkinje fibres, hypothesized that if chloride is distributed in accordance

with the constant field equation (Hodgkin & Katz, 1949), assuming constant chloride permeability (P_{Cl}), then the chloride equilibrium potential (E_{Cl}) should be in the neighbourhood of the mean membrane potential. They calculated that, for a Purkinje fibre beating at 1 Hz, $E_{Cl} = -50$ mV. The measured values of chloride noted above give calculated values of E_{Cl} somewhat more positive but that could be explained if the intracellular activity coefficient is lower than the extracellular one. To test the Hutter–Noble hypothesis it is necessary to know the intracellular chloride activity, a^i_{Cl}.

With the development of liquid ion exchanger micro-electrodes, it has become possible to measure a^i_{Cl} in intact heart muscle cells. The results of such measurements in frog heart are reported here. Preliminary reports of some of this work have been published elsewhere.

METHODS

Frogs (*Rana pipiens*) were pithed and the hearts removed. For experiments with sinus venosus the pacemaker region was identified under a dissecting microscope and this region dissected free from the rest of the heart. For the other experiments a strip approximately 1 mm wide was dissected from either the atrium or the ventricle. In all cases the piece of tissue was then fixed, endocardial surface up, with pins in a tissue bath. The preparation was superfused with frog Ringer solution of the following composition: 110 mM-NaCl, 2·5 mM-KCl, 1·5 mM-$CaCl_2$, 2·5 mM-$MgSO_4$, 5·5 mM glucose, 5 mM-HEPES, NaOH to adjust pH to 7·4. The experiments were conducted at room temperature, 22–24° C.

The sinus preparations beat spontaneously, usually at a frequency of about 0·6 Hz, but the atrial and ventricular preparations were quiescent except when driven with a stimulator. When stimulated the atrial and ventricular strips were driven at a frequency of 0·5 Hz.

Chloride liquid ion exchanger micro-electrodes were prepared as previously described (Walker, 1971). Ag–AgCl micro-electrodes were constructed as described by Nield & Thomas (1973). Both types of electrodes were calibrated in solutions of known chloride activity immediately before and after intracellular measurements were made. If the before and after calibrations were not the same, the intracellular measurements were discarded.

Membrane potential measurements were made with 3 M-KCl filled micropipettes having resistances in the range of 12–15 MΩ when the tips were immersed in frog Ringer. The KCl micro-electrodes were made by pulling micropipettes from 1 mm o.d. Pyrex (Corning code 7740) capillaries on a Kopf vertical pipette puller. The pipettes were taken from the puller and the tips immersed in glass distilled water for approximately 15 min, during which time the terminal 300–400 μm of the tip filled with water. The pipettes were taken one at a time from the distilled water and 3 M-KCl was injected as far down into the shoulder as possible with a 30-gauge needle attached to a Millipore filter (0·45 μm pores) and a hypodermic syringe. The pipette was then placed horizontally on the movable stage of a compound microscope and a solid glass needle was inserted into the pipette. The needle was advanced rapidly by hand and under visual control (100 × magnification) until the needle touched the column of water in the tip. This procedure caused the KCl to flow down into the pipette and displace the air bubble upward so it could easily be flushed out

with the 30-gauge needle. The filled pipettes were then stored with their tips in 3 M-KCl for about 1 h before being used. Micro-electrodes filled in this manner were found to have very reproducible impedances and essentially no tip potentials. Fresh micro-electrodes were filled on the morning of every experiment.

When the chloride in the Ringer solution was reduced it was by replacing it, on a molar basis, with glucuronate. The chloride activity in all solutions was measured with an Ag–AgCl electrode using a calomel or Ag–AgCl electrode with a saturated KCl bridge as the reference electrode.

RESULTS

When a chloride electrode is introduced into a cell the potential difference, ΔE (mV), that is measured is the sum of the membrane potential, E_M (mV), and the potential difference due to the difference of chloride activities outside, a^o_{Cl} (mM), and inside, a^i_{Cl} (mM), the cell. This is shown in eqn. (1):

$$\Delta E = E_M + \frac{nRT}{F} \log_e \frac{a^o_{Cl}}{a^i_{Cl}}. \quad (1)$$

R, T and F have their usual meanings and n is an empirical constant (dimensionless) that is necessary because the slopes of the chloride electrodes are usually less than predicted by the Nernst equation. n is a constant for any one electrode and is in the range of $0{\cdot}95 \leqslant n \leqslant 1{\cdot}0$.

Because the frog heart muscle cells are too small to insert two electrodes into the same cell the following procedure was adopted. Five to ten ΔE measurements were made with a chloride electrode and then five to ten E_M measurements were made. The two kinds of measurements were made as close together in time as possible. The average values of the two sets of

TABLE 1. a^i_{Cl} measurements in frog sinus venosus. Each row contains the results of measurements in one preparation. The values of ΔE and E_D are the means and S.E. of the means. The numbers in parentheses are the numbers of measurements from which the means and S.E. were calculated. The values of a^i_{Cl} are calculated from the mean values of ΔE and E_D. The values of E_{Cl} are calculated from a^i_{Cl} and the known value of a^o_{Cl}. The error estimates for a^i_{Cl} and E_{Cl} are calculated from the S.E. of ΔE and E_D using a propagation of error method. The bottom row is the means and S.E. obtained from the entries in the Table

ΔE (mV)	E_D (mV)	a^i_{Cl} (mM)	E_{Cl} (mV)
$-28{\cdot}0 \pm 1{\cdot}0$ (5)	$-55{\cdot}0 \pm 2{\cdot}6$ (5)	$29{\cdot}2 \pm 3{\cdot}5$	$-28{\cdot}8 \pm 3{\cdot}0$
$-24{\cdot}2 \pm 1{\cdot}7$ (5)	$-53{\cdot}0 \pm 2{\cdot}2$ (4)	$27{\cdot}1 \pm 3{\cdot}1$	$-30{\cdot}8 \pm 2{\cdot}9$
$-27{\cdot}4 \pm 0{\cdot}5$ (5)	$-52{\cdot}4 \pm 1{\cdot}6$ (5)	$32{\cdot}9 \pm 2{\cdot}3$	$-25{\cdot}7 \pm 1{\cdot}8$
$-37{\cdot}7 \pm 0{\cdot}7$ (6)	$-58{\cdot}0 \pm 1{\cdot}8$ (4)	$39{\cdot}8 \pm 3{\cdot}1$	$-20{\cdot}9 \pm 2{\cdot}0$
$-30{\cdot}8 \pm 1{\cdot}3$ (5)	$-51{\cdot}0 \pm 3{\cdot}2$ (5)	$39{\cdot}4 \pm 5{\cdot}6$	$-21{\cdot}2 \pm 3{\cdot}6$
$-32{\cdot}8 \pm 1{\cdot}9$ (6)	$-61{\cdot}2 \pm 1{\cdot}3$ (6)	$27{\cdot}5 \pm 2{\cdot}7$	$-30{\cdot}3 \pm 2{\cdot}5$
$-34{\cdot}3 \pm 0{\cdot}6$ (6)	$-48{\cdot}4 \pm 1{\cdot}8$ (4)	$50{\cdot}6 \pm 3{\cdot}9$	$-14{\cdot}8 \pm 2{\cdot}0$
$-37{\cdot}3 \pm 2{\cdot}9$ (3)	$-53{\cdot}0 \pm 2{\cdot}8$ (6)	$46{\cdot}8 \pm 7{\cdot}9$	$-16{\cdot}8 \pm 4{\cdot}3$
$-33{\cdot}6 \pm 0{\cdot}8$ (10)	$-69{\cdot}0 \pm 2{\cdot}1$ (10)	$22{\cdot}2 \pm 2{\cdot}0$	$-35{\cdot}8 \pm 2{\cdot}3$
$-31{\cdot}8 \pm 1{\cdot}5$	$-55{\cdot}7 \pm 2{\cdot}1$	$35{\cdot}0 \pm 3{\cdot}2$	$-25{\cdot}0 \pm 2{\cdot}3$

measurements and the known value of a^{o}_{Cl} were used to calculate a^{i}_{Cl}. The error estimates of the derived data, a^{i}_{Cl} and E_{Cl}, were obtained from the S.E. of the means of ΔE and E_M by a propagation of error method (Deming, 1964). The results of the experiments with sinus venosus, atrium and ventricle are shown in Tables 1–3 respectively. The entries in the bottom row of each table are means computed from the values in the body of that table.

TABLE 2. a^{i}_{Cl} measurements in frog atrium

ΔE (mV)	E_D (mV)	a^{i}_{Cl} (mM)	E_{Cl} (mV)
−53·8 ± 3·6 (5)	−88·4 ± 3·4 (5)	21·8 ± 4·4	−36·3 ± 5·2
−50·1 ± 1·9 (10)	−90·1 ± 1·4 (10)	18·0 ± 1·7	−41·2 ± 2·4
−46·6 ± 1·5 (10)	−91·2 ± 1·5 (10)	15·4 ± 1·3	−45·2 ± 2·1
−48·7 ± 1·8 (10)	−87·6 ± 1·2 (10)	18·8 ± 1·7	−40·1 ± 2·3
−44·4 ± 1·1 (10)	−90·2 ± 2·0 (10)	13·8 ± 1·3	−48·0 ± 2·4
−49·9 ± 1·7 (10)	−88·1 ± 1·4 (10)	19·3 ± 1·7	−39·4 ± 2·3
−48·5 ± 1·3 (10)	−90·3 ± 1·7 (10)	16·2 ± 1·4	−43·8 ± 2·3
−47·6 ± 1·2 (20)	−92·0 ± 1·6 (10)	14·6 ± 1·2	−46·6 ± 2·0
−46·6 ± 1·4 (10)	−93·4 ± 1·8 (10)	14·4 ± 1·3	−47·4 ± 2·4
−46·8 ± 1·5 (11)	−87·4 ± 1·9 (10)	17·0 ± 1·6	−42·6 ± 2·5
−53·2 ± 2·2 (10)	−88·7 ± 2·1 (10)	21·0 ± 2·6	−37·2 ± 3·2
−48·7 ± 0·9	−89·8 ± 0·6	17·3 ± 0·8	−42·5 ± 1·2

TABLE 3. a^{i}_{Cl} measurements in frog ventricle

ΔE (mV)	E_D (mV)	a^{i}_{Cl} (mM)	E_{Cl} (mV)
−49·5 ± 1·1 (10)	−88·0 ± 2·2 (10)	18·6 ± 1·9	−40·4 ± 2·6
−49·8 ± 1·4 (10)	−91·6 ± 1·9 (7)	16·7 ± 1·6	−43·1 ± 2·5
−46·6 ± 1·8 (10)	−90·6 ± 1·6 (10)	15·8 ± 1·5	−44·6 ± 2·5
−45·8 ± 0·9 (10)	−87·2 ± 1·6 (10)	17·0 ± 1·3	−42·7 ± 1·9
−53·2 ± 2·6 (10)	−90·7 ± 2·1 (10)	19·3 ± 2·6	−39·3 ± 3·5
−51·7 ± 1·5 (10)	−95·2 ± 0·8 (10)	15·1 ± 1·1	−45·6 ± 1·8
−56·7 ± 2·1 (10)	−89·4 ± 2·3 (10)	23·6 ± 3·0	−34·3 ± 3·3
−52·1 ± 1·5 (10)	−90·7 ± 2·1 (10)	18·0 ± 2·0	−41·2 ± 2·8
−49·4 ± 0·9 (10)	−91·5 ± 2·7 (10)	15·5 ± 1·8	−45·0 ± 3·0
−47·1 ± 2·0 (10)	−89·4 ± 2·0 (10)	15·9 ± 1·8	−44·4 ± 2·9
−53·7 ± 1·7 (10)	−88·9 ± 1·6 (10)	21·3 ± 2·1	−36·9 ± 2·5
−48·6 ± 1·2 (10)	−92·2 ± 3·3 (10)	15·0 ± 2·2	−45·7 ± 3·7
−50·4 ± 0·9	−90·4 ± 0·6	17·6 ± 0·8	−41·9 ± 1·0

Because the chloride liquid ion exchanger has poor selectivity with respect to other anions (Walker, 1971), a control experiment was done using an Ag–AgCl micro-electrode of the design described by Nield & Thomas (1973). This experiment was done in a ventricle because that is the easiest of the three tissues in which to make cell penetrations. The results of that experiment were $\Delta E = -57{\cdot}5 \pm 1{\cdot}3$ (7) for the liquid ion exchanger electrode and $\Delta E = -59{\cdot}8 \pm 2{\cdot}6$ (8) for the Ag–AgCl electrode. Since the slopes of the two electrodes were the same, and assuming a

constant diastolic membrane potential, E_D, it can be concluded that the liquid ion exchanger chloride electrode measures only chloride inside of these cells.

The values of a^i_{Cl} reported in Tables 1–3 are obviously too high for chloride to be passively distributed at the diastolic membrane potential, E_D. It might, however, be passively distributed at some higher potential determined by the constant field equation as hypothesized by Hutter & Noble (1961). If their hypothesis is correct, then in preparations which are not beating, i.e. E_M is constant and equal to E_D, a^i_{Cl} should decrease until $E_{Cl} = E_D$. The results of experiments with non-beating atrial and ventricular strips are presented in Tables 4 and 5. It can be seen that there was no significant decrease from the control value of a^i_{Cl} in either tissue.

TABLE 4. a^i_{Cl} measurements in non-beating frog atrium. Each pair of rows is results from a single preparation. The top row of each pair is the control values (preparation being driven at 0·5 Hz) and the bottom row of each pair is the values when the preparation is not beating. The last pair of rows is the means and S.E. of the entries in the Table. The top row of that pair is averages of the control values and the bottom row is averages of the non-beating values

Non-beating (hr)	ΔE (mV)	E_D (mV)	a^i_{Cl} (mM)	E_{Cl} (mV)
0	−58·9 ± 1·0 (9)	−91·8 ± 2·1 (9)	22·8 ± 2·2	−35·1 ± 2·5
5	−59·4 ± 1·5 (5)	−95·1 ± 3·4 (7)	20·3 ± 3·1	−38·1 ± 3·9
0	−58·9 ± 1·7 (8)	−87·6 ± 2·0 (8)	28·3 ± 3·0	−29·6 ± 2·7
4½	−56·5 ± 2·4 (8)	−93·5 ± 1·1 (6)	19·7 ± 2·2	−38·8 ± 2·8
0	−57·0 ± 1·5 (8)	−92·6 ± 1·6 (8)	22·0 ± 1·9	−36·1 ± 2·2
3½	−53·4 ± 1·5 (8)	−91·6 ± 1·5 (8)	18·3 ± 1·6	−40·8 ± 2·2
0	−56·2 ± 1·0 (9)	−94·1 ± 1·7 (9)	20·1 ± 1·6	−38·4 ± 2·0
4	−55·1 ± 1·7 (9)	−92·6 ± 1·7 (7)	18·8 ± 1·9	−40·0 ± 2·6
0	−53·1 ± 1·4 (7)	−93·4 ± 2·0 (7)	17·7 ± 1·8	−41·5 ± 2·5
4	−53·6 ± 1·1 (8)	−95·3 ± 1·3 (8)	16·8 ± 1·2	−43·0 ± 1·8
0	−55·1 ± 1·5 (8)	−91·8 ± 1·6 (9)	21·1 ± 1·9	−37·2 ± 2·3
18	−60·9 ± 1·9 (8)	−96·3 ± 1·8 (7)	21·6 ± 2·3	−36·5 ± 2·7
0	−56·5 ± 0·9	−91·9 ± 0·9	22·0 ± 1·4	−36·3 ± 1·6
	−56·5 ± 1·3	−94·1 ± 0·7	19·2 ± 0·7	−39·5 ± 0·9

It might be argued that the chloride permeability, P_{Cl}, is so low in non-beating preparations that the chloride efflux rate is too low to bring about an observable decrease in a^i_{Cl} during the time allowed for the experiments. To test this possibility a^o_{Cl} was reduced by as much as 90 % by replacement with glucuronate to see if a^i_{Cl} could be made to decrease. These experiments were done with non-beating preparations. Glucuronate was chosen because it is an impermeant anion in frog skeletal muscle (J. W. Woodbury, personal communication) and it is not 'seen' by the chloride liquid ion

exchanger. Even when 90 % of the chloride is replaced by glucuronate the glucuronate does not affect the chloride liquid ion exchanger microelectrode in any way. Table 6 shows the results of these experiments.

Because of the technical problems in making a^i_{Cl} measurements, no kinetic studies of the rate of change of a^i_{Cl} were done when a^o_{Cl} was changed. However, in several experiments, lasting 18–24 hr, a^i_{Cl} was measured at different times during the experiments. The earliest measurements were 1 hr after reducing a^o_{Cl} and by that time a^i_{Cl} had reached its new steady-state value. In no experiment during which multiple measurements were made was there a significant change in a^i_{Cl} after the first measurement following the reduction of a^o_{Cl}. In one experiment in which a^o_{Cl} was reduced to 10 % and later returned to 100 %, a^i_{Cl} had returned to the control value when it was measured 1·5 hr after the return to 100 %.

TABLE 5. a^i_{Cl} measurements in non-beating frog ventricle

Non-beating (hr)	ΔE (mV)	E_D (mV)	a^i_{Cl} (mM)	E_{Cl} (mV)
0	−53·6 ± 1·4 (8)	−94·6 ± 2·3 (8)	16·5 ± 1·8	−43·1 ± 2·8
3½	−52·6 ± 1·3 (7)	−98·4 ± 2·9 (7)	13·5 ± 1·8	−48·3 ± 3·4
8	−62·8 ± 1·4 (5)	−97·8 ± 3·4 (5)	21·1 ± 3·2	−36·8 ± 3·8
0	−54·0 ± 1·8 (7)	−85·6 ± 1·3 (7)	24·1 ± 2·2	−33·7 ± 2·4
3½	−55·9 ± 2·1 (8)	−89·9 ± 2·1 (8)	22·3 ± 2·7	−35·6 ± 3·1
0	−54·9 ± 1·7 (9)	−91·6 ± 1·1 (9)	20·0 ± 1·6	−38·5 ± 2·1
3½	−53·7 ± 1·2 (9)	−96·9 ± 1·2 (8)	16·6 ± 1·1	−43·8 ± 1·7
0	−48·0 ± 2·4 (6)	−86·7 ± 1·9 (7)	18·4 ± 2·3	−40·6 ± 3·2
5	−48·8 ± 1·0 (8)	−88·6 ± 1·9 (8)	17·6 ± 1·5	−41·7 ± 2·2
0	−49·0 ± 2·0 (8)	−90·2 ± 2·2 (6)	17·1 ± 2·0	−42·5 ± 3·0
4	−52·9 ± 1·6 (8)	−94·1 ± 2·3 (8)	17·6 ± 1·9	−41·7 ± 2·8
0	−50·2 ± 1·1 (9)	−92·6 ± 1·9 (8)	16·8 ± 1·4	−42·9 ± 2·2
18	−52·3 ± 1·6 (8)	−93·0 ± 1·7 (6)	17·5 ± 1·6	−41·9 ± 2·4
0	−51·6 ± 1·2	−90·2 ± 1·4	18·8 ± 1·2	−40·2 ± 1·5
	−53·6 ± 1·2	−93·4 ± 1·5	18·2 ± 0·8	−41·2 ± 1·2

It should be noted that altering a^o_{Cl} does not affect E_D. This is in accordance with the observation that frog ventricle behaves as a nearly perfect potassium electrode (Walker & Ladle, 1973).

The experiments where a^o_{Cl} was reduced also serve as another test of whether chloride is passively distributed. If chloride is passively distributed then, when a^o_{Cl} was reduced, a^i_{Cl} should have decreased so that E_{Cl} remained constant. In fact, as shown in Fig. 1, where E_{Cl} is plotted as a function of $\log_{10} a^o_{Cl}$, the data points can be fitted with a straight line (eqn. (2)) with a slope of −38·5 mV and a correlation coefficient of −0·97:

$$E_{Cl} = -38{\cdot}5 \log_{10} a^o_{Cl} + 30{\cdot}2. \quad (2)$$

TABLE 6. a_{Cl}^{i} measurements in frog ventricle when a_{Cl}^{o} is reduced by replacing chloride with glucuronate. For each of the thirteen experiments the control values (a_{Cl}^{o} = 100 %) of E_D and a_{Cl}^{i} are given along with at least one set of values when a_{Cl}^{o} < 100 %. The time in hours is the length of time the preparation was exposed to reduced a_{Cl}^{o} before the measurements were made. In the last experiment in the Table, a_{Cl}^{o} was returned to 100 % after 2 hr at 10 %

a_{Cl}^{o} (%)	E_D (mV)	a_{Cl} (mM)	Time (hr)
100	−94·5 ± 0·9	18·2 ± 1·2	
50	−96·7 ± 1·7	8·3 ± 0·6	7½
100	−92·8 ± 1·3	19·5 ± 1·3	
50	−100·7 ± 3·2	12·0 ± 2·0	9
50	−92·2 ± 3·1	15·4 ± 2·7	22
100	−96·0 ± 1·3	18·7 ± 1·8	
50	−96·8 ± 1·9	16·0 ± 1·4	2½
100	−99·4 ± 2·1	15·7 ± 1·6	
50	−98·8 ± 2·0	16·5 ± 1·4	4½
100	−99·4 ± 2·4	17·2 ± 2·3	
35	−97·1 ± 1·8	13·4 ± 1·1	3½
100	−95·0 ± 1·6	17·2 ± 1·3	
35	−98·2 ± 1·1	9·6 ± 0·5	2
100	−98·3 ± 1·2	17·3 ± 1·1	
20	−92·5 ± 0·5	10·3 ± 0·9	2½
20	−96·0 ± 0·4	8·8 ± 0·6	20
100	−96·6 ± 1·8	17·2 ± 1·6	
20	−96·8 ± 2·7	8·7 ± 1·1	1½
20	−94·3 ± 1·3	8·4 ± 0·5	18
100	−98·3 ± 2·0	15·5 ± 1·5	
20	−96·6 ± 1·7	9·1 ± 0·7	1
20	−96·0 ± 0·4	8·6 ± 0·5	18
100	−96·7 ± 3·5	17·8 ± 2·5	
10	−95·7 ± 0·7	8·4 ± 0·4	1
100	−101·2 ± 2·7	13·9 ± 1·8	
10	−97·0 ± 0·6	6·0 ± 0·7	4
100	−96·4 ± 2·3	19·5 ± 2·2	
10	−93·8 ± 1·7	8·3 ± 0·8	3½
10	−92·8 ± 0·5	7·8 ± 0·2	10½
10	−96·0 ± 1·7	7·0 ± 0·7	24
100	−95·0 ± 0·7	17·2 ± 1·5	
10	−96·0 ± 1·5	5·6 ± 0·4	2
100	−101·6 ± 1·6	15·8 ± 1·6	1½

DISCUSSION

To compare the measured values of E_{Cl} with the values predicted by the Hutter–Noble hypothesis, it is necessary to integrate the equation for chloride current, I_{Cl}, obtained from the constant field equation. Assuming

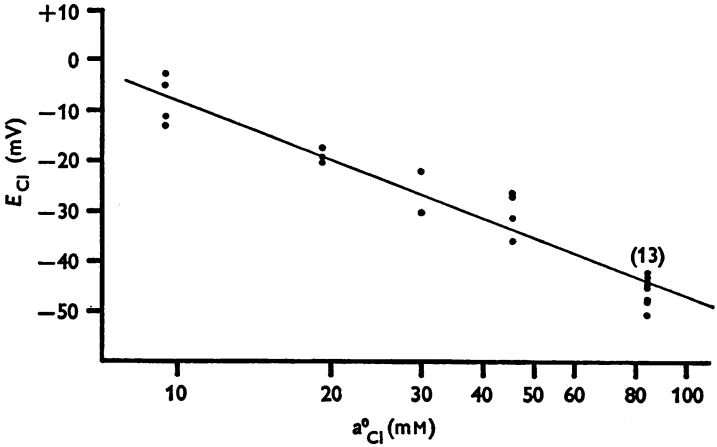

Fig. 1. E_{Cl} plotted as a function of $\log_{10} a^o_{Cl}$. The values of a^i_{Cl} in Table 6 were used to calculate the values of E_{Cl}. The line is the least-squares linear regression fit to the data $E_{Cl} = -38{\cdot}5 \log_{10} a^o_{Cl} + 30{\cdot}2$ with a correlation coefficient of $-0{\cdot}97$.

that chloride is passively distributed and that P_{Cl} is constant, the integral of I_{Cl} over one cardiac cycle is set equal to zero as shown in eqn. (3):

$$\int_0^T I_{Cl}\,dt = \int_0^T E_M \frac{1-\exp\left[(E_{Cl}-E_M)F/Rt\right]}{1-\exp\left(-E_M F/RT\right)} dt = 0. \tag{3}$$

This integration is carried out substituting different values of E_{Cl} until the value is found that makes the integral equal to zero.

If instead of assuming constant P_{Cl} one assumes constant g_{Cl}, a different value is predicted for E_{Cl}. Again the integral of I_{Cl} for one cardiac cycle is set equal to zero as shown in eqn. (4):

$$\int_0^T I_{Cl}\,dt = \int_0^T (E_M - E_{Cl})\,dt = 0. \tag{4}$$

In this case E_{Cl} is equal to the mean value of E_M during the cardiac cycle. For the special case of non-beating preparation, $E_{Cl} = E_M$ under both assumptions. The values of E_{Cl} predicted under the two assumptions and the observed values from Tables 1, 4 and 5 are presented in Table 7.

For sinus venosus the predicted values of E_{Cl} were calculated for the two extreme frequencies that were observed. The average observed value of E_{Cl} is positive by a significant amount ($P = 0{\cdot}0005$) with respect to both of the predicted values. Two possible interpretations of this result are that chloride is passively distributed, but the assumption of constant g_{Cl} or constant P_{Cl} is not valid or chloride is actively transported into the cells. On the basis of the data presented here it is not possible to choose between these two alternatives.

In atrial strips beating at 0·5 Hz, the observed value of E_{Cl} is significantly more negative ($P = 0{\cdot}0005$) than the predicted value of E_{Cl} assuming constant P_{Cl}. It is, however, in good agreement with the value predicted assuming constant g_{Cl}. It is tempting on the basis of this data to conclude that chloride is passively distributed at the mean membrane potential. However, in non-beating preparations where the mean membrane potential is equal to E_D, E_{Cl} is not significantly different from that in

TABLE 7. Comparison of observed and predicted values of E_{Cl} in frog sinus venosus, atrium and ventricle

	Frequency (Hz)	E_{Cl} (mV) g_{Cl} = constant	E_{Cl} (mV) P_{Cl} = constant	E_{Cl} (mV) Observed
Sinus venosus	0·6	−46	−38	−25·0 ± 2·3
	0·75	−41	−35	—
Atrium	0	−94	−94	−36·3 ± 1·6
	0·5	−40	−32	−39·5 ± 0·9
Ventricle	0	−93	−93	−40·2 ± 1·5
	0·5	−43	−32	−41·2 ± 1·2

the beating preparation where the mean membrane potential is −40 mV. This observation in the non-beating preparation would lead one to think that chloride might be actively transported into the cells and agreement with the predicted E_{Cl} assuming constant g_{Cl} in the beating preparation is fortuitous. It is clear that the constant P_{Cl} assumption does not predict the observed E_{Cl}.

The results in ventricle are essentially the same as in atrium. In the beating preparation the observed E_{Cl} is negative with respect to the value predicted assuming constant P_{Cl} ($P = 0{\cdot}0005$) and the same as the value predicted under the constant g_{Cl} assumption. As in atrium there is no difference in the values of E_{Cl} in the beating and non-beating preparations. In ventricle, however, the chloride replacement experiments make it clear that, in non-beating preparations, the membrane is quite permeable to chloride. Since a large change in the average membrane potential does not cause a shift in E_{Cl}, even though the membrane is demonstrably permeable to chloride, we conclude that chloride is actively transported into frog ventricle cells.

Walker & Ladle (1973) estimated, from measurements of intracellular potassium activity, that the permeability ratio P_{Cl}/P_K is ten times higher in frog atrium than in frog ventricle. Because of this estimate and the experimental finding that ventricle is quite permeable to chloride, our speculation is that chloride is also actively transported into frog atrial cells. Our conclusion that chloride is actively transported into ventricle

and the observations presented here concerning E_{Cl} in sinus venosus also lead us to think that chloride is actively transported into the latter.

The Hutter–Noble hypothesis, that E_{Cl} can be predicted from the constant field equation assuming constant P_{Cl}, does not apply to any of the three types of frog heart muscle.

At this time we do not know the functional significance of chloride being actively transported into frog heart cells. At least in ventricle, substantial replacement of extracellular chloride with an impermeant anion has no effect on the action potential. Since chloride current has been suggested to be of importance in the slow diastolic depolarization of pace-maker cells (Hutter & Noble, 1961; DeMello, 1963), it will be of considerable interest to pursue the relationship between E_{Cl} and E_M in such tissues.

Relationship between a^i_{Cl} and a^o_{Cl}

The results of the chloride replacement experiments with ventricle have been used to try and find a relationship between a^i_{Cl} and a^o_{Cl} without much satisfaction. Combining eqn. (2) with the equation defining E_{Cl}, an equation (eqn. (5)) can be derived to describe the relation between a^i_{Cl} and a^o_{Cl}.

$$a^i_{Cl} = 3{\cdot}2(a^o_{Cl})^{\frac{1}{3}}. \tag{5}$$

When the data are plotted with a^i_{Cl} as a function of $(a^o_{Cl})^{\frac{1}{3}}$, the least-squares linear regression fit for the data is described by eqn. (6):

$$a^i_{Cl} = 4{\cdot}3(a^o_{Cl})^{\frac{1}{3}} - 2{\cdot}3. \tag{6}$$

The correlation coefficient is 0·90. The difference between the observed and predicted slopes is significant ($P = 0{\cdot}005$).

When a^i_{Cl} is plotted as a linear function of a^o_{Cl}, the data can again be fitted with a straight line, eqn. (7), with a correlation coefficient of 0·90:

$$a^i_{Cl} = 0{\cdot}12a^o_{Cl} + 6{\cdot}8. \tag{7}$$

The slope of the line is significantly different from zero ($P = 0{\cdot}005$). Since the data can be described equally well by both linear and cubic equations, it is not possible at this time to draw any conclusion about the relationship between a^i_{Cl} and a^o_{Cl}.

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