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THE IONIC BASIS OF THE RESTING POTENTIAL IN A CROSS-STRIATED MUSCLE OF THE AQUATIC SNAIL *LYMNAEA STAGNALIS*

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SUMMARY

The mean resting potential in the heart ventricle muscle cells of the freshwater snail *Lymnaea stagnalis* was found to be $-61{\cdot}2 \pm 3{\cdot}5$ (..) mV (ranging from -56 mV to -68 mV). The average intracellular potassium concentration was estimated to be $51{\cdot}5 \pm 14{\cdot}6$ (..) m (ranging from 27·8 m to 77·3 m). The membrane of the heart ventricle muscle cells appears to be permeable to both potassium and chloride, as changes in the extracellular concentration of either of these ions resulted in a change in the membrane potential. A ten-fold change in the extracellular potassium concentration was associated with a $50{\cdot}4 \pm 3{\cdot}8$ (..) mV slope when the potassium concentration was above about 6 m. Deviations from the straight-line relation predicted for a potassium electrode could be accounted for by introducing a term for sodium permeability. The ionic basis of the membrane potential in these cells can be described by a modified form of the Goldman-Hodgkin-Katz equation.

INTRODUCTION

The ionic basis of the membrane potential in molluscan muscle cells has received little detailed attention. In the few cases examined so far, the membrane potential appears to be a function, to a greater or lesser extent, of the extracellular potassium concentration (Hill, Greenberg, Irisawa & Nomura, 1970; Burnstock, Greenberg, Kirby & Willis, 1967; Twarog, 1967). Chloride ions may also be involved (Shigeto, 1970).

Resting potentials of between -27 mV and -65 mV have been reported for different molluscan muscles (Kater, Heyer & Hegmann, 1971; Kidokoro, Hagiwara & Henkart, 1974; Florey & Kriebel, 1969; Kobayashi & Shigenaka, 1978; Twarog, 1967; Shigeto, 1970). These resting potentials are low compared to those reported for vertebrate muscles (cf. -92 mV for frog sartorius muscle; Adrian, 1956). This could reflect either damage to the sarcolemma during impalement, or a genuine difference. In some cases the resting potential declined rapidly following impalement (e.g. Kidokoro *et al.* 1974) which would suggest that the membrane had been damaged. The values for resting potentials determined by high resistance gap voltage clamp

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techniques, on the other hand, incorporate other errors. For example, Sugi & Yamaguchi (1976), using an oil gap voltage clamp, reported a resting potential of −12 mV to −25 mV for the ABRM of *Mytilus edulis* (cf. −65 mV; Twarog, 1967). However, the resting potentials of the muscles of different invertebrates, e.g. *Ascaris lumbricoides*, may be genuinely low (−30 mV to −40 mV; Brading & Caldwell, 1964; del Castillo, de Mello & Morales, 1964*a*,*b*; Toida, Kuriyama, Tashiro & Ito, 1975). The apparently low resting potentials of some molluscan muscles, therefore, does not represent a unique situation. This could reflect the comparatively low values of the potassium equilibrium potential calculated for some molluscan muscles. For example, the K^+ equilibrium potential of myocardial muscle fibres of *Crassostrea gigas* was calculated from measurements of intracellular K^+ concentration to be −68 mV (Shigeto, 1970), which is considerably less negative than the K^+ equilibrium potential of about −100 mV in vertebrate skeletal muscles (Adrian, 1956; Hodgkin & Horowicz, 1959).

If muscle cell membranes are freely permeable to K^+ and Cl^-, the Donnan equilibrium theory holds that under conditions of a constant $[K^+]_e \times [Cl^-]_e$ product (Boyle & Conway, 1941), the membrane potential is given by:

$$V_m\ (mV) = 58 \log \frac{[K^+]_e}{[K^+]_i} \qquad (1)$$

where a decade change in the extracellular K^+ concentration should be associated with a 58 mV change in the membrane potential on the condition that the membrane is not significantly permeable to ion species other than K^+ or Cl^- (cf. Hodgkin & Horowicz, 1959).

In the present investigation we have examined the ionic basis of the resting potential in heart ventricle muscle cells of the aquatic snail, *Lymnaea stagnalis*.

MATERIALS AND METHODS

The experimental animals

Two subspecies of the freshwater snail *Lymnaea stagnalis* were used in these investigations. *L. stagnalis appressa* were collected locally while *L. stagnalis stagnalis* were imported from England (Griffin & George Ltd., Gerrard Biological Centre, East Preston, West Sussex, U.K.). The animals were kept in large aquaria containing standing, aerated water and were fed lettuce leaves *ad libitum*. During the course of the experiments no intersubspecific physiological differences were observed, and the two subspecies were used interchangeably.

The preparation and apparatus

The heart was removed from an unanaesthetized, de-shelled snail. The atrium and aorta of the isolated heart were pinned to a small Sylgard block (Sylgard 184 Silicone Elastomer, Dow Corning Corp., Midland, Michigan) to immobilize the preparation. The ventricle was opened by cutting along its long axis with fine scissors. After cutting away the atrium and aorta, the ventricle was spread apart and its edges were pinned to a small Sylgard mounting block with miniature tungsten staples, exposing the inner

Table 1. *Composition of the constant $[K^+]_e \times [Cl^-]_e$ product salines ($[K^+] \times [Cl^-] = 60$ mM)*

K^+	Na^+	Ca^{2+}	Mg^{2+}	Ac^-	Glu^-	Cl^-	HEPES
1·0	41·65	0·5	10·0	0·2	1·0	60·0	5·0
2·0	40·8	0·5	10·0	0·4	2·0	30·0	5·0
4·0	39·1	0·5	10·0	0·8	4·0	15·0	5·0
6·0	37·4	0·5	10·0	1·2	6·0	10·0	5·0
8·0	35·7	0·5	10·0	1·6	8·0	7·5	5·0
10·0	34·0	0·5	10·0	2·0	10·0	6·0	5·0
15·0	29·75	0·5	10·0	3·0	15·0	4·0	5·0
20·0	25·5	0·5	10·0	4·0	20·0	3·0	5·0
25·0	21·25	0·5	10·0	5·0	25·0	2·4	5·0
30·0	17·0	0·5	10·0	6·0	30·0	2·0	5·0
40·0	8·5	0·5	10·0	8·0	40·0	1·5	5·0

Concentrations given in mM.
Glu = glutamate, Ac = acetate (note elevated Mg^{2+} and reduced Ca^{2+} concentrations).
HEPES was adjusted to pH 7·6 with NaOH. 5 mM HEPES added 2·35 mM-Na^+ to the salines.

face. The Sylgard block with attached ventricle was placed in a preparation chamber (volume = 1 ml) in which the superfusion fluid could be readily exchanged *via* a nine-way non-return valve (see Brezden & Gardner, 1983).

The membrane potential of the heart muscle cells was measured using 3 M-KCl-filled intracellular glass microelectrodes and standard electrophysiological techniques, employing a WPI model KS 700 microelectrode amplifier (WPI Instruments Co., Ridgewood, New Jersey). Permanent recordings of membrane potential were made on a Linear Instruments model 395 chart recorder (Linear Instruments Corp., Irvine, California).

The saline solutions

Salines with K^+ concentrations of 1 mM to 40 mM were prepared such that the product of the K^+ and Cl^- concentrations was constant at 60 mM (Table 1). These salines contained elevated Mg^{2+} and reduced Ca^{2+} concentrations; the reason for this is discussed below.

In addition to the above salines, normal *Lymnaea* saline (1·6 mM-K^+), low Cl^- (5 mM) saline, Na^+-free saline and Na^+-free saline with low Ca^{2+} and high Mg^{2+} concentrations were used (Table 2).

The experimental regime

In these experiments, the response of the membrane potential of individual *Lymnaea* heart ventricle muscle cells to changes in the extracellular K^+ and Cl^- concentrations was measured. The K^+ concentration was varied in a random sequence to reduce systematic experimental error. After each change in the K^+ concentration the preparation was returned to 1 mM-K^+ saline (baseline membrane potential). The experiment was considered successful only if a complete recovery of the baseline membrane potential was achieved after each change in the extracellular K^+ concentration. However, in initial experiments elevated K^+ concentrations produced a contracture of the heart

Table 2. *Composition of normal and substituted* Lymnaea *salines*

Salt	Normal	Na^+-free	Na^+-free, low Ca^{2+}, high Mg^{2+}	Low Cl^-
NaCl	50·0	0	0	5Cl, 45 G
KCl	1·6	0	0	1·6 G
$CaCl_2$	3·5	3·5	0·5	3·5 A
$MgCl_2$	2·0	2·0	10·0	2·0 A
HEPES-Na	5·0	0	0	5·0
HEPES-K	0	5·0	5·0	0
BDAC	0	50·0	42·5	0

Concentrations given in mM.

BDAC = bis(2-hydroxyethyl) dimethyl ammonium chloride.

HEPES was adjusted to pH 7·6 with 10 M-NaOH or 10 M-KOH (the latter was used in Na^+-free saline). 5 mM-HEPES-Na added 2·35 mM-Na^+ to the saline while 5 mM-HEPES-K added 2·72 mM-K^+ (i.e., the Na^+-free saline contained 2·72 mM-K^+).

In the low Cl^- saline, Cl = chloride salt, G = glutamate salt and A = acetate salt.

ventricle muscle cells. As a result, it was not possible to maintain successful microelectrode insertion. Various mechanical means (e.g. 'floating' microelectrodes, more rigid preparation mounting, etc.) were not successful in eliminating this problem, probably because of the extremely fragile nature of this tissue. Therefore, the Mg^{2+} concentration was elevated to 10 mM and the Ca^{2+} concentration was reduced to 0·5 mM to inhibit contraction. Following this procedure, successful impalement could be maintained through many solution changes.

A further criterion for an acceptable experiment was that the membrane potential in 1 mM-K^+ be more negative than, or equal to, −55 mV; cells with more positive resting potentials were judged to be damaged.

In the following text, '±x' denotes the standard deviation of the given value.

RESULTS

The resting potential

The mean resting potential of *Lymnaea* heart ventricle muscle cells in normal saline was found to be $-61{\cdot}2 \pm 3{\cdot}5$ mV ($N = 30$) (ranging from −56 mV to −68 mV).

The potassium and chloride dependence of the resting potential

A reduction of the extracellular Cl^- concentration from 62·6 mM to 5 mM resulted in a transient depolarization of the membrane potential. This depolarization was of the order of 6 mV in magnitude and the membrane potential reverted to a level which was 2 mV less negative than the original resting potential after about 3 min in the reduced Cl^- saline. Restoration of the original Cl^- concentration induced a hyperpolarization of about 7 mV, followed by a repolarization of the membrane potential to a stable level about 1 mV more negative than the original resting potential (Fig. 1).

A straight-line relationship between the membrane potential and the extracellular K^+ concentration (K-slope) was observed at K^+ concentrations above about 6 mM, where a decade change in the extracellular K^+ concentration was associated with an average slope of $50{\cdot}4 \pm 3{\cdot}8$ mV ($N = 20$), ranging from 44 mV to 59 mV (e.g. Fig. 2).

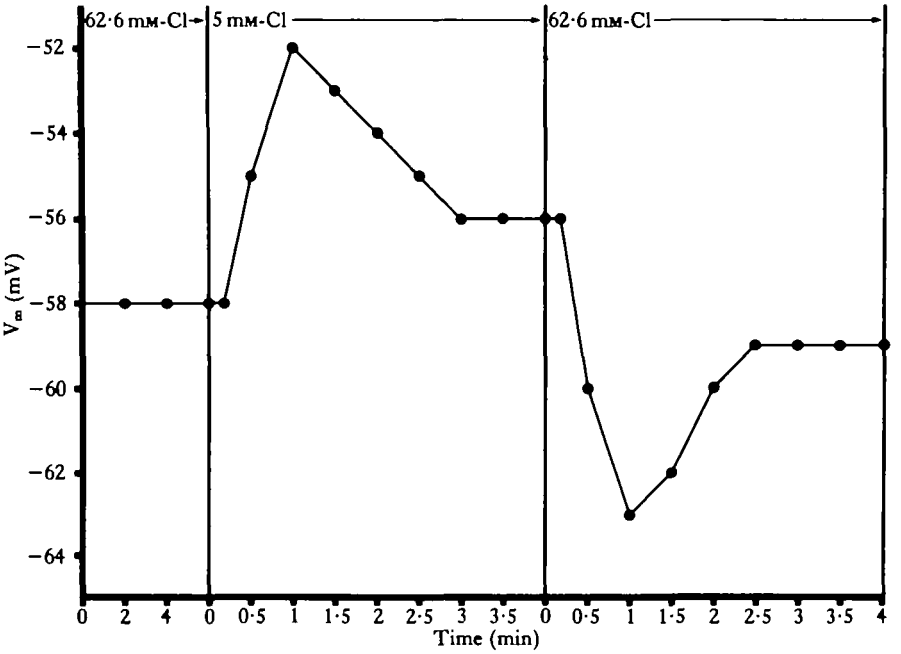

Fig. 1. Effect of reduced chloride concentration on the membrane potential of a *Lymnaea* ventricle muscle cell. Note change in time scale. *N* 8. Data from a single cell; one of eight experiments.

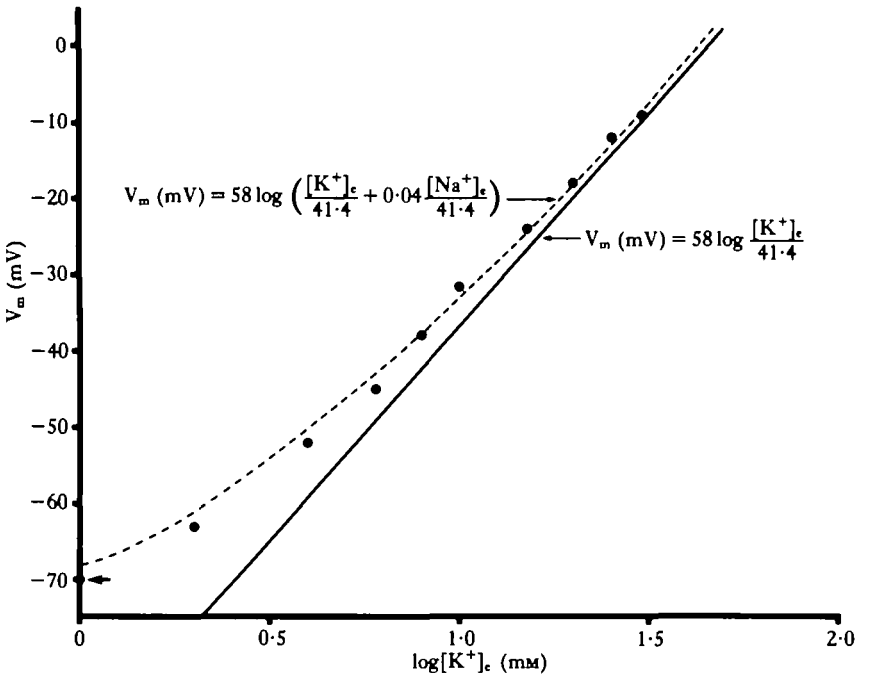

Fig. 2. Relation between the membrane potential and the extracellular potassium concentration in a *Lymnaea* heart ventricle muscle cell. The filled circles are the experimentally determined points. The solid line denotes the expected results using the intracellular potassium concentration calculated from the data in Fig. 3 and equation (1) whereas the dashed line was calculated using equation (2). See text for further explanation. Data are taken from a single experiment.

In an attempt to account for the observed deviation from an ideal potassium electrode, a modified form of the Goldman-Hodgkin-Katz equation (Hodgkin & Katz, 1949) as applied by Moreton (1968) was used. This equation is given by:

$$e^{VF/RT} = \frac{[K^+]_e}{[K^+]_i} + \frac{P_{Na}[Na^+]_e}{P_K[K^+]_i} \quad (2)$$

and predicts a linear relationship between $e^{VF/RT}$ and $[K^+]_e$ if $[K^+]_i$ and the ratio P_{Na}/P_K remain unchanged (cf. Moreton, 1968). The inverse of the slope of the line gives an estimate of $[K^+]_i$, and the value of the term P_{Na}/P_K can be calculated from the Y-intercept. The data from Fig. 2 were plotted using equation (2) giving a value for $[K^+]_i$ of 41·4 mM, and a P_{Na}/P_K ratio of 0·04 (Fig. 3). Substituting these values in equation (2) and rearranging to solve for V (where $[Na^+]_e$ was known) gave the values for the dashed line in Fig. 2.

The above calculations were performed for each of the 20 successful experiments. The mean estimated intracellular K^+ concentration was 51·5 ± 14·6 mM (ranging from 27·8 mM to 77·3 mM) and the mean ratio of P_{Na}/P_K was 0·07 ± 0·003 (ranging from 0·01 to 0·14). The experimental points were very close to those predicted by equation (2) (Table 3).

The effect of sodium-free saline on the membrane potential

The exposure of heart ventricle muscle cells to Na^+-free saline resulted in a gradual depolarization of the membrane potential and eventual contraction of the muscle cells.

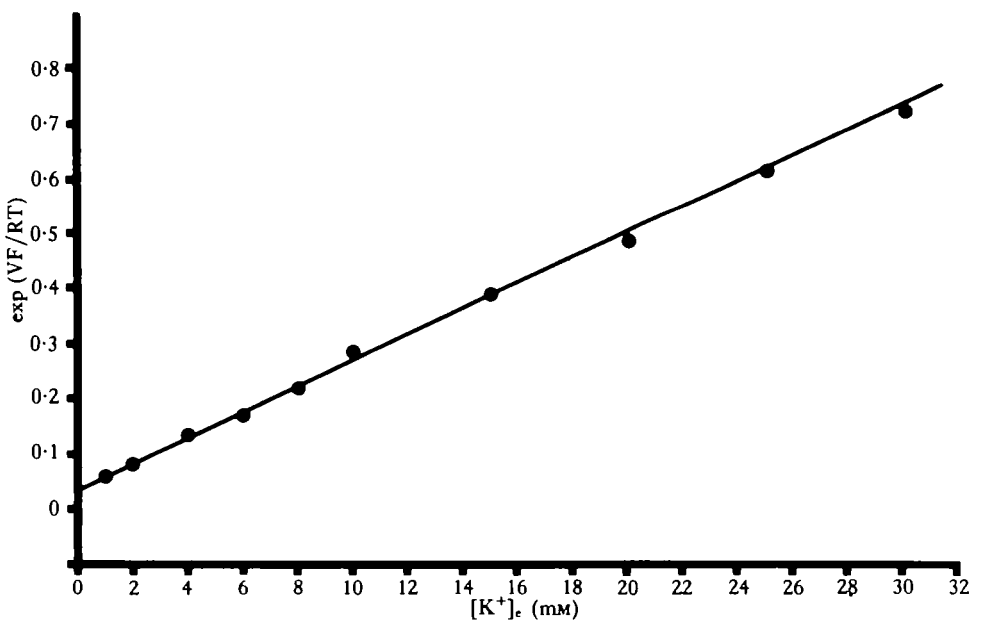

Fig. 3. Relationship between $e^{VF/RT}$ and the extracellular potassium concentration in a *Lymnaea* ventricle muscle cell. Coefficient of correlation = 0·99 (linear regression analysis). The data for this figure are from the same experiment used to plot the dashed line in Fig. 2. See text for explanation.

Table 3. *Deviation between predicted and observed values for the membrane potential of* Lymnaea *heart ventricle muscle cells at various extracellular potassium concentrations*

$[K^+]$ (mM)	Observed V_m (± S.D.)	Expected V_m (± S.D.)
1	−62·2 (5·2)	−63·0 (9·8)
2	−58·7 (5·4)	−55·3 (14·0)
4	−52·5 (5·4)	−51·1 (7·9)
6	−45·1 (5·5)	−44·4 (7·0)
8	−38·4 (5·8)	−40·3 (6·7)
10	−31·6 (6·0)	−34·2 (6·4)
15	−24·8 (6·6)	−27·0 (5·9)
20	−19·5 (6·3)	−22·7 (5·6)
25	−15·5 (6·3)	−17·5 (6·2)
30	−11·0 (6·3)	−12·9 (6·0)
40	−5·5 (5·9)	−5·3 (6·3)

The predicted values are from equation (2). $N = 20$.

This depolarization could be reversed and the contraction prevented if the cells were exposed to normal saline shortly after the onset of depolarization (Fig. 4). However, if the cells were pre-treated with 0·5 mM-Ca^{2+}/10 mM-Mg^{2+} saline, the subsequent exposure to 0 mM-Na^+/0·5 mM-Ca^{2+}/10 mM-Mg^{2+} saline resulted in a transient hyperpolarization of the membrane potential (Fig. 4). Thus it would appear that the

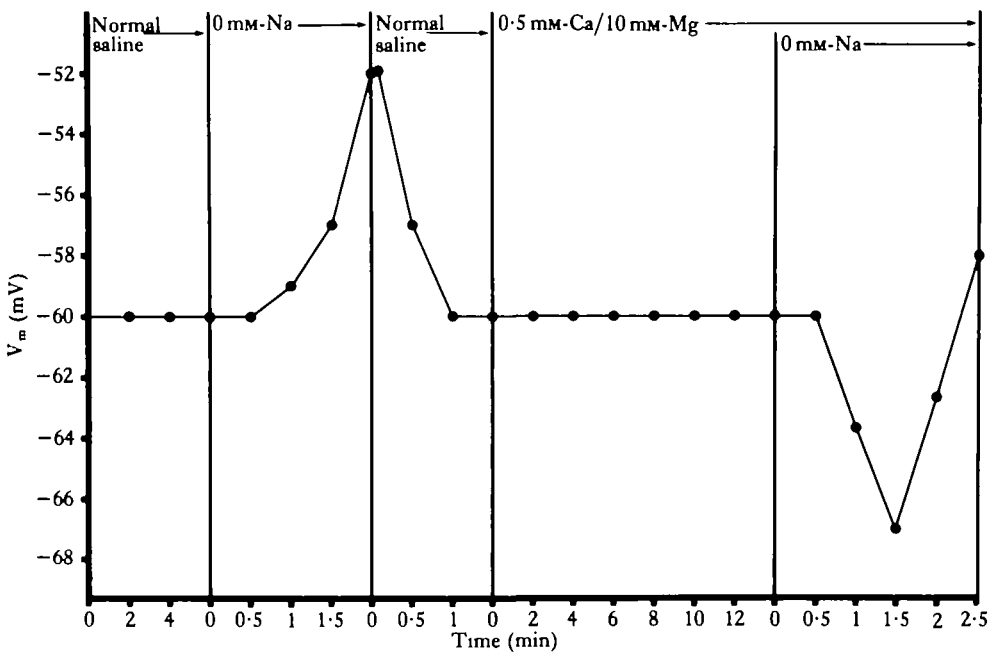

Fig. 4. Effect of sodium-free saline on the membrane potential of a *Lymnaea* heart ventricle muscle cell under normal conditions and in the presence of low calcium-high magnesium saline. Data are from a single cell; one of eight experiments. Note changes in time scale. $N = 8$.

removal of extracellular Na^+ results in a hyperpolarization only if the influx of Ca^{2+} is prevented, although even 0·5 mM-Ca^{2+}/10 mM-Mg^{2+} saline did not prevent the contraction induced by the removal of extracellular Na^+.

DISCUSSION

The chloride dependence of the resting potential

The membrane potential of *Lymnaea* heart ventricle muscle cells did not change by the predicted amount during the reduction or subsequent restoration of the original extracellular Cl^- concentration. In the experiment presented in Fig. 1, for example, the membrane potential should have changed from −58 mV to −26 mV when the Cl^- concentration was reduced from 62·6 mM to 5 mM (cf. Hodgkin & Horowicz, 1959), whereas only a 6 mV depolarization was observed. However, the full magnitude of the expected change can only be measured when the extracellular Cl^- concentration is changed very rapidly (Hodgkin & Horowicz, 1959). In the present experiments about 1 min was required to exchange the bath contents completely (faster flow rates dislodged the microelectrodes).

Hodgkin & Horowicz (1959) reported that the resting potential in frog skeletal muscles was quickly restored following changes induced by lowering or restoring the extracellular Cl^- concentration, and attributed the restoration of the resting potential to an insignificant change in the intracellular K^+ concentration during variations in the extracellular Cl^- concentration. However, a hysteresis in the value of the membrane potential following a change in the extracellular Cl^- concentration was observed in all of the *Lymnaea* muscle cells examined, i.e., within the 2–3 min time frame of the experiment the membrane potential always stabilized at a level about 2 mV less negative (following a reduction in the extracellular Cl^- concentration) and about 1 mV more positive (following the restoration of the original Cl^- concentration) than the original resting potential. These results suggest the possibility that the intracellular K^+ concentration may have changed by as much as 4 mM in these cells during alterations in the extracellular Cl^- concentration. If the intracellular K^+ concentration is low (as may be the case in *Lymnaea* ventricle muscle cells) the Donnan equilibrium theory [and using equation (2)] predicts that this change in the intracellular K^+ concentration may be sufficient to account for the observed changes in the resting potential.

The potassium dependence of the resting potential

The membrane potential of *Lymnaea* heart ventricle muscle cells was found to be relatively insensitive to changes in extracellular K^+ concentrations below about 6 mM. An average slope of 50·4 ± 3·8 mV per decade change in the extracellular K^+ concentration was obtained between 6 mM and 40 mM. This is about 8 mV lower than predicted by equation (1), suggesting that these cells are permeable to some ion(s) other than K^+ and Cl^-. It is unlikely that changes in the intracellular concentrations of K^+ or Cl^- contributed to the reduction in the K-slope, since constant $[K^+]_e$ × $[Cl^-]_e$ product salines were always employed.

The sodium dependence of the resting potential

In all the muscle cells examined, the resting potential was considerably lower than that predicted by equation (1). This type of deviation could be explained if the cell membrane has a resting permeability to Na^+. However, upon removal of extracellular Na^+, the expected hyperpolarization (cf. Sattelle, 1974) was only observed if the magnesium concentration was elevated and the calcium concentration reduced. It would seem, therefore, that under normal conditions, the depolarization was a direct or indirect result of Ca^{2+} entry into the cell, and that this entry could be prevented by elevating the extracellular Mg^{2+} concentration and lowering the extracellular Ca^{2+} concentration. Removal of extracellular Na^+ inhibits, or possibly reverses the Na^+-Ca^{2+} exchange mechanism (Baker, 1972), and increases the influx of Ca^{2+} into the cell (Coraboeuf, Gautier & Guiraudon, 1981). Whether or not an elevation of free intracellular Ca^{2+} levels alone could be responsible for the observed depolarization cannot be determined on the basis of the available evidence. Nevertheless, under appropriate conditions the removal of extracellular Na^+ does cause a hyperpolarization of the heart ventricle muscle cell membrane, an observation which is consistent with the proposal that this membrane has a resting permeability to Na^+.

A quantitative description of the membrane potential

The mean resting potential of $-61{\cdot}2 \pm 3{\cdot}5$ mV in *Lymnaea* heart ventricle muscle cells is substantially less negative than that in vertebrate cardiac muscle (-90 mV to -100 mV; Weidmann, 1956). Also, the mean estimated intracellular K^+ concentration of $51{\cdot}5 \pm 14{\cdot}6$ mM is considerably lower than that of vertebrate cardiac muscles (>100 mM; Kunze & Russell, 1981; Walker, 1981). Apart from the relatively low level of resting potential and of intracellular K^+ concentration, the ionic basis of the resting potential in *Lymnaea* heart ventricle muscle cells was found to be similar to that of frog skeletal muscle (cf. Hodgkin & Horowicz, 1959): the heart ventricle muscle cell membrane appears to be permeable to both K^+ and Cl^- since changes in the extracellular concentration of either of these ions resulted in a change in the membrane potential. They also appear to have a resting permeability to Na^+.

In conclusion, the membrane potential (assuming P_{Na}/P_K does not change) can be defined by equation (2). This equation produced values for the membrane potential which approximated those observed at extracellular potassium concentrations between 1 mM and 40 mM in *Lymnaea* heart ventricle muscle cells.

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