CHLORIDE EQUILIBRIUM POTENTIAL IN SQUID GIANT AXON

by

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Résumé

Le potentiel d'équilibre des ions Cl^- a été mesuré dans l'axone géant du Calmar à l'aide de micro-électrodes à échangeur d'ions liquide. Les résultats obtenus sont comparables à ceux obtenus par d'autres auteurs utilisant des électrodes AgCl ou effectuant l'analyse chimique d'échantillons d'axoplasme. Ils montrent que l'activité intracellulaire des ions Cl^- est plus élevée qu'on ne l'attendrait d'une distribution passive.

L'activité intracellulaire des ions Cl^- n'est pas modifiée par une augmentation de la concentration extracellulaire de K^+, l'addition de tétraéthylammonium, de procaïne, d'ions ammonium — substances qui modifient l'activité intracellulaire des ions Cl^- dans d'autres systèmes.

La perméabilité aux ions Cl^- de l'axone de Calmar au repos semble très faible.

Introduction

In many neurones, chloride ions do not seem to be passively distributed across the membrane, i.e. the chloride equilibrium potential, E_{Cl}, is different from the membrane potential, E_m. The squid giant axon is the classical example of a cell in which the intracellular chloride activity (a_{Cli}) is higher than expected from a passive distribution (Mauro, 1954; Keynes, 1963). The neurones of another marine mollusc, *Aplysia,* are a good example of the inverse situation in which a_{Cli} is lower than expected (Kehoe, 1972; Russell and Brown, 1972; Ascher, Kunze and Neild, 1976).

The high values of internai chloride in squid axon could be questioned for a variety of technical reasons. Most of the results corne from analyses of extruded axoplasm, and show the chloride *concentration* to be between 100 and 150 mM/kg (Keynes, 1963) although some lower values have been reported (see Brinley, 1965). These methods necessarily include any bound chloride and cannot detect

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differences between the chloride concentration of various separate intracellular compartments, should they exist.

These problems were avoided by Mauro (1954), who was the first to measure chloride *activity* in the squid axon. By showing the presence of a potential difference between chlorided silver wires placed inside and outside the axon, he demonstrated that E_{Cl} was less negative than E_m, and therefore that a_{Cli} was higher than permitted for a passive distribution. This conclusion was strengthened by the work of Keynes (1963).

However, recent observations by Neild and Thomas (1974) could throw some doubt on the validity of these measurements. These authors showed that Ag/AgCl microelectrodes are sometimes unreliable and can give overestimates of a_{Cli}. We therefore decided to measure a_{Cli} in the squid giant axon with another type of electrode, the liquid ion-exchange resin microelectrode devised by Walker (1971). Although these electrodes do not distinguish between chloride and other anions as well as Ag/AgCl electrodes, they have given reliable results when used to measure chloride in *Aplysia* neurones (Russell and Brown, 1972; Ascher *et al.,* 1976) and frog skeletal muscle (Feltz, Large and Rodeau, 1975).

The measurements that we present below show that a_{Cli} in the squid giant axon is high, and thus confirm the observations of Mauro (1954) and Keynes (1963). In addition, several experimental manipulations, which have been shown to markedly change a_{Cli} in *Aplysia* neurones (Ascher *et al.,* 1976), were found to be completely ineffective in changing a_{Cli} of the squid axon.

Methods

Squids (*Loligo forbesi)* were caught individually using hand lines equipped with special lures ("turlutes" or "jigs") brought from Newfoundland by R. Boucher-Rodoni. They were kept in an inflatable rubber boat ("Bombard") filled with sea-water, and normally lived for several days.

A ligature was made around the stellar nerve about 6 to 8 cm from the stellate ganglion and the nerve and ganglion were removed from the animal. Small nerve fibres were dissected away to expose the giant axon for a length of about 1 cm.

The giant axon was penetrated with two microelectrodes; one filled with 0.6 M K_2SO_4 to record E_m, and the other filled with chloride-specific liquid ion-exchange resin (Corning 477315) to measure E_{Cl}. Details of the properties and calibration of these chloride-sensitive electrodes and the subsequent calculation of E_{Cl} and a_{Cli} are given elsewhere (Brown, Walker and Sutton, 1970; Russell and Brown, 1972; Ascher *et al.,* 1976). As isethionate has been found in relatively large amounts (165 mM) in squid axoplasm (Koechlin, 1955; Deffner, 1961) the response of the chloride-sensitive electrodes to isethionate was checked during the calibration procedure. The mean interference coefficient ($K_{Cl\ .\ Ise}$, see Brown *et al.,* 1970 or Moody and Thomas,

1971) was found to be 0.11, which means that 165 mM isethionate in the axoplasm would cause the internal chloride to be overestimated by 0.11 X 165 = 18.2 mM.

The composition of the solutions is given in Table 1. Unless otherwise stated, the pH was adjusted to 7.8 with 10 mM Tris Cl buffer. The solutions were calculated to be iso-osmolar using tables in the Handbook of Chemistry and Physics (1975).

All experiments were performed at room temperature (18 °C).

TABLE 1

Composition of solutions. Concentrations are in mM/l.

	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	Isethionate	HCO_3^-	SO_4^{2-}	Sucrose	Tris Cl pH 7.8
A	**480**	**10**	**10**	**50**	**620**	—	—	—	—	**10**
B	**390**	**100**	**10**	**50**	**620**	—	—	—	—	**10**
C	**480**	**10**	**10**	**50**	**140**	**480**	—	—	—	**10**
D	**480**	**10**	—	**60**	**130**	—	**480**	—	—	—
E	**484**	**10**	**10**	**50**	**40**	—	—	**297**	**389**	**10**

RESULTS

From 14 experiments, the average membrane potential was found to be — 55 mV ± 0.6 mV (S.E. of mean).

Chloride equilibrium potential

The potential recorded from the chloride-sensitive électrode after subtraction of the membrane potential varied between — 22 and — 29 mV. After each reading had been corrected using the calibration curve for the électrode, the mean value of E_{Cl} was calculated to be —27 mV ± 1.0 (S.E. of mean). Assuming that the activity coefficients were the same inside and outside the cell, the intracellular chloride concentration can be calculated to be 212 mM/1. After correction for isethionate in the axoplam, this is reduced to 194 mM/1 and the corrected value of E_{Cl} is — 29 mV.

Effects of changes of K_o

When K_o was increased from 10 to 100 mM/1 the membrane potential changed to — 25 mV and stabilized at this value. On returning to ASW (K_o = 10 mM/1) the membrane repolarised to its initial potential at a rate comparable to that of the depolarisation (half time about 4 mn).

There was not detectable change of Cl_i in the high K^+ sea-water, even after a period of 1.5 h.

Nominally K^+-free sea-water produced a slight hyperpolarisation (3 mV), and no change in Cl_i.

Effects of low Cl_0

None of the low Cl_0 media (see Table 1) produced a depolarisation of the axon. They all appeared to cause a slight hyperpolarisation, but this was probably due to a change of the junction potential at the sea-water/agar bridge used as the extracellular reference electrode.

None of the low Cl_0 solutions caused any detectable change in Cl_i when applied for a period of 30 mn. In one experiment in which Cl_0 was reduced to 40 mM/l (solution E, Table 1) for 80 mn, the change of E_{Cl} was 4mV (Cl_i, reduced from 212 mM/l to 178 mM/l).

In one experiment, the axon was cut at a distance of 2 mm from the recording electrodes, while in low Cl_0 sea-water (solution E, Table 1). After 4.9 hours E_{Cl}, had changed by 17 mV, i.e. the internal Cl^- activity at the recording site had fallen to half its initial value. Assuming that the cut axon was an impermeable cylinder open at one end and that the diffusion coefficient was the same everywhere in the system, the apparent diffusion coefficient for Cl^- ions was calculated to be $44 \times 10^{-6}.s^{-1}$. This is about a quarter the value for free Cl^- ions in solution, but the difference is probably not significant and a better agreement could have been obtained if the contraction of the axon at the cut had been taken into account.

Effects of procaine and TEA

These two compounds have been shown by Ascher *et al.* (1976) to induce a fall of $C1_i$ in *Aplysia* neurones. They induced a small depolarisation but no change in Cl_i when applied to the squid axon at concentrations that are effective for *Aplysia* neurones (procaine 20 mM/l, TEA 380 mM/l).

Effects of NH_4^+

It has been shown that in *Aplysia* neurones when ASW containing 10 mM $(NH_4)_2SO_4$ at pH 9.0 is applied for 10 mn and then replaced by normal ASW there is a fall of Cl_i (Ascher *et al.,* 1976).

Fig. 1 shows that this effect is not observed in squid axon. The pH of the ASW was first changed from 7.8 to 9.0, without significant effect. The addition of 10 mM $(NH_4)_2SO_4$ caused a depolarisation which was only slowly reversed when the NH^+ was removed. Cl_i remained unchanged throughout the experiment.

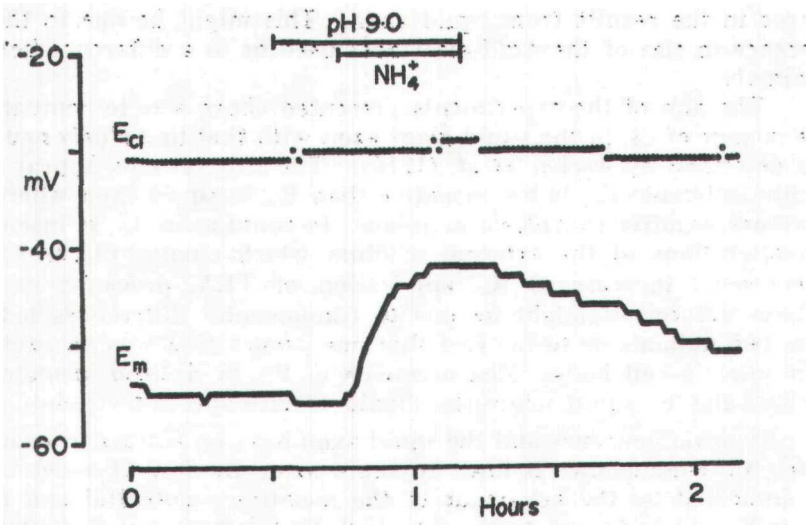

FIG. 1.

The effects of low external pH and of NH_4^+ on E_{Cl} and E_m. The original traces were measured at 1 mn intervals and replotted to give a more convenient time scale. Measurements were made to the nearest millivolt, which accounts for the step like changes in the replotted lines. The value of E_{Cl} has been corrected for the presence of isethionate.

Measurement of E_K

In one experiment, E_K was measured using a K^+-sensitive microelectrode (made in the same way as a Cl^--sensitive electrode but with Corning exchanger 477317), and the value was found to be — 82 mV. This is close to the value measured by Hodgkin and Huxley (1952) and implies an internal K^+ concentration of 260 mM/l, assuming that the activity coefficient is the same inside and outside the cell and that the external K^+ concentration is 10 mM/l. The value of 260 mM/l is close to that found by Brinley and Mullins (1967) in *Loligo pealei,* but lower than the currently quoted value of 400 mM/l which seems to originate from Hodgkin (1951). The explanation of this discrepancy proposed by Hodgkin and Huxley (1952) — that the extracellular K^+ concentration close to the membrane is higher than in sea-water — does not.account for the value of a_{Ki}. measured with the K^+ électrode.

DISCUSSION

Our measurements of E_{Cl}, made with liquid ion-exchange resin microelectrodes, agree entirely with those made by Mauro (1954) and Keynes (1963) using AgCl microelectrodes. It has been shown by Neild and Thomas (1974) that AgCl microelectrodes did not give a true estimate of Cl_i in snail neurones, but there appears to be no such

error in the results from squid axon. This might be due to the difference in size of the AgCl electrodes used or to a difference between animals.

The aim of the experiments presented above was to compare the behaviour of Cl_i in the squid giant axon with that in *Aplysia* neurones as described by Ascher *et al.* (1976). The two systems appear to be quite different; E_{Cl} is less negative than E_m in squid axon whereas it is more negative in *Aplysia* neurones. In squid axon, Cl_i is insensitive to alterations of the external medium which change Cl_i in *Aplysia* neurones.: increase in K_o, application of TEA, procaine, or NH_4^+. These differences might be due to fundamental differences between the two animals or to the fact that one preparation was an axon and the other a cell body. Measurements of E_{Cl} in *Aplysia* axons or the cell bodies of squid neurones should resolve the latter point.

Aplysia neurones and the squid axon have on feature in common: their Cl^- conductance is low. In squid axon, the low Cl^- conductance is indicated by the behaviour of the membrane potential and Cl_i in high K_o or low Cl_0 solutions. The high K_o solution caused a relatively abrupt depolarisation which showed no secondary phase corresponding to KCl and water entry, and the low Cl_0 solutions did not induce a depolarisation. The changes of Cl_i are negligible in both cases. Although it was suggested that the Cl^- conductance of squid axon was quite high (Hodgkin and Katz, 1949), more recent data have in general indicated that it is low (e.g. Freeman, Reuben, Brandt and Grundfest, 1966).

The isotopic fluxes of Cl^- across the squid axon membrane are small (Keynes, 1963; Brinley and Mullins, 1965), but exchange of Cl^- with other anions might provide a way of regulating Cl_i. However, the absence of changes of Cl_i when Cl_0 was replaced by isethionate or bicarbonate, or when the external pH was increased, gives no support to such a hypothesis. The mechanisms which maintain the high level of internal Cl^- remain to be understood.

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Summary

The chloride equilibrium potential was measured in squid giant axons by means of liquid ion-exchange resin microelectrodes. The results were comparable to those obtained by other authors who used AgCl electrodes or who made chemical analyses of samples of extruded axoplasm and indicate that the intracellular chloride activity is higher than would be expected for a passive distribution.

The intracellular chloride activity was not changed by high external K^+, tetraethylammonium, procaine, or ammonium ions — substances which alter internal chloride in other systems.

The resting chloride permeability of the squid axon appears to be very low.

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