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DIRECT ESTIMATES OF CHLORIDE ACTIVITY IN MUSCLE FIBRES DEPOLARIZED BY CARBACHOL

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Intracellular chloride activities have been measured in muscle fibres with chloride-sensitive electrodes. It was found that the depolarization induced by carbachol produces an influx of chloride ions, the accumulation of which leads to further sustained depolarization. The present study thus confirms the evidence put forward by Jenkinson & Terrar (1973) of an influence of chloride ions on the membrane potential after a prolonged application of carbachol.

It has recently been shown by Jenkinson & Terrar (1973) that the time course of the depolarization of the end-plate caused by carbachol does not necessarily reflect the direct action of the drug. The evidence they obtained strongly suggested that, as a consequence of the initial depolarization (primarily due to the inward current of cations at the end-plate, Takeuchi & Takeuchi, 1960; Jenkinson & Nicholls, 1961), chloride ions entered the fibre, increasing the internal chloride concentration, $(Cl^-)_i$. Since the membrane potential depends upon the value of $(Cl^-)_i$, larger values than normal corresponding to depolarization, the removal of the direct effect of carbachol, either by the removal of the drug or by desensitization, will not by itself abolish the depolarization.

The evidence put forward by Jenkinson & Terrar (1973) came from a comparison of the effects of carbachol in normal Ringer solution with those observed in various conditions in which no chloride movements could have occurred. In the present experiments an attempt has been made to follow directly the relationship between $(Cl^-)_i$ and membrane potential during and after an application of carbachol, taking advantage of the recent development of chloride-sensitive microelectrodes (Walker, 1971). For this purpose simultaneous recordings were made of the membrane potential with a conventional intracellular electrode and of the Cl-potential with a second intracellular Cl-sensitive microelectrode, both inserted into a muscle fibre at the end plate. From the determination of the chloride potential, the internal chloride concentration could be simply evaluated.

Methods Sartorius muscles of *Rana esculenta* were mounted in a perspex chamber and superfused at a constant rate with frog Ringer solution at pH 7.4 (mM): NaCl, 115; $CaCl_2$, 1.8; KCl, 2.5; Na_2HPO_4, 1.12; NaH_2PO_4, 0.4), or at pH 5 (NaCl, 115; $CaCl_2$, 1.8; potassium hydrogen phthalate, 2.5; the pH was adjusted to 5 with NaOH). Carbachol (carbamyl-choline chloride) was diluted in the saline. In some experiments, tetrodotoxin (TTX) was added at a concentration of 10^{-7} M.

The two electrodes were inserted at the end-plate within 100 μm of one another. The membrane potential (E_m) was measured between the conventional 3M KCl microelectrode and an indifferent bath electrode; the Cl-potential (E_{Cl}) was calculated from the difference of potential (V_{Cl}) between the conventional and the chloride-sensitive electrode. A pen recorder (Brush MK 280) provided a permanent control of both E_m and V_{Cl}. The recorded potential V_{Cl} differed slightly from E_{Cl} since the Cl^- electrodes displayed a change (v) of between 44 and 52 mV as compared to the expected 58 mV for a tenfold change in activity within the range 1 mM to 1 M. A correction factor had then to be introduced and E_{Cl} was evaluated as $E_{Cl} = V_{Cl} \times 58/v$. The internal chloride activity was derived directly from a calibration curve constructed for each electrode. The activity scale has been drawn on the right hand side of the figure. The intracellular chloride concentration can be easily calculated by assuming that the activity coefficient of intracellular chloride is the same as that for extracellular chloride, 0.77 (Robinson & Stokes, 1959).

Results and Discussion Figure 1 shows the results from a typical experiment in which carbachol (5×10^{-5} M) was added to the bath for two minutes. Impalement with the chloride-sensitive electrode, and previous drug application, reduced the membrane potential to -76 mV as observed at the beginning of the recording of Figure 1. Before the application of the drug, it can be seen that the chloride ions appear to be passively distributed: the measured internal

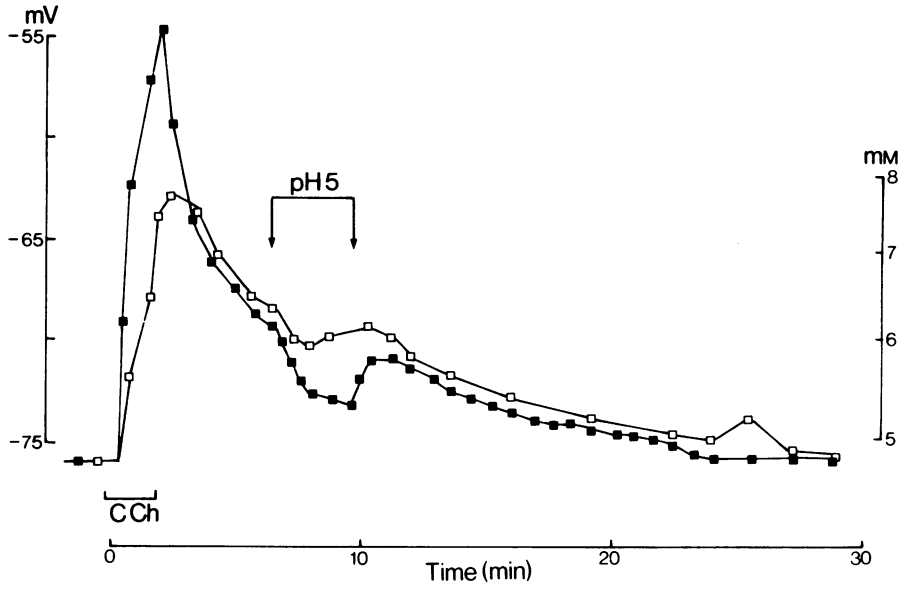

Fig. 1 Simultaneous recording of the membrane potential (■) and the chloride potential (□) in a sartorius muscle fibre. Internal chloride activity (□) can be read from the scale on the right. Carbachol (CCh; 5×10^{-5} M) was added for 2 min to the bath already containing 10^{-7} M tetrodotoxin. Between the arrows, the pH of the medium was changed from 7.4 to 5.0.

chloride activity of 4.7 mM corresponds to equal values of E_m and E_{Cl} As predicted by Jenkinson & Terrar (1973), during the rapid depolarization which occurs in the presence of carbachol, chloride ions enter the fibre with a slight delay, causing a significant increase in activity up to 7.8 mM. When the carbachol is washed out of the bath, the internal chloride activity returns only gradually to the initial level, with a corresponding slow recovery of the membrane potential. The membrane may be repolarized more rapidly by a reduction in pH (see Jenkinson & Terrar, 1973). That this effect is due to a reduced contribution of the chloride to the membrane potential is shown in Fig. 1 since during the repolarization of the membrane caused by a change in pH from 7.4 to 5.0 the efflux of chloride was halted.

The difference between E_m and E_{Cl} is indicative of the relative contribution of the chloride permeability to the total membrane permeability. Cationic permeability is dominant as long as carbachol is present, whereas on repolarization the membrane behaves almost as a chloride electrode. The Cl^- contribution can be lowered by reducing the external pH, since Hutter & Warner (1967a,b; 1972) demonstrated that low pH decreased the muscle membrane permeability to Cl^- without affecting the K^+ permeability.

If it is assumed that in the absence of carbachol, the membrane potential depends only on the internal and external concentrations of K^+ (K_i and K_o) and Cl^-(Cl_i and Cl_o) and on the ratio of their permeability, P_K and P_{Cl}, so that

$$E_m = -\frac{RT}{F} \ln \frac{P_K/P_{Cl}(K_i) + (Cl_o)}{P_K/P_{Cl}(K_o) + (Cl_i)}$$

(Hodgkin & Horowicz, 1959) it should be possible to estimate P_K/P_{Cl} from the results of Fig. 1, if K_i is assumed to have remained constant throughout the experiment at 140 mM (Hodgkin & Horowicz, 1959).

During the slow phase of the repolarization, anomalous rectification (Katz, 1949: Hodgkin & Horowicz, 1959; Adrian & Freygang, 1962) effectively reduces the outward flow of K^+, thus maintaining E_m close to E_{Cl}. In the experiment illustrated, from the data obtained at pH 7.4, a P_K/P_{Cl} value of 0.05 can be calculated throughout the slow recovery phase. At pH 5, P_K/P_{Cl} is higher, and at the maximum repolarization point on Fig. 1, it was calculated to be 0.2. These results are in good agreement with values from other experiments for similar conditions. Thus Hodgkin & Horowicz (1959) estimated P_K for outward going potassium to be around 0.1×10^{-6} cm/s and

Hutter & Warner (1967b) observed in acid pH a fourfold to tenfold decrease in P_{Cl} from an average value of 1.76×10^{-6} cm/s at alkaline pH.

In conclusion, a direct estimate of changes in internal chloride activity by the use of chloride-sensitive electrodes confirms the interpretation of Jenkinson & Terrar (1973) on the time course of the carbachol effect. After epolarization produced by carbachol, a chloride accumulation slows the repolarization of the membrane even though the carbachol effect has subsided.

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