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Calcium conductance of acetylcholine-induced endplate channels

SEVERAL lines of evidence indicate that an influx of Ca^{2+} ions accompanies transmitter activation of the postsynaptic membrane at the neuromuscular junction[1-5]. We report here that we have now investigated the elementary characteristics of the Ca^{2+} current by bathing muscles in $CaCl_2$ solutions containing no Na^+ ions, so that Ca^{2+} is the only ion available to carry any appreciable inward current[1,3,6,7]. The single channel current (i) and mean lifetime (τ) of the postsynaptic channels were then determined by the technique of noise analysis[8,9]. It was found that τ in the Ca^{2+} solution was shorter than in normal Ringer, but that the voltage dependence of τ was unchanged. The elementary current showed a non-linear voltage dependence, unlike the linear dependence in normal solution[8].

Experiments were carried out on the frog (*Rana temporaria*) sartorius muscle, at a temperature of 5–6 °C. The muscle was strongly stretched to reduce contraction and its associated artefacts. The normal bathing solution contained (in mM); NaCl, 120; KCl, 2; $CaCl_2$, 1.8; HEPES, 4 (*p*H 7.2). Calcium solutions contained: $CaCl_2$, 82 or 160; KCl, 2; HEPES, 4 (*p*H 7.2): care was taken to avoid contamination with Na^+. A standard two-point voltage clamp was used at the endplates, and a third external micropipette filled with 2 M acetylcholine (ACh) was used to apply ACh ionophoretically to the endplate. A fast Fourier transform method was used to analyse the frequency components of the current fluctuations during steady ACh application, and i and τ were determined as previously described[8,9].

Records were usually obtained from several fibres in normal Ringer solution, and then from the same, and additional fibres, after changing to Ca^{2+} solution. Resting membrane potentials were higher in isotonic and hypertonic Ca^{2+} solutions (mean values: normal Ringer 85.1 ± 5.8 mV, Ca^{2+} solution 103 ± 7.2 mV: all deviations are ±1 s.d. unless otherwise stated), and the input resistance of the fibres was increased (normal Ringer ~350 kΩ; Ca^{2+} solution 1.32 ± 0.38 MΩ). Pipettes with large tip diameters were used to reduce background noise, and the values

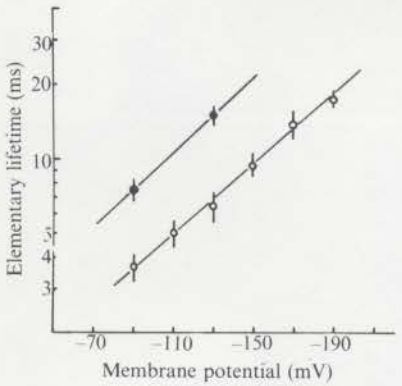

Fig. 1 Membrane potential dependence of the ACh-induced single channel lifetime, τ, measured from muscles bathed in normal Ringer solution (●) and in isotonic or hypertonic Ca^{2+} solutions (○). No differences were seen in τ between isotonic and hypertonic Ca^{2+} solutions, and the results shown are pooled data from both. Lines were fitted by eye, and error bars give ±1 s.d. Five endplates were examined in Ringer solution, and seven in $CaCl_2$ solutions. Values were obtained during steady ionophoretic application of ACh to voltage-clamped endplates, by fitting the membrane current fluctuation spectra to a lorentzian curve. Background spectra were subtracted before fitting. τ was calculated from the half power frequency f_c of the spectra as $\tau = 1/(2\pi f_c)$. About 15 noise segments of 512 points were obtained at each potential, at a digitisation rate of 500 or 1,000 Hz, and were used to compute an average spectrum.

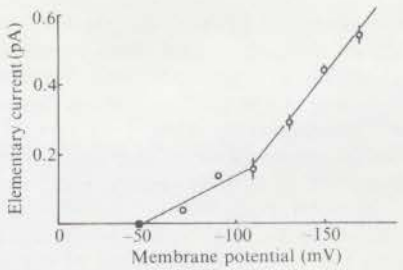

Fig. 2 Membrane potential dependence of the single channel current i, measured from fibres bathed in 160 mM $CaCl_2$ solution. Lines were fitted by eye. Points shown with error bars are means from five fibres, and bars are ±1 s.e.m. Points at −90 and −70 mV are measurements from single fibres. The equilibrium potential (●) was measured from the reversal potential of the ACh response in seven fibres, and was −44.4 mV (s.d. ± 1.1 mV). Elementary currents were calculated from the total spectrum variance, after subtraction of background variance. The alternative technique of computing i from the zero frequency asymptote of the spectra gave values in good agreement.

given above may be artificially low because of fibre damage during penetration. The frequency of miniature endplate potentials was elevated after changing to Ca^{2+} solution, but fell to nearly zero after 2–3 h, when ACh noise recordings could be made. Miniature endplate currents (m.e.p.cs) were reduced in duration and size in Ca^{2+} solution (see also ref. 3), mean amplitudes at −130 mV being 2.86 ± 0.39 nA in normal Ringer, and 0.97 ± 0.37 nA in Ca^{2+} solution.

In normal Ringer, ionophoretic application of large doses of ACh to the endplate caused local contractions, and these became much larger in isotonic Ca^{2+} solution. For example, a barely visible contraction, involving two sarcomeres, was seen with a synaptic charge flux of about 1 nC. Because of this difficulty in avoiding movement artefacts, some experiments were carried out in hypertonic Ca^{2+} solution (160 mM $CaCl_2$), which helped to reduce contraction. To guard against the possibility that part of the synaptic current was carried by an influx of Cl^- ions[7], recordings were made from two fibres in a bathing solution of 82 mM Ca-cyclamate. M.e.p.cs were recorded in this solution, with the same amplitudes as in 82 mM $CaCl_2$ solution, and there was little difference in reversal potential. Also K^+ ion influx made no appreciable contribution to the synaptic current, as no significant changes in equilibrium potential or single channel currents were seen in fibres bathed in a solution containing only 160 mM $CaCl_2$.

A variety of divalent cations in addition to Ca^{2+} are able to carry a synaptic current. We have recorded m.e.p.cs and ACh-induced currents from fibres bathed in isotonic solutions of $MgCl_2$, $MnCl_2$, $SrCl_2$ and $CoCl_2$ (all solutions also contained 2 mM KCl and 4 mM HEPES). This contrasts with the behaviour in several other systems, where Mg^{2+}, Mn^{2+} and Co^{2+} are known to block Ca^{2+} fluxes[10-13]. The drug D600 has also been shown to block Ca^{2+} fluxes in a variety of preparations[11-13]. We found that at a concentration of 200 μg ml^{-1} D600 decreased the frequency of m.e.p.cs in isotonic Ca^{2+}, and did not abolish the ACh sensitivity of the muscle membrane. However, with ionophoretic ACh application only brief transient responses could be recorded, and a period of a few minutes was required for recovery of the response to a second pulse. This effect of D600, which was also present in normal Ringer, may be attributable to a blocking of open channels, or to an increase in desensitisation.

Figure 1 shows the voltage dependence of mean lifetime (τ) of ACh-induced channels in Ca^{2+} solution, plotted on semi-logarithmic coordinates. The data are fitted well by a straight line, indicating that τ increases exponentially with membrane hyperpolarisation. The voltage constant (potential shift to give an e-fold change in τ) was 62 mV. τ increases exponentially with

hyperpolarisation in normal Ringer[8], and the Ca^{2+} data closely parallel a line drawn through control measurements in normal Ringer, although shifted in a hyperpolarised direction by about 46 mV. The shortening of τ in Ca^{2+} solution is surprising, as an increase in Ca^{2+} concentration from 1 to 10 mM has been reported to slow the decay phase of m.e.p.cs[14]. However, we have been unable to confirm these results, and found that this increase in Ca^{2+} concentration produced in some experiments a shortening of m.e.p.c. decay, and in others no detectable change.

Figure 2 shows the voltage dependence of the elementary current, derived from noise analysis, in hypertonic Ca^{2+} solution. The relationship is not linear throughout, indicating that the elementary conductance (γ) varies with membrane potential. Closely similar behaviour was seen in experiments using isotonic Ca^{2+} solution. This is unlike the situation in normal Ringer, where γ is independent of potential[8], and in our experiments had a value of 29.3 ± 2.7 pS (s.e.m.). In hypertonic Ca^{2+} solution the voltage dependence of i was approximately linear at potentials more hyperpolarised than -110 mV, and the slope of this segment gave a value for γ of 6.25 pS. Noise measurements at potentials between equilibrium and -100 mV were very difficult to obtain, but an average value for γ of $\sim$2.5 pS was estimated for this segment.

Our results confirm previous observations[1–5] showing that activation of the postsynaptic membrane with ACh causes an increase in permeability to Ca^{2+} ions, and further indicate that Mg^{2+}, Mn^{2+}, Sr^{2+} and Co^{2+} ions are also able to cross the channels induced by ACh. However, the conductances for these ions are small, and at -90 mV, for example, the elementary conductances in both isotonic $MgCl_2$ and $CaCl_2$ solutions are about 10 times less than in normal Ringer.

Divalent ions might cross the endplate membrane (1) nonspecifically through the 'ordinary' channels normally used by Na^+ and K^+, or (2) through a separate Ca^{2+}-specific channel. The apparently identical voltage dependence of τ for Ca^{2+} and Na^+ currents, together with the failure of Mg^{2+}, Mn^{2+}, Co^{2+} and D600 to block the m.e.p.cs, favour (1), but do not entirely exclude (2). The reason for the differences in conductance properties in Na- or Ca-Ringer is not clear, and the non-linear dependence of the elementary Ca^{2+} current on membrane potential is not predicted by the Goldman–Hodgkin–Katz model of ion permeation[15–18]. However, differences also exist between various divalent ions themselves, and we find that, unlike Ca^{2+}, the elementary Mg^{2+} current has a linear voltage dependence over the range examined (-50 to -130 mV).

The decrease in channel lifetime in Ca^{2+} solutions is in the opposite direction to that expected from the effect of Ca^{2+} ions in screening negative surface charges on the muscle membrane[14], and suggests that Ca^{2+} ions have an additional effect on the channel. It may be that in addition to direct effects of various ions on the receptors and their environment, the lifetime of transmitter-induced channels is influenced by the nature of the ions which permeate them[19].

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