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PROPERTIES OF MINIATURE EXCITATORY JUNCTIONAL CURRENTS AT THE LOCUST NERVE–MUSCLE JUNCTION

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SUMMARY

1. Miniature excitatory junctional currents (m.e.j.c.s) were examined in conditions where inward current was carried mainly by Na^+ (i.e. in normal medium, Ca^{2+}-free medium and Cl^--free medium). M.e.j.c.s were also examined in isotonic Ca^{2+} where the inward post-synaptic current was carried mainly by Ca^{2+}.

2. In normal medium, mean m.e.j.c. amplitude = $2{\cdot}34 \pm 0{\cdot}05$ nA. The decay time constant of m.e.j.c.s (excluding a small percentage with abnormal shapes) was $\tau_{m.e.j.c.} = 2{\cdot}62 \pm 0{\cdot}11$ msec ($V_m = -80$ mV, $T = 22$ °C). Decay-time was not markedly changed in Ca^{2+}-free or Cl^--free medium. $\tau_{m.e.j.c.}$ approaches the life-time of glutamate activated junctional channels.

3. Excitatory junctional currents, evoked by nerve impulses, decayed slightly faster than m.e.j.c.s obtained in the same fibres. Extracellularly recorded m.e.j.c.s and voltage-clamped m.e.j.c.s were similar in time course.

4. $\tau_{m.e.j.c.}$ decreased exponentially with membrane hyperpolarization. An *e*-fold change was produced by $182{\cdot}1 \pm 24{\cdot}8$ mV change in V_m.

5. The dependence of mean m.e.j.c. amplitude on clamp potential showed a slight non-linearity at hyperpolarized levels. The equilibrium potential for transmitter action was close to 0 mV in normal solution as well as in Ca^{2+}-free and Cl^--free solutions.

6. The kinetics of junctional channels are altered in isotonic Ca^{2+}. M.e.j.c. amplitude was reduced to about one-third normal size; mean m.e.j.c. = $0{\cdot}74 \pm 0{\cdot}03$ nA. The decay time becomes markedly briefer, $\tau_{m.e.j.c.} = 1{\cdot}01 \pm 0{\cdot}08$ msec, indicating a reduction in mean channel life-time ($V_m = -80$ mV, $T = 22$ °C).

7. A population of slow time course and composite m.e.j.c.s appear when muscle fibres are hyperpolarized in isotonic Ca^{2+}, thus producing a prolongation in mean $\tau_{m.e.j.c.}$. This results from an influence of post-synaptic membrane potential on presynaptic transmitter release. If such m.e.j.c.s are ignored the voltage dependence of $\tau_{m.e.j.c.}$ of the remaining events is abolished or even reversed indicating that voltage sensitivity of channel life-time is altered in isotonic Ca^{2+}. The equilibrium potential for transmitter action may be slightly more positive than normal.

8. We estimate that a single packet of neurally released transmitter normally opens, on average, 250 ion channels at these junctions.

INTRODUCTION

The decay time of miniature end-plate currents reflects the life-time of transmitter induced channels at the vertebrate end-plate (Katz & Miledi, 1973*a*; Anderson & Stevens, 1973) and also at the crab, locust and crayfish nerve–muscle junctions (Crawford & McBurney, 1976*b*; Anderson, Cull-Candy & Miledi, 1976; Cull-Candy & Miledi, 1980*b*; Stettmeier, Finger & Dudel, 1978) where the transmitter is thought to be glutamate or a closely related substance (Takeuchi & Takeuchi, 1964; Usherwood & Machili, 1968; Beránek & Miller, 1968).

To obtain information about synaptic channels opened by the neurally released transmitter we have analysed miniature excitatory junctional currents (m.e.j.c.s) under voltage clamp in conditions where the inward synaptic current is carried mainly by Na^+ ions in the presence and absence of Ca^{2+} ions. We have also examined miniature currents in conditions where any inward post-synaptic current caused by transmitter action is expected to result mainly from an influx of Ca^{2+} ions through synaptic channels. A preliminary report of some of these results has appeared (Cull-Candy & Miledi, 1980*a*).

METHODS

Preparation and solutions. Experiments were made on distal fibre bundles of the extensor tibiae muscle of the metathoracic leg of the adult locust *Schistocerca gregaria* (Hoyle, 1955; Cochrane, Elder & Usherwood, 1972). The muscle was dissected essentially as previously described (Cull-Candy, 1976) with some minor modifications.

Fibres were examined in Cl^--free medium in which their input resistance was 5–10 MΩ, or in normal locust medium in which the input resistance was about 1 MΩ. In some experiments isotonic Ca was used and in this medium the input resistance was about 3–4 MΩ (see Results).

Cl^--free medium contained (mM): $NaMeSO_4$, 180; $KMeSO_4$, 2; NaH_2PO_4, 4; Na_2HPO_4, 6; Ca propionate, 2; pH 6·8 (Cull-Candy, 1976). In some experiments, phosphate buffer was replaced by 10 mM-HEPES buffer, especially when preparations were going to be examined subsequently in isotonic Ca^{2+}. Normal medium contained (mM): NaCl, 180; KCl, 2; NaH_2PO_4, 4; Na_2HPO_4, 6; $CaCl_2$, 2. Isotonic Ca^{2+} medium contained (mM): $CaCl_2$, 120; KCl, 2; HEPES, 10; pH 6·8. Ca^{2+}-free medium was either normal medium or Cl^--free medium in which Ca^{2+} was replaced by (mM): $MgCl_2$, 5 or $MgSO_4$, 5 and EGTA, 1.

Voltage clamping. Voltage-recording micro-electrodes (3 M-KCl) and current-passing micro-electrodes (2 M-K acetate or citrate) were placed within 100 μm of each other in the middle region of the muscle fibre. Voltage-clamp technique was essentially as previously described (Anderson, Cull-Candy & Miledi, 1978). M.e.j.c.s were recorded through an active virtual earth (with 500 Hz or 1 kHz) low pass filter, and data were collected on analogue tape (band width 0–1200 Hz).

Analysis of m.e.j.c.s. Analogue records were fed via an active filter (Kemo) into a PDP-11 and digitized at 0·5–0·1 msec sample interval. The time constant of decay of miniature currents was obtained by several methods. Digitized averaged or single m.e.j.c.s were plotted semi-logarithmically and a least squares line was fitted to the decay phase. Usually more than fifty m.e.j.c.s were analysed to give an estimate of $\tau_{m.e.j.c.}$. Records were digitized at 0·5 msec sample interval after low pass filtering at 1000 Hz ($1/f^8$ roll-off). The Fast Fourier Transform was calculated for 512 point segments to give the spectral density function, $S(f)$, of m.e.j.c.s. Background noise, calculated from segments of noise occurring between m.e.j.c.s, was subtracted. Finally, filmed m.e.j.c.s were traced by hand and $\tau_{m.e.j.c.}$ measured for comparison with other estimates.

RESULTS

Miniature currents in normal medium and Ca^{2+}-free medium

Typical examples of m.e.j.c.s recorded under voltage clamp from locust nerve–muscle junctions are illustrated in Fig. 1*A*. Normally, m.e.j.c.s rise rapidly and decay exponentially although, as previously described for other synapses, a small percentage

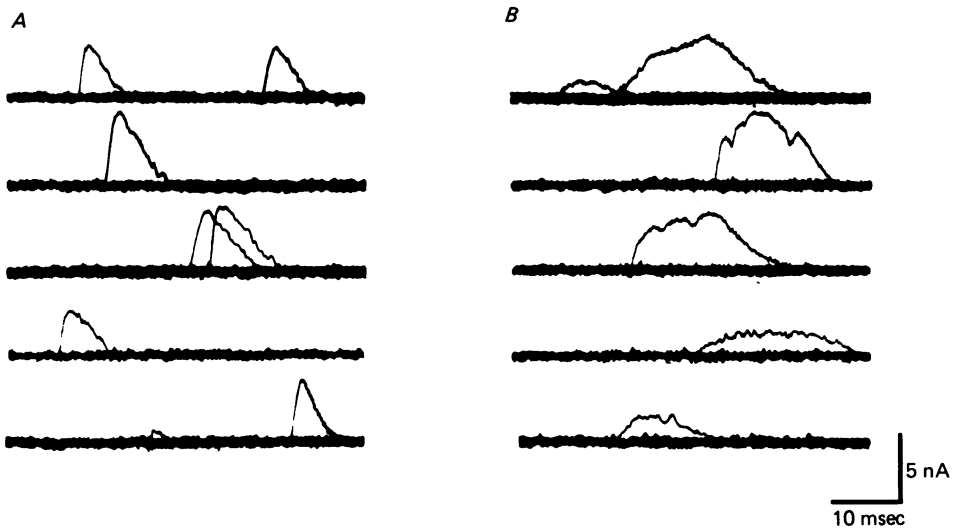


Fig. 1. Miniature excitatory junctional currents recorded from muscle fibres in normal bathing medium. *A*, examples of typical m.e.j.c.s as well as a small m.e.j.c. *B*, selected examples of m.e.j.c.s with abnormal time courses. M.e.j.c.s shown include those with prolonged rise or decay phases as well as composite events. Each trace is of several superimposed sweeps (inward current upwards). Clamp holding potential −100 mV, $T = 22$ °C.

of currents do not fit this pattern (see Green, Miledi, Perez & Vincent, 1975; Crawford & McBurney, 1976*b*; Cull-Candy, Miledi & Trautmann, 1979; Magazanik & Vyskocil, 1979). Examples of m.e.j.c.s with abnormal rising and falling phases are shown in Fig. 1*B*.

Amplitude of m.e.j.c.s

In normal medium and in Ca^{2+}-free (EGTA) medium m.e.j.c.s are well above the noise level of the recording system and amplitude histograms usually approximate to a Gaussian distribution. However, as can be seen in Fig. 2*A* (see also Fig. 1*A*) a population of small m.e.j.c.s is sometimes detectable (Hodgkiss & Usherwood, 1978) similar to that found at vertebrate end-plates (Kriebel & Gross, 1974; Bevan, 1976). In normal bathing medium the mean m.e.j.c. amplitude at a clamp potential of −80 mV = 2·34 ± 0·05 nA (mean ± S.E. of the mean, for twenty-four muscle fibres); in Cl^- free medium, the mean m.e.j.c. amplitude at −80 mV = 2·26 ± 0·19 nA (for ten fibres) at $T = 22$ °C. These m.e.j.c. amplitude distributions were essentially unchanged in Cl^- or Ca^{2+}-deficient conditions.

Time course of m.e.j.c.s and e.j.c.s

In normal bathing medium the rise-time of m.e.j.c.s was $1{\cdot}11 \pm 0{\cdot}05$ msec (ten fibres), and no evidence was found for a consistent voltage sensitivity of the rise-time.

The time constant of decay of m.e.j.c.s, $\tau_{m.e.j.c.}$, was obtained by one of several methods; from a semi-logarithmic plot of the decay phase of individual or averaged m.e.j.c.s (Fig. 5*B*); from spectral analysis of m.e.j.c.s (Fig. 9); or from individual

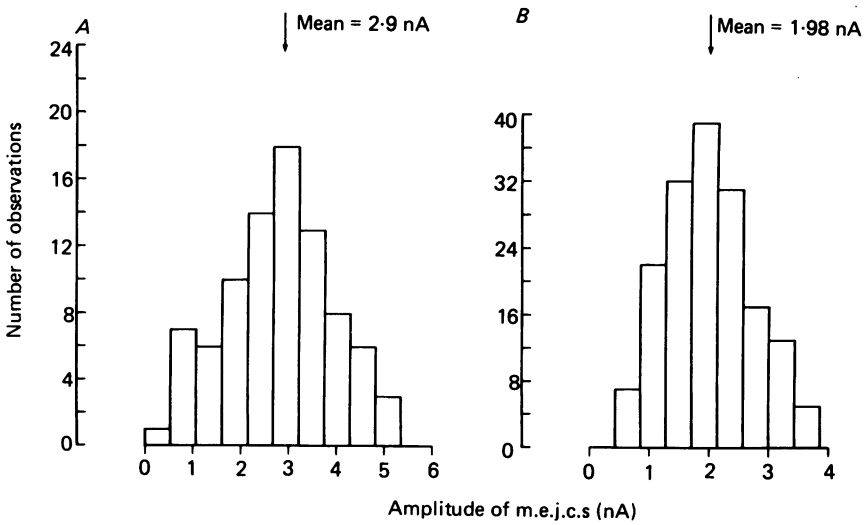


Fig. 2. Amplitude distributions of m.e.j.c.s in voltage-clamped muscle fibres. *A*, normal medium, mean m.e.j.c. = 2·90 nA at a clamp holding potential $V_m = -110$ mV. *B*, Ca^{2+}-free (EGTA) medium, mean m.e.j.c. = 1·98 nA at a clamp holding potential $V_m = -80$ mV. M.e.j.c.s in normal and Ca^{2+}-free medium have an approximately Gaussian distribution. In *A* some small m.e.j.c.s are present, $T = 22$ °C.

m.e.j.c.s traced by hand. When the same population of m.e.j.c.s was analysed these methods showed reasonable agreement for m.e.j.c.s in normal medium. In practice the small percentage of m.e.j.c.s of abnormally slow time course (Fig. 1*B*), which were often excluded from the analysis (see later), were not so readily excluded from spectral analysis, so that $\tau_{m.e.j.c.}$ estimated from the spectra tends to be slightly longer than that obtained with the other methods.

The time constant of decay of m.e.j.c.s obtained in normal medium was $\tau_{m.e.j.c.} = 2{\cdot}98 \pm 0{\cdot}14$ msec (for ten muscle fibres). In Ca^{2+}-free (EGTA) medium $\tau_{m.e.j.c.} = 2{\cdot}76 \pm 0{\cdot}18$ msec (for ten fibres) at $V_m = -80$ mV, $T = 22$ °C. Similar values were also obtained in Cl^--free medium, which suggests that the mean life-time of the transmitter-induced channel was not markedly altered by the various conditions.

Fig. 3*A* and *B* shows the distribution of $\tau_{m.e.j.c.}$ obtained from a single muscle fibre clamped at -80 mV and -110 mV. The histograms are unimodal and skew with more events of prolonged time course than expected of a Gaussian distribution. Excitatory junctional currents (e.j.c.s), elicited by nerve stimulation usually appeared slightly briefer in decay time than m.e.j.c.s obtained in the same fibre when compared

at the same clamp potential. A striking example of this is illustrated in Fig. 3 *A* and *B* where the arrows above the histograms indicate the mean values of $\tau_{m.e.j.c.}$ and $\tau_{e.j.c.}$. In this fibre $\tau_{m.e.j.c.}$ $(-80\ mV) = 3{\cdot}0 \pm 0{\cdot}87$ msec ($\pm$S.D.), $\tau_{m.e.j.c.}$

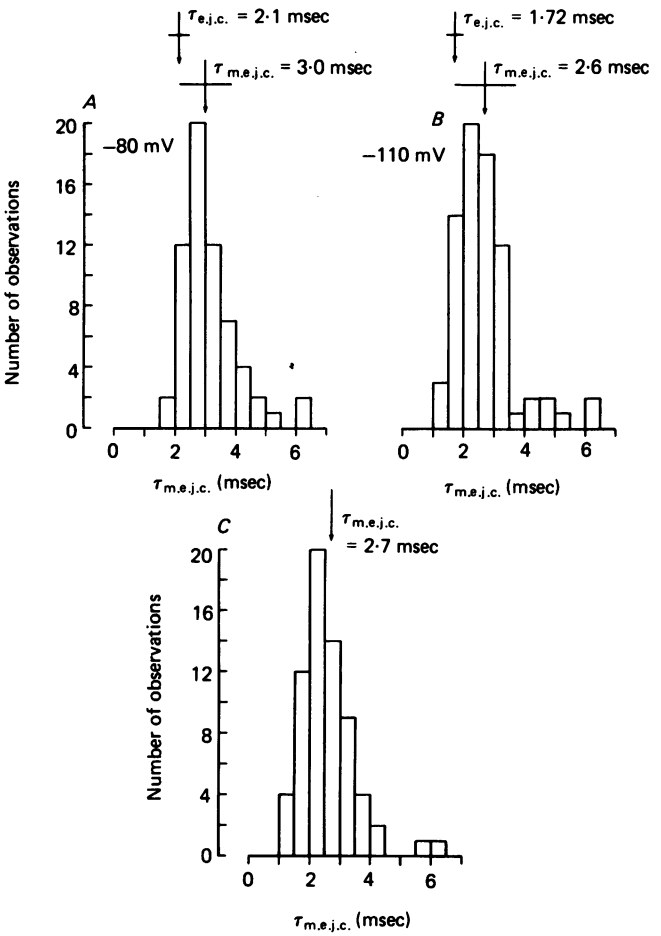


Fig. 3. *A*, *B*, distribution of values for $\tau_{m.e.j.c.}$ in a single muscle fibre under voltage clamp at two potentials (*A*, −80 mV; *B*, −110 mV). *C*, distribution of values for $\tau_{m.e.j.c.}$ obtained with a focal extracellular pipette from a different muscle fibre at −60 mV. In all histograms the distribution of $\tau_{m.e.j.c.}$ is approximately normal except for a small population of events with prolonged time course. In *A* and *B* the arrows above the histograms indicate the mean (and S.D.) of $\tau_{m.e.j.c.}$ and $\tau_{e.j.c.}$. Values for $\tau_{e.j.c.}$ are obtained from nerve-impulse evoked e.j.c. in the same fibre. Note that $\tau_{e.j.c.}$ is briefer than $\tau_{m.e.j.c.}$

$(-110\ mV) = 2{\cdot}60 \pm 0{\cdot}96$ msec; whereas $\tau_{e.j.c.}$ $(-80\ mV) = 2{\cdot}1 \pm 0{\cdot}32$ msec, $\tau_{e.j.c.}$ $(-110\ mV) = 1{\cdot}72 \pm 0{\cdot}27$ msec.

M.e.j.c.s identified visually as being of abnormal time course (i.e. prolonged rise or decay-time or composite wave form) amounted to as much as 10% of the total m.e.j.c. population. If these events were excluded then $\tau_{m.e.j.c.}$

(−80 mV) = 2·62±0·11 msec (for ten fibres) at T = 22 °C, which approaches previous estimates of approximately 2·5 msec for the time constant underlying the e.j.c. and glutamate noise at V_m = −80 mV (Anderson, Cull-Candy & Miledi, 1977, 1978; Cull-Candy & Miledi, 1980*b*).

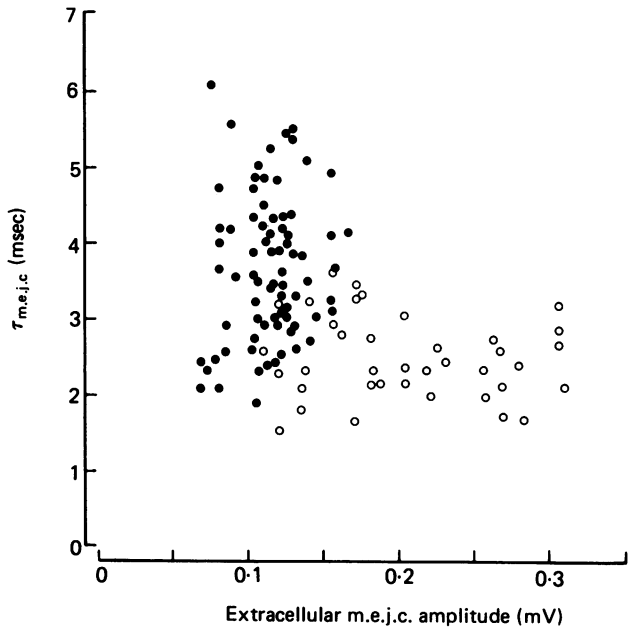


Fig. 4. Scatter plot of the time constant of decay of m.e.j.c.s (recorded with an extracellular micropipette) against m.e.j.c. amplitude. Events are separated into two groups (●, ○) recorded at two extracellular sites separated by less than 5 μm. The population of m.e.j.c. represented by (○) had the largest mean amplitude obtained at this junction. $\tau_{m.e.j.c.}$ (○) = 2·49 msec; $\tau_{m.e.j.c.}$ (●) = 3·67 msec, T = 21 °C.

The muscle fibres studied have distributed innervation so that some m.e.j.c.s originate at a distance from the recording and current-passing electrodes. Because the fibres are short they can be considered to be effectively space-clamped when the input resistance of the fibre is reasonably high. However, the space constant of the fibres decreases as the frequency of the command signal increases so that some filtering of high frequencies will occur if events originate far from the recording site. The rising phase of the m.e.j.c. will therefore be more affected than the decay phase. Indeed, when care was taken to avoid exerting undue pressure with an external recording electrode, $\tau_{m.e.j.c.}$ obtained with focal external recording and with voltage clamp were similar, suggesting that measurements of $\tau_{m.e.j.c.}$ with voltage clamp were not obviously distorted. Fig. 3*C* shows the distribution of values of $\tau_{m.e.j.c.}$ for events recorded with a focal extracellular micropipette. The histogram, which is unimodal and skew, resembles distributions of intracellularly recorded m.e.j.c.s in locust fibres (Fig. 3*A* and *B*).

On some occasions there was variation in the mean decay time of externally

recorded m.e.j.c.s. Fig. 4 shows a scatter plot of the amplitude of external m.e.j.c.s *vs.* time constant of decay, obtained from two neighbouring sites on the membrane. The m.e.j.c. decay was slower in the region where smaller events were seen. At this site the m.e.j.c.s were presumably being recorded at some distance from their origin. The prolonged decay could result from retarded clearance of transmitter from the cleft perhaps due to a 'compression artifact' (see Katz & Miledi, 1973*a*; Anderson *et al.* 1976), although other possibilities are not discounted.

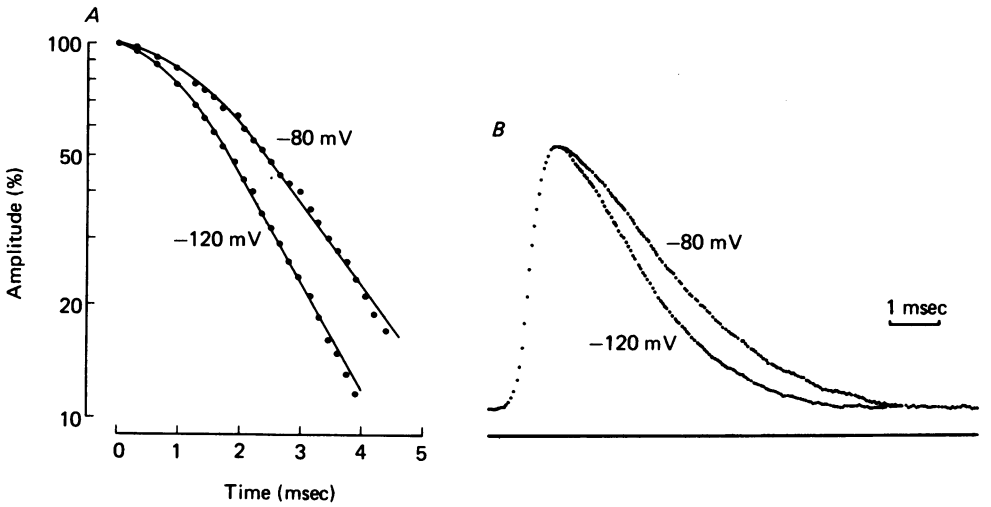


Fig. 5. *A*, decay phases of the m.e.j.c.s shown in *B* plotted on semi-logarithmic co-ordinates. Straight lines drawn through the points were fitted by the least squares method to values between 60 and 20% of the m.e.j.c. amplitude to give the decay time constant. $\tau_{m.e.j.c.}$ (−80 mV) = 2·0 msec, $\tau_{m.e.j.c.}$ (−120 mV) = 1·5 msec. The dependence of $\tau_{m.e.j.c.}$ on clamp potential was calculated according to: $(V_1 - V_2)/\ln(\tau_1/\tau_2)$. The membrane potential change required to produce an *e*-fold change in $\tau_{m.e.j.c.}$ was 139 mV. *B*, Examples of averaged m.e.j.c.s illustrating the effect of membrane potential on $\tau_{m.e.j.c.}$. M.e.j.c.s have been recorded from the same muscle fibre at two clamp potentials. Digitized m.e.j.c. at V_m = −80 mV is the average of twenty-four m.e.j.c.s; digitized m.e.j.c. at V_m = 120 mV is the average of nineteen m.e.j.c.s. Amplitudes of m.e.j.c.s have been normalized to allow comparison of the decay phases. The rising phase shown is that of the m.e.j.c. at −80 mV. The mean amplitude of m.e.j.c.s changed from 2·90 nA at −80 mV to 3·3 nA at −120 mV (not shown). T = 22 °C.

Membrane potential dependence of $\tau_{m.e.j.c.}$

The decay time of m.e.j.c.s decreased with membrane hyperpolarization as previously described in this preparation (Anderson *et al.* 1976; Cull-Candy & Miledi, 1980*b*) and at glutamate-sensitive neuromuscular junctions of the crayfish (Dudel, 1974; Onodera & Takeuchi, 1978). The membrane potential dependence of m.e.j.c. decay is illustrated in Fig. 5, which shows examples of averaged m.e.j.c.s obtained from the same muscle fibre at two clamp potentials. The m.e.j.c. amplitudes have been normalized to allow comparison of the decay times which, in the example shown, decrease from τ = 2·0 msec at V_m = −80 mV to τ = 1·5 msec at V_m = −120 mV. As can be seen from the semi-logarithmic plots of the decay phases, the m.e.j.c.s decayed

approximately exponentially between about 70 and 20 % of their peak amplitude (Fig. 5*A*).

The relationship between $\tau_{m.e.j.c.}$ and membrane potential is shown in Fig. 6 for two muscle fibres in normal (Cl^--free) medium (Fig. 6*A*) and for two muscle fibres

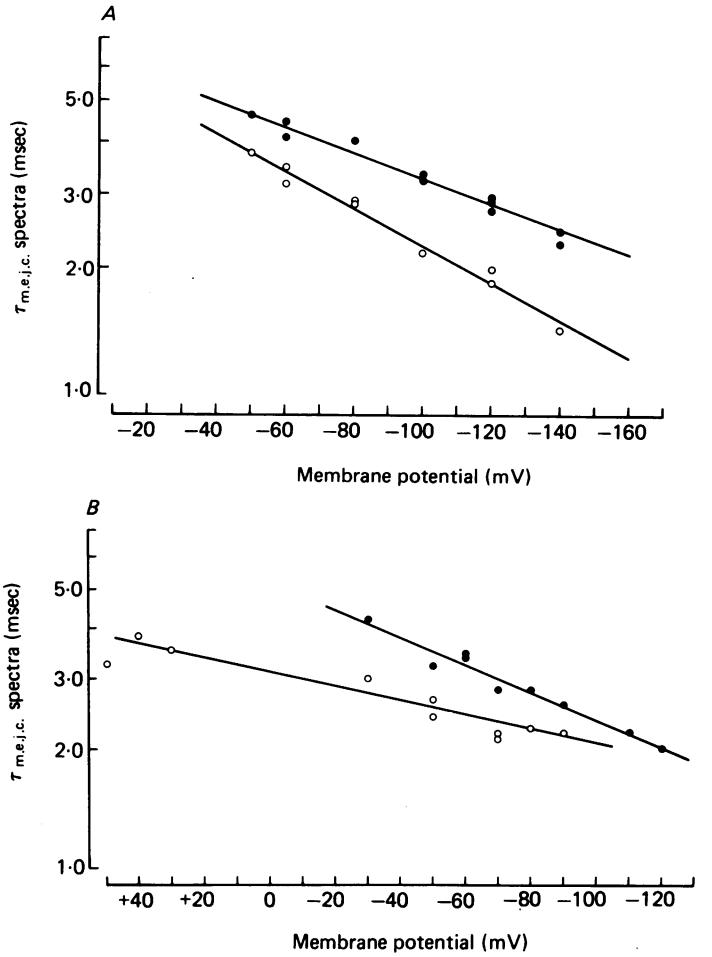


Fig. 6. Dependence of the time constant of decay of m.e.j.c.s ($\tau_{m.e.j.c.}$) on clamped membrane potential. The lines fitted to the points represent the relationship $\tau(V_1) = \tau(V_2)$. $\exp(V/H)$. *A*, two muscle fibres in Cl^--free medium. Using slope values $H(\bigcirc) = 98{\cdot}4$ mV, $H(\bullet) = 142{\cdot}8$ mV. *B*, two muscle fibres in Ca^{2+}-free (EGTA) medium, $H(\bigcirc) = 244{\cdot}8$ mV, $H(\bullet) = 141{\cdot}7$ mV. $T = 22$ °C.

in calcium-free medium (Fig. 6*B*). $\tau_{m.e.j.c.}$ appeared to be exponentially dependent on V_m over a wide range of membrane potentials (+70 to −140 mV) and was similar in normal medium and in Ca^{2+}-free medium. The membrane potential change required to produce an e-fold change in $\tau_{m.e.j.c.}$, obtained according to: $(V_1 - V_2)/\ln(\tau_1/\tau_2)$, was $182{\cdot}1 \pm 24{\cdot}8$ mV ($\pm$S.E. of the mean for ten fibres) in normal medium, and $183{\cdot}31 \pm 15{\cdot}6$ mV (for ten fibres) in Ca^{2+}-free medium. This is similar

to the membrane potential dependence of the mean channel life-time of junctional channels opened by glutamate in locust (Anderson *et al.* 1976) and crayfish fibres (Stettmeier *et al.* 1978).

Membrane potential dependence of m.e.j.c. amplitude

Fig. 7 *A* and *B* shows the dependence of m.e.j.c. amplitude on clamp potential in normal and Ca^{2+}-free medium. Mean m.e.j.c. amplitudes were obtained by measuring at least fifty m.e.j.c.s at a given membrane potential. Clamp potential was then altered, usually in steps of 10 or 20 mV, and the process repeated. As can be seen in Fig. 7 the relationship varies considerably from fibre to fibre. The equilibrium potential, E_{eq}, of the m.e.j.c. was either extrapolated or interpolated from a least squares fit to the peak m.e.j.c. amplitudes. In Cl^--free medium, $E_{eq} = +1{\cdot}42 \pm 0{\cdot}95$ mV (for six fibres); in Ca^{2+}-free (EGTA) medium $E_{eq} = +0{\cdot}26 \pm 0{\cdot}56$ mV (for five fibres). These values are similar to those previously obtained for glutamate and the transmitter (see for example, del Castillo, Hoyle & Machne, 1953; Beránek & Miller, 1968; Anwyl & Usherwood, 1974; Cull-Candy, 1976; Anderson *et al.* 1978).

The main feature of interest was that the relationship, although linear over part of its range, appeared to be markedly non-linear at hyperpolarized membrane potentials. Two possible explanations for this phenomenon are that the transmitter-induced channel rectifies at hyperpolarized membrane potentials or that the number of channels opened by a transmitter packet is decreasing. These possibilities are being examined.

Miniature currents in isotonic Ca^{2+}

Miniature potentials and currents can be recorded for many hours (> 12 hr) from fibres soaked in isotonic Ca^{2+}. Although reduced in size, they are clearly visible at the muscle fibre resting potential. In high Ca^{2+} the frequency of m.e.j.c.s was usually slightly elevated, but varied considerably from cell to cell. Bursts of high frequency discharge are interspersed with periods of near normal frequency. This may reflect some failure over the control of free intracellular calcium in the nerve terminals, when they are bathed in a medium containing a high level of Ca^{2+}, in the absence of Na^+ (Heuser, Katz & Miledi, 1971; Baker, Hodgkin & Ridgway, 1971; Thieffry, Bruner & Personne, 1980).

The input resistance of muscle fibres was obtained by measuring the feed-back current required to step the holding potential from -60 to -80 mV. The input resistance in isotonic Ca^{2+} was $R_{in} = 3{\cdot}05 \pm 0{\cdot}3$ MΩ; resting potentials of fibres increased from approximately -60 mV in normal bathing medium to $-92{\cdot}9 \pm 4{\cdot}68$ mV (twenty-five fibres in six muscles) in isotonic Ca^{2+}. Resting potentials in excess of -100 mV were common. Some muscle fibres were still capable of generating spikes in response to a depolarizing current pulse as expected if inward regenerative current is carried mainly by Ca^{2+}. However, most fibres were incapable of generating spikes in isotonic Ca^{2+} even when depolarized to close to 0 mV from a holding potential of -120 mV. Contraction occurs at a threshold of -30 to -40 mV. The problem concerning the lack of a spike in isotonic Ca^{2+} requires further investigation.

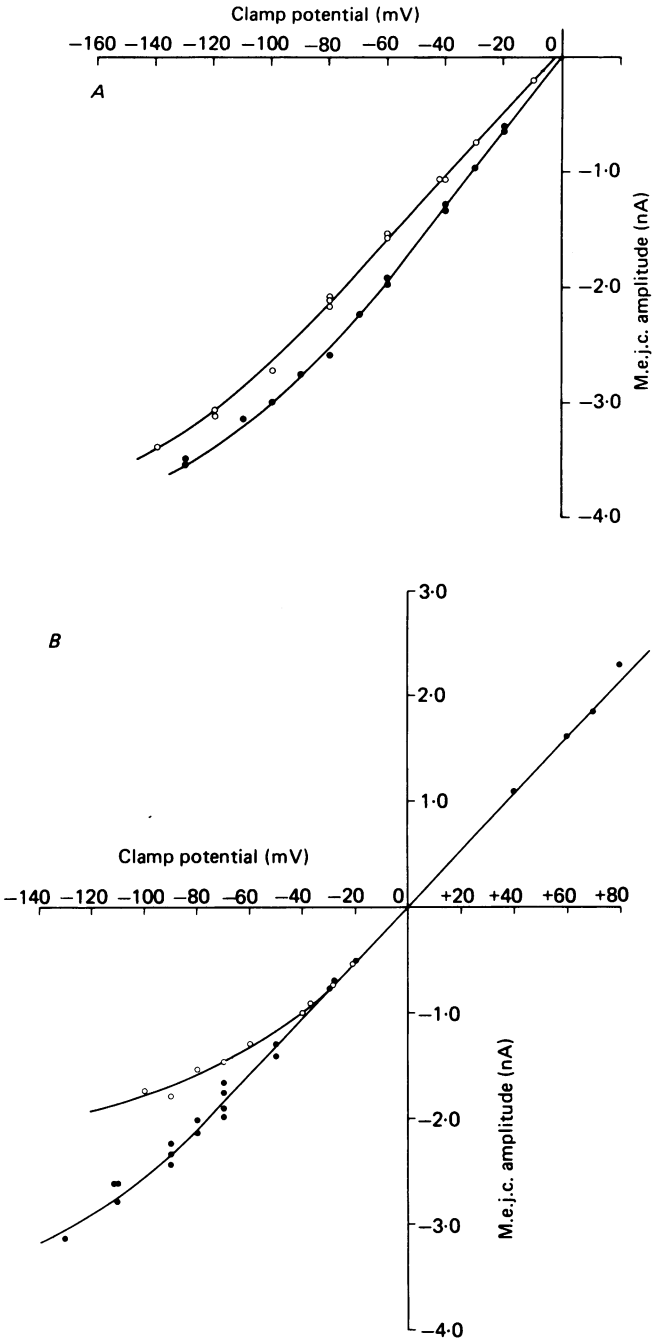


Fig. 7. Relationship between m.e.j.c. amplitude and clamp holding potential. Each point represents the averaged amplitude of at least fifty m.e.j.c.s at each clamp potential. The equilibrium potential for the m.e.j.c.s is near 0 mV in all fibres. Note the non-linear relationship at hyperpolarized levels. *A*, two muscle fibres in normal medium, E_{eq} (○) = 2·5 mV, E_{eq} (●) = 0 mV. *B*, two muscle fibres in Ca^{2+}-free medium, E_{eq} close to 0 mV.

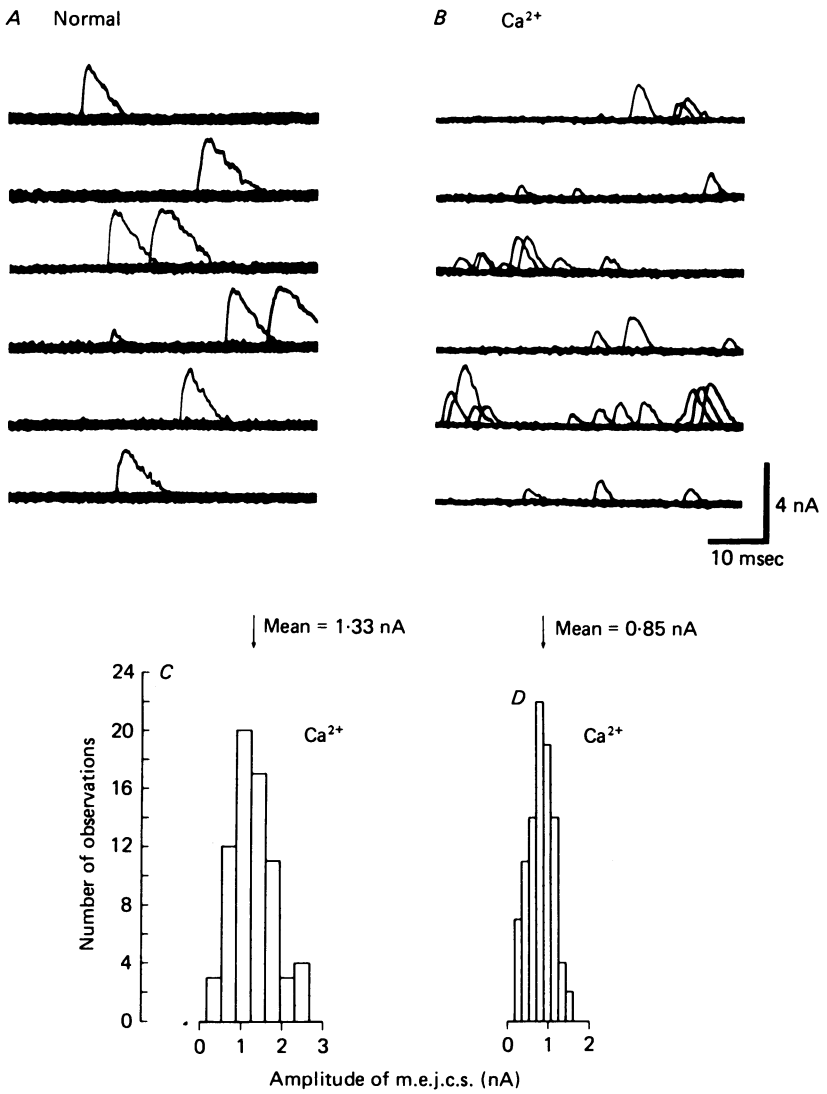
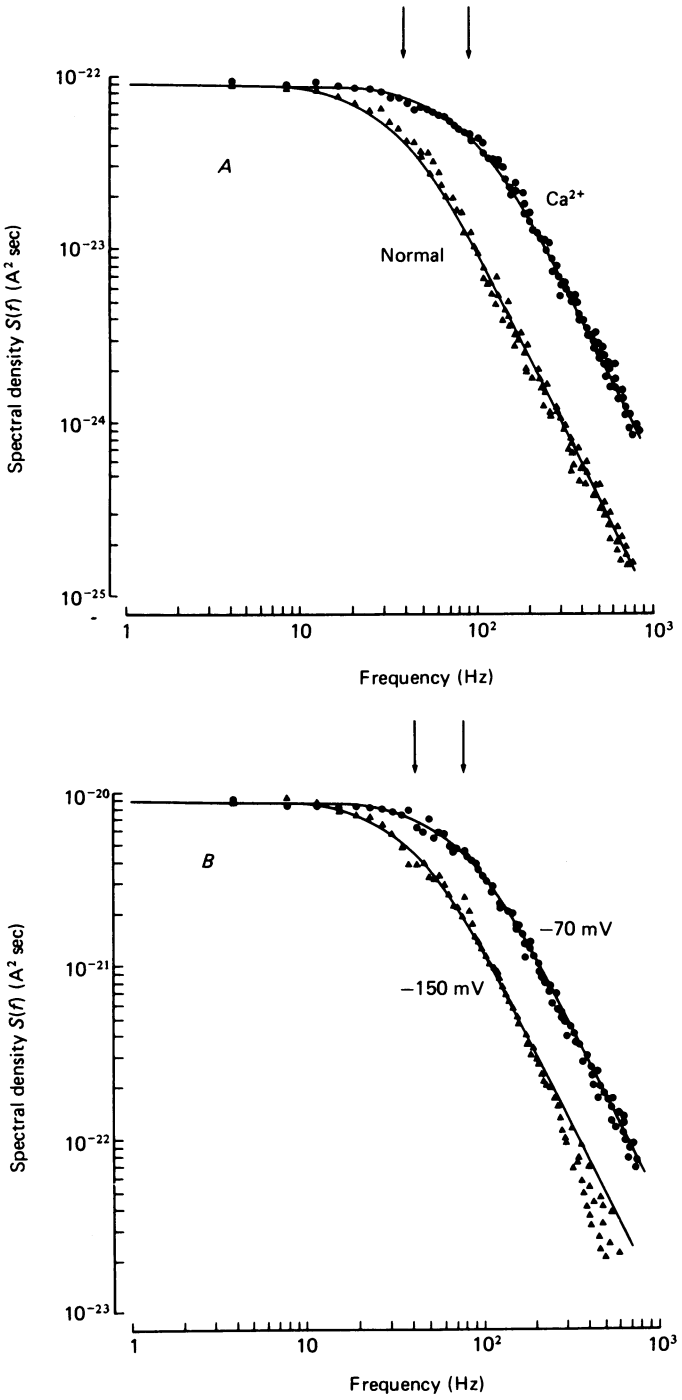


Fig. 8. Examples of miniature excitatory junctional currents from a single muscle fibre *A*, in normal medium and *B*, after soaking for several hours in isotonic Ca^{2+}, at a clamp potential $V_m = -110$ mV and $T = 22$ °C. Each trace shows several superimposed current sweeps. In normal medium (*A*) m.e.j.c.s are well above the noise level, apart from a small m.e.j.c. present on the fourth trace down. In isotonic Ca^{2+} (*B*) m.e.j.c.s are reduced to approximately one-third normal size with some events disappearing into the background noise. The decay time of m.e.j.c.s is markedly briefer in isotonic Ca^{2+}. Note the increased frequency of m.e.j.c.s and their apparent greater variation in size in isotonic Ca^{2+}. *C*, *D*, amplitude distributions of m.e.j.c.s in voltage-clamped muscle fibres (different experiment from *B*) bathed in isotonic Ca^{2+}. *C*, mean m.e.j.c. = 1·33 nA at a clamp holding potential $V_m = -110$ mV. *D*, mean m.e.j.c. = 0·85 nA at $V_m = -110$ mV. M.e.j.c.s in *D* show a skew distribution. Background noise masks events less than approximately 0·2 nA, therefore the smallest m.e.j.c.s are not seen in these muscle fibres. $T = 22$ °C.


A
Ca2+
Normal
Spectral density S(f) (A2 sec)
10-22
10-23
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1
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102
103
Frequency (Hz)
B
−70 mV
−150 mV
Spectral density S(f) (A2 sec)
10-20
10-21
10-22
10-23
1
10
102
103
Frequency (Hz)

Fig. 9.

Amplitude of m.e.j.c.s in isotonic Ca^{2+}

Fig. 8*A* and *B* shows examples of m.e.j.c.s recorded from the same muscle fibre (at a clamp potential of −110 mV) in normal medium and after 3 hr exposure to isotonic Ca^{2+}. In high Ca^{2+} the m.e.j.c. amplitude is reduced to about one-third of the normal size with many smaller currents being barely visible above the noise level. Furthermore, the m.e.j.c. decay time becomes markedly shorter as can be seen in Fig. 8*B*.

Amplitude histograms of m.e.j.c.s, obtained at a holding potential of −110 mV from fibres soaked for several hours in isotonic Ca^{2+}, are shown in Fig. 8*C* and *D* (different experiment from that shown in Fig. 8*A* and *B*). The histograms are often slightly skew indicating that smaller events are being lost in the background noise. The size of the currents in isotonic Ca^{2+} is therefore over-estimated. At −80 mV the mean m.e.j.c. amplitude = 0·74 ± 0·03 nA (for eleven fibres) at $T = 22$ °C.

Time course of m.e.j.c.s in isotonic Ca^{2+}

Fig. 9*A* shows examples of power density spectra of m.e.j.c.s obtained from a muscle fibre in normal bathing medium and in isotonic Ca^{2+}, at $V_m = -100$ mV. These spectra can be fitted by a single Lorentzian component, $S(f) = S(0)/(1+(f/f_c)^2)$. However, in many experiments the high frequency components of the spectra roll-off faster than predicted by the $1/f^2$ curve (see Fig. 9*A*), as expected if the rise-time of the m.e.j.c. is not instantaneous. The spectrum gives an estimate of $\tau_{m.e.j.c.}$ according to $\tau_{m.e.j.c.} = 1/(2\pi f_c)$, where f_c is the frequency at which the power is reduced to half the plateau level (Katz & Miledi, 1972).

The m.e.j.c. spectra obtained in high Ca^{2+} are similar to those obtained in normal medium, but are shifted towards higher frequencies indicating that the m.e.j.c. decay-time is briefer in isotonic Ca^{2+}. For the examples shown in Fig. 9*A* the time constant shifts from 3·98 msec in normal medium to 1·8 msec in isotonic Ca^{2+}. Typically, $\tau_{m.e.j.c.}$ at −100 mV is reduced to about half the normal duration when

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Fig. 9. *A*, spectral density of m.e.j.c.s recorded in normal medium (▲), and in isotonic Ca^{2+} (●). Both spectra are obtained from the same cell clamped at $V_m = -100$ mV, $T = 20$ °C. Spectrum in normal medium (▲) is the average of sixty-five spectra (> one m.e.j.c./spectrum), cut-off frequency indicated by arrow is, $f_c = 40$ Hz, hence $\tau_{m.e.j.c.} = 3{\cdot}98$ msec, $S(O) = 1{\cdot}8 \times 10^{-21}$ A² sec. Spectrum in isotonic Ca^{2+} (●) is the average of eighty-eight spectra (> one m.e.j.c./spectrum), cut-off frequency indicated by arrow is, $f_c = 88$ Hz, hence $\tau_{m.e.j.c.} = 1{\cdot}81$ msec, $S(O) = 9 \times 10^{-23}$ A² sec. Vertical axis applies to spectrum in isotonic Ca^{2+}. The spectrum in normal medium has been shifted along the vertical axis to allow comparison between curves. Multiplication of ordinate values by twenty gives spectral density values for the normal spectrum. *B*, effect of clamp potential on spectral density of m.e.j.c.s in isotonic Ca^{2+}. Both spectra are obtained from the same muscle fibre at clamp membrane potential $V_m = -70$ mV (●) and $V_m = -150$ mV (▲), $T = 20$ °C. Spectrum at −70 mV (●) is the average of 100 spectra (> one m.e.j.c./spectrum), cut-off frequency indicated by arrow is $f_c = 79$ Hz, hence $\tau_{m.e.j.c.} = 2{\cdot}01$ msec, $S(O) = 1{\cdot}9 \times 10^{-22}$ A² sec. Spectrum at −150 mV (▲) is the average of seventy-seven spectra (> one m.e.j.c./spectrum), cut-off frequency indicated by arrow is $f_c = 40$ Hz, hence $\tau_{m.e.j.c.} = 3{\cdot}98$ msec, $S(O) = 9 \times 10^{-21}$ A² sec. Vertical axis applies to the −150 mV curve, multiplication of the ordinate values by 0·021 gives spectral density values for −70 mV curve.

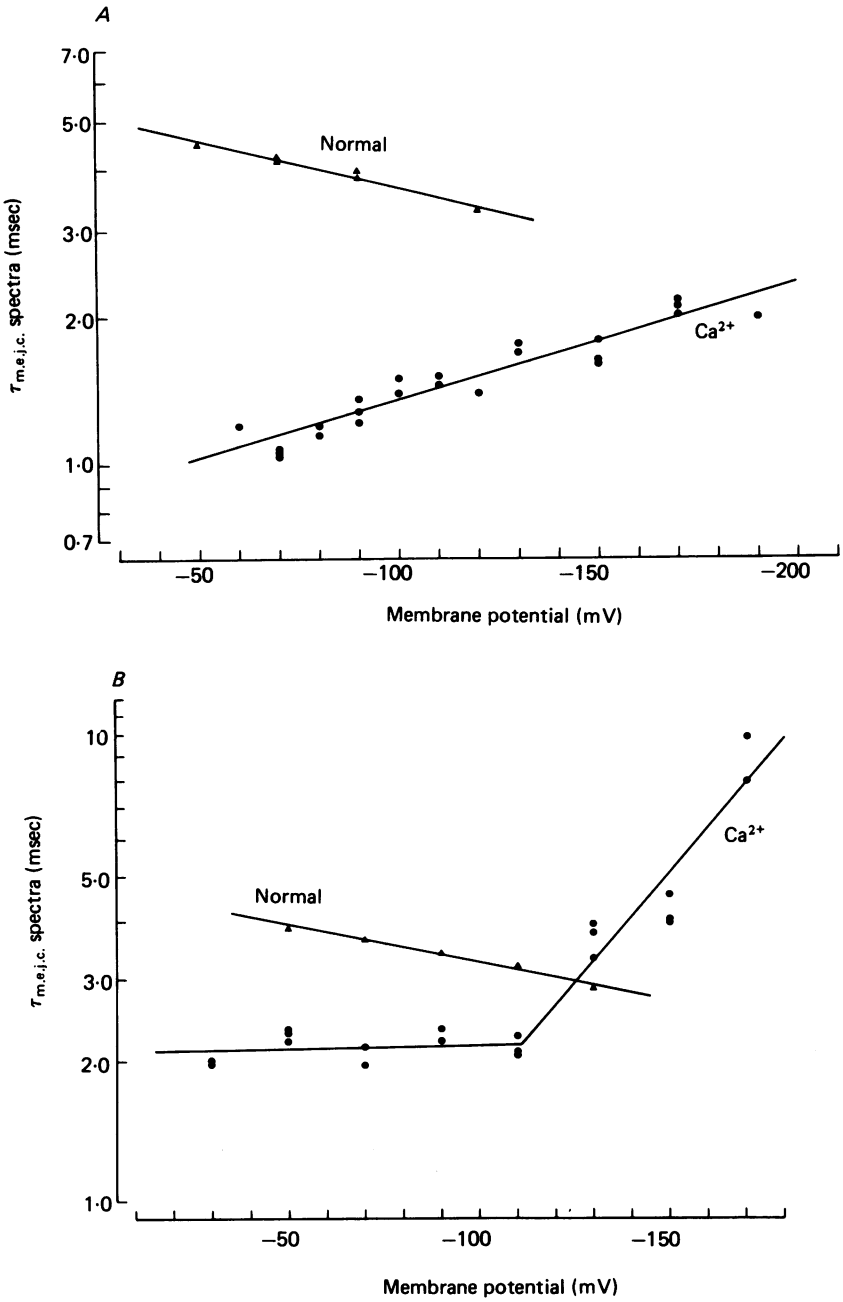

Fig. 10. Dependence of the time constant of m.e.j.c. spectra on clamped membrane potential. Straight lines drawn through the points are fitted by the least squares method (on log–linear co-ordinates) and drawn according to the equation $\tau(V) = \tau(0) \exp(V/H)$. *A*, plots for two cells (in the same muscle) in normal medium (▲), and after soaking the muscle for several hours in isotonic Ca^{2+} (●). When the fibre is transferred to isotonic Ca^{2+} m.e.j.c. spectra become briefer, the effect being more noticeable at depolarized levels; in addition m.e.j.c. spectra are now prolonged by membrane hyperpolarization. Slope

the main permeant cation changes from Na^+ to Ca^{2+}. In isotonic Ca^{2+} medium $\tau_{m.e.j.c.} = 1{\cdot}58 \pm 0{\cdot}08$ msec (for ten fibres) $V_m = -80$ mV. M.e.j.c.s. of abnormal time course are present in isotonic Ca^{2+}. If such events were excluded from the analysis then $\tau_{m.e.j.c.}$ $(-80$ mV$) = 1{\cdot}01 \pm 0{\cdot}08$ msec (for fourteen fibres) at $T = 22$ °C.

Membrane potential dependence of $\tau_{m.e.j.c.}$ in isotonic calcium

Power spectra of m.e.j.c.s obtained at two potentials in a fibre soaked in isotonic Ca^{2+} are illustrated in Fig. 9*B*. Hyperpolarization of the membrane causes the spectrum to shift to lower frequencies, indicating $\tau_{m.e.j.c.}$ is sensitive to membrane potential in isotonic Ca^{2+}. However, $\tau_{m.e.j.c.}$ was prolonged rather than shortened by hyperpolarization suggesting an increase in the life-time of the transmitter-activated channel.

The phenomenon has been investigated over a range of membrane potentials. In some fibres the voltage sensitivity of $\tau_{m.e.j.c.}$ obtained from the half-power frequency of the m.e.j.c. spectra was studied in normal medium as well as an isotonic Ca^{2+} (see Fig. 10). The semi-logarithmic plot of $\tau_{m.e.j.c.}$ as a function of clamp potential demonstrates that $\tau_{m.e.j.c.}$ both in normal medium and in isotonic Ca^{2+} appears to depend on clamp potential. However, in Ca^{2+} the voltage sensitivity of $\tau_{m.e.j.c.}$ is the reverse of that seen in normal medium. In Fig. 10*A* an *e*-fold change in $\tau_{m.e.j.c.}$ is produced by a 225 mV change in membrane potential in normal medium and a 216 mV change in the presence of isotonic Ca^{2+}. The mean change in membrane potential required to produce an *e*-fold change in $\tau_{m.e.j.c.}$ in isotonic Ca^{2+} was 195 ± 90 mV (for five fibres).

In some muscle fibres the voltage sensitivity showed a sudden change in intensity at approximately -100 to -120 mV (Fig. 10*B*). The voltage sensitivity of $\tau_{m.e.j.c.}$ was usually small at depolarized levels (-40 to -110 mV). At hyperpolarized membrane potentials the voltage sensitivity showed a marked increase becoming larger than in fibres where the voltage dependence followed a single exponential.

These results were obtained by spectral analysis of sectors of records containing m.e.j.c.s and the possibility remained that changing the membrane potential altered the population of m.e.j.c.s being examined, since we noted that hyperpolarization increased m.e.j.c. frequency (see below). Therefore, to examine further the voltage dependence of $\tau_{m.e.j.c.}$ in isotonic Ca^{2+} the time constant of decay of individual m.e.j.c.s was measured. Fig. 11*A* and *B* shows the distributions of $\tau_{m.e.j.c.}$ values obtained from a fibre clamped at -80 mV and at -150 mV. The histogram at -80 mV is approximately normal but with more long events than predicted for a Gaussian distribution, resembling the observations made in normal bathing medium (see Fig. 3*A* and *B*). In this fibre the main peak of the histogram, which occurs at

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values for *H* (normal) = 225 mV, *H* (Ca^{2+}) = 216 mV. *B*, plots for two cells in the same muscle (different muscle from that shown in *A*) in normal medium (▲), and after several hours in isotonic Ca (●). In this fibre m.e.j.c. spectra shows only slight voltage dependence at depolarized potentials (more positive than -100 mV) with a sharp increase in slope at hyperpolarized potentials (more negative than -110 mV). Over both portions of the curve voltage dependence is the reverse of that in normal medium. Slope values of *H*(normal) = 262·2 mV, *H*(Ca^{2+}, < -110 mV) = 2700 mV, *H*(Ca^{2+}, > -110 mV) = 45·4 mV; $T = 20$ °C.

about 1·2 msec, shifts to slightly longer values at −150 mV (Fig. 11*B*). If all measurements of $\tau_{m.e.j.c.}$ in this cell are included in the mean then $\tau_{m.e.j.c.}$ (−80 mV) = 1·32±0·79 msec (±S.D.) and $\tau_{m.e.j.c.}$ (−150 mV) = 1·95±1·42 msec

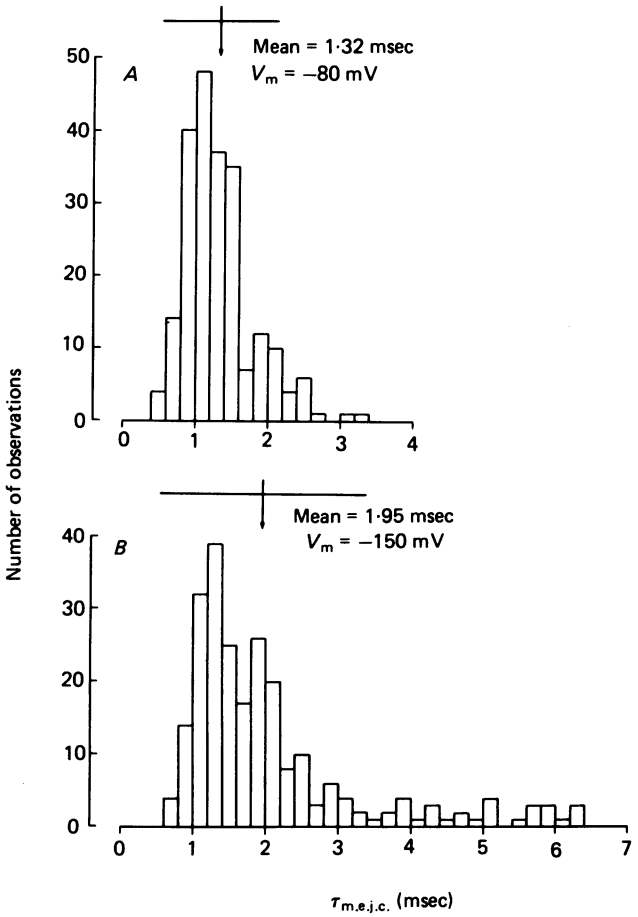

Fig. 11. Distribution of values for $\tau_{m.e.j.c.}$ in isotonic Ca^{2+} obtained at two clamp potentials from the same muscle fibre; *A*, V_m = −80 mV; *B*, V_m = −150 mV. Arrows above the histograms indicate the mean (and S.D.) of $\tau_{m.e.j.c.}$. At −80 mV (*A*) the distribution of $\tau_{m.e.j.c.}$ is approximately normal except for a small population of events with prolonged time course. At −150 mV (*B*) the main peak of the histogram is shifted slightly to longer values. The second peak (at about 2 msec), which corresponds to the population of m.e.j.c.s with prolonged time courses, becomes more pronounced at hyperpolarized potentials. Events with time constants between 3 and 6 msec are also apparent at −150 mV. *A*, mean $\tau_{m.e.j.c.}$ (−80 mV) = 1·32±0·79 msec (±S.D), n = 223 m.e.j.c.s. *B*, mean $\tau_{m.e.j.c.}$ (−150 mV) = 1·95±1·42 msec, n = 240 m.e.j.c.s T = 21 °C.

(±S.D.). However, it is clear from the histogram that the mean increase in $\tau_{m.e.j.c.}$ at −150 mV results in part from an increase in the population of events falling outside the normal distribution. If the population of slow time course m.e.j.c.s is excluded, the mean value for the normally distributed events increases

from $\tau_{m.e.j.c.}$ (-80 mV) = $1{\cdot}1 \pm 0{\cdot}03$ msec ($\pm$S.E. of the mean) to $\tau_{m.e.j.c.}$ (-150 mV) = $1{\cdot}45 \pm 0{\cdot}04$ msec ($\pm$S.E. of the mean). However, in a majority of fibres the main peak of the histogram was not markedly potential sensitive.

An example of the increase in the population of prolonged m.e.j.c.s which occurs with hyperpolarization can be seen in Fig. 12 which illustrates m.e.j.c.s recorded from the same fibre at -110 and -150 mV. For most fibres examined in isotonic Ca^{2+}

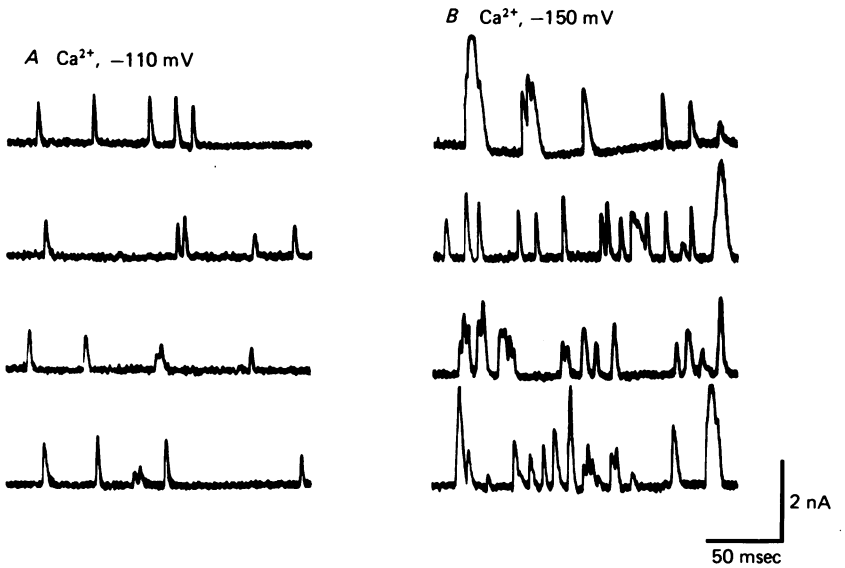

Fig. 12. Miniature excitatory junctional currents recorded at two clamp potentials from a muscle fibre bathed in isotonic Ca^{2+}. *A*, m.e.j.c.s at a clamp potential $V_m = -110$ mV. *B*, m.e.j.c.s at a clamp potential $V_m = -150$ mV. The frequency as well as the mean amplitude of events increases with membrane hyperpolarization. The occurrence of large composite m.e.j.c.s, of variable time course, also increases. These composite events presumably result from simultaneous release of several transmitter packets. $T = 22$ °C.

membrane hyperpolarization markedly increased both m.e.j.c. frequency and the occurrence of long events. The large prolonged events appear to be composite m.e.j.c.s resulting from the simultaneous action of a number of transmitter packets on the post-synaptic membrane. Because of the wide variability in m.e.j.c. frequency observed in isotonic Ca^{2+} we have not attempted to quantify the effect of the clamp potential on spontaneous transmitter release although it was found to be readily reproducible in many fibres. Furthermore, it was noted that the increase in spontaneous transmitter release only became noticeable at potentials more negative than -110 to -120 mV and the extent of the effect varied in different fibres.

The influence of membrane hyperpolarization on spontaneous transmitter release can at least partly account for the prolongation of the time constant underlying the m.e.j.c. spectra, which occurs in Fig. 10*A* and *B*. Furthermore, it can also explain the sharp change in potential sensitivity of $\tau_{m.e.j.c.}$ obtained from spectra, which occurs at potentials more negative than -110 mV in some fibres (Fig. 10*B*). There is evidence that a similar phenomenon occurs to a lesser extent in normal bathing medium. In these conditions fibres are not readily held at potentials more negative

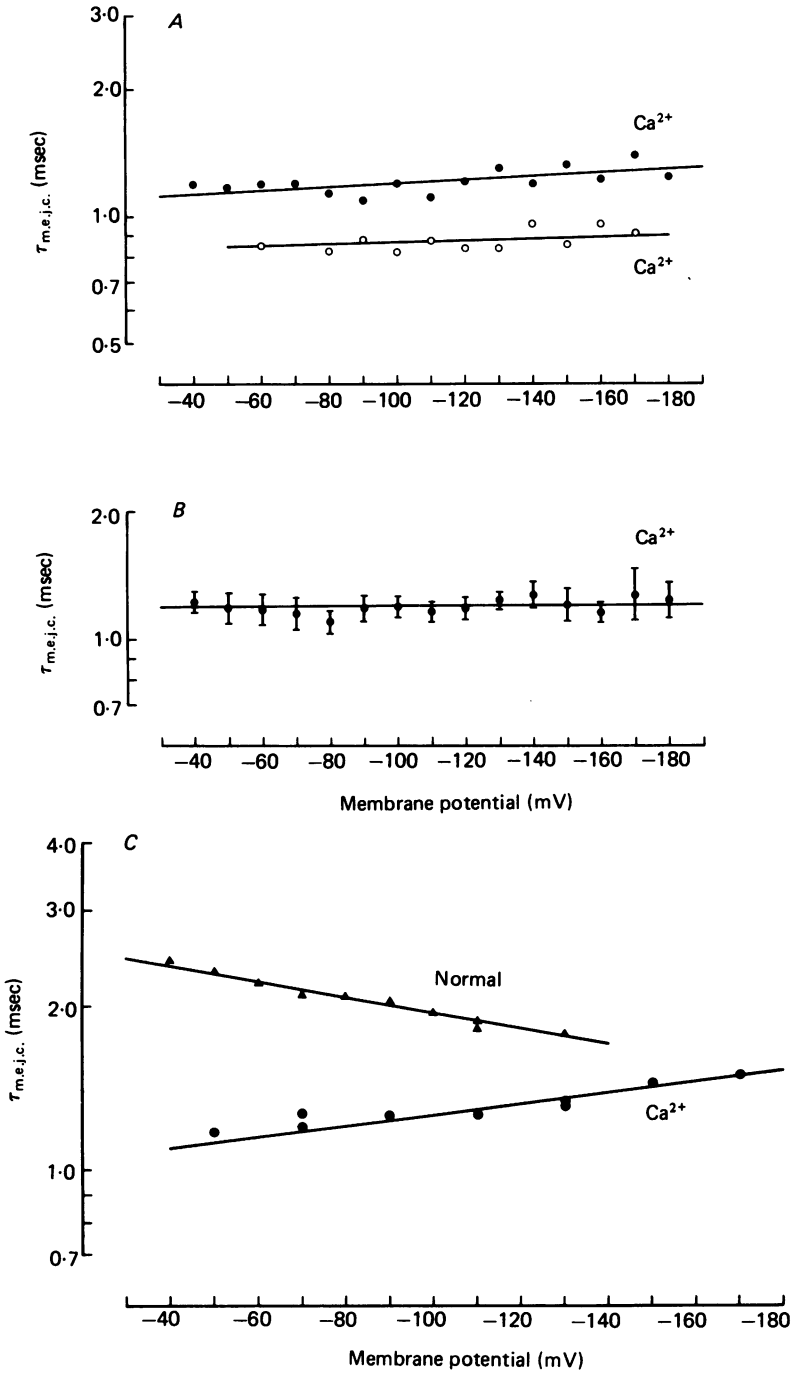
A
3·0
2·0
1·0
0·7
0·5
$\tau_{m.e.j.c.}$ (msec)
Ca^{2+}
Ca^{2+}
−40 −60 −80 −100 −120 −140 −160 −180
B
2·0
1·0
0·7
$\tau_{m.e.j.c.}$ (msec)
Ca^{2+}
−40 −60 −80 −100 −120 −140 −160 −180
Membrane potential (mV)
C
4·0
3·0
2·0
1·0
0·7
$\tau_{m.e.j.c.}$ (msec)
Normal
Ca^{2+}
−40 −60 −80 −100 −120 −140 −160 −180
Membrane potential (mV)

Fig. 13.

than −110 to −120 mV due to electrical break-down of the fibre membrane. It was, however, possible to step the potential briefly to −160 or −170 mV for durations of about 500 msec by applying brief command pulses. This appeared to increase the occurrence of abnormal and composite m.e.j.c.s.

Since the slow and composite m.e.j.c.s are not expected to reflect the mean life-time of the post-synaptic channel these were excluded from analysis where the dependence of $\tau_{m.e.j.c.}$ on clamp potential was studied in isotonic Ca^{2+} (Fig. 13). The plots in Fig. 13*A*, which represent data obtained from two muscles, show a slight prolongation of $\tau_{m.e.j.c.}$ with hyperpolarization. Averaged data obtained from fifteen fibres in six muscles are represented by the plot in Fig. 13*B*. The mean values of $\tau_{m.e.j.c.}$ obtained in these fibres shows no significant potential sensitivity between −40 and −180 mV in isotonic Ca^{2+}. On the other hand Fig. 13*C* shows an example of $\tau_{m.e.j.c.}$ *vs.* clamp potential obtained from a fibre which exhibited marked potential sensitivity in isotonic Ca^{2+}. In this fibre $\tau_{m.e.j.c.}$ was significantly prolonged by hyperpolarization even though $\tau_{m.e.j.c.}$ was derived from measurements of individual m.e.j.c.s.

Membrane potential dependence of m.e.j.c. amplitude in isotonic Ca^{2+}

The dependence of m.e.j.c. amplitude on clamp potential in isotonic Ca^{2+} is shown in Fig. 14. Because m.e.j.c.s are already markedly reduced in amplitude in isotonic Ca^{2+}, and since they are difficult to reverse, accurate measurement becomes increasingly difficult as the equilibrium potential for transmitter action is approached. It has been necessary to estimate the null point for transmitter action in isotonic Ca^{2+} by a long extrapolation. With this limitation in mind the extrapolated equilibrium potential in high Ca^{2+} appears to be shifted to more positive values than normal (Fig. 14*A* and *B*). The mean value for the equilibrium potential in isotonic Ca^{2+} was $V_{eq} = +30 \pm 4{\cdot}6$ mV (for five fibres).

In some fibres a more complex relation between m.e.j.c. amplitude and clamp potential was encountered. Fig. 14*B* illustrates an experiment in which m.e.j.c. amplitude is non-linearly dependent on membrane potential. In this example the mean conductance change produced by a transmitter packet increased as the cell was hyperpolarized. This contrasts with the behaviour in normal medium where the conductance usually decreased with hyperpolarization (as described above). However, when the cell was depolarized the relationship was approximately linear.

Fig. 13. Dependence of the time constant of decay, $\tau_{m.e.j.c.}$, on clamped membrane potential in the presence of isotonic Ca^{2+}. *A*, plots obtained from two muscles in isotonic Ca^{2+}. ●, pooled data from six cells. Estimates from a given cell contributing to a point are from more than fifty m.e.j.c.s. Slope value for $H = 1008$ mV. ○, pooled data from seven cells. Slope value for $H = 1925$ mV. Straight lines through the points are fitted by the least squares method. Note that $\tau_{m.e.j.c.}$ shows weak voltage sensitivity (compare with Fig. 10). *B*, plot of averaged data from six muscles in isotonic calcium. Each point is the mean value (±S.E. of the mean) from twelve to fifteen muscle fibres (more than fifty events in each cell at each potential). The line fitted to the points shows no voltage sensitivity of $\tau_{m.e.j.c.}$. *C*, plots from two cells (in the same muscle) in normal medium (▲) and after soaking the muscle in isotonic Ca^{2+} (●). When the cell is transferred to isotonic Ca^{2+} $\tau_{m.e.j.c.}$ is reduced at depolarized potentials. In this example $\tau_{m.e.j.c.}$ is prolonged by hyperpolarization in isotonic Ca^{2+}. Slope values for H (normal) = 304·8 mV; H (Ca^{2+}) = 440·9 mV. Data have been selected to exclude slow time course events from the final values $T = 21$ °C.

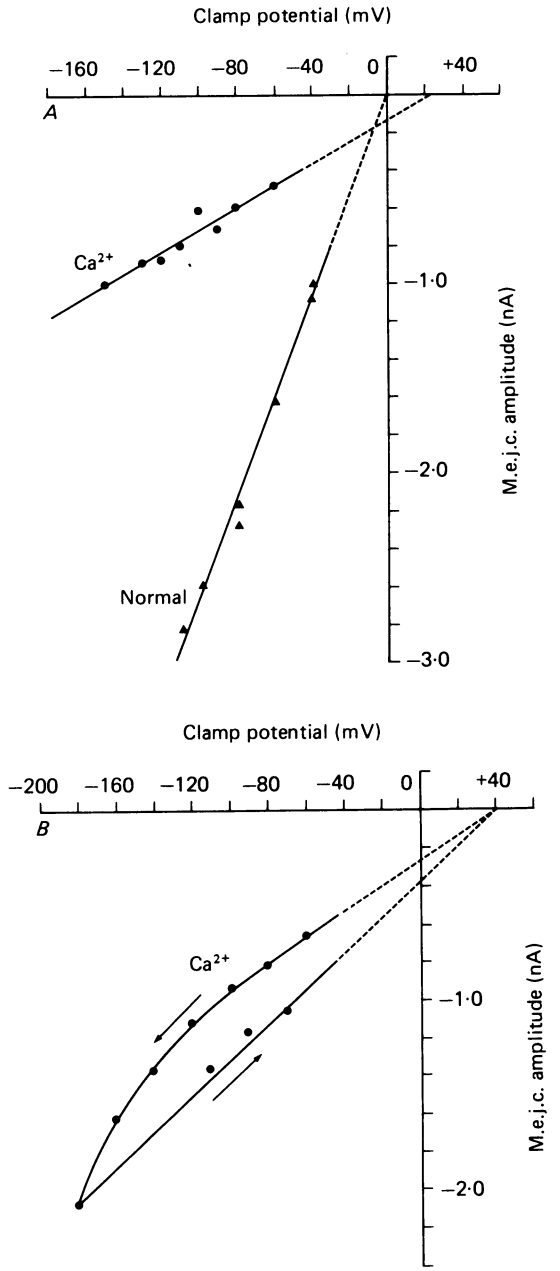

Fig. 14. Relationship between m.e.j.c. amplitude and clamp holding potential; *A*, in a single muscle fibre examined in normal medium (▲), and after soaking for several hours in isotonic Ca^{2+}(●). The extrapolated equilibrium potential in normal medium (▲) is close to 0 mV; extrapolated equilibrium potential in isotonic Ca^{2+} is +23 mV. *B*, a single muscle fibre in isotonic Ca^{2+} (different muscle fibre from that used in *A*). The fibre was first hyperpolarized in 20 mV steps from −60 to −180 mV during which m.e.j.c. amplitude shows a non-linear dependence on clamp potential. The fibre was then depolarized in steps to −70 mV during which m.e.j.c. amplitude is linearly dependent on clamp potential. The extrapolated equilibrium potential for transmitter action is approximately +42 mV.

DISCUSSION

Normal m.e.j.c.s

Since the time course and amplitude of m.e.j.c.s is similar in normal medium and in Ca^{2+}-free and Cl^--free media it appears that these changes in the ionic composition of the external medium do not alter the characteristics of the junctional channels. Furthermore, the effective binding of transmitter to receptors is presumably unchanged. This is of interest as glutamate binding to hippocampal membranes of the C.N.S. is markedly affected by Ca^{2+} levels and EGTA (Baudry & Lynch, 1979).

$\tau_{m.e.j.c.}$ is expected to give an accurate estimate of the life-span of the transmitter-induced channel only if the transmitter concentration during the miniature current subsides rapidly, compared with the life-time of the channel (Katz & Miledi, 1973*a*; Anderson & Stevens, 1973). At various synapses $\tau_{m.e.p.c.}$ often slightly exceeds the mean channel life-time (obtained from noise analysis) because of the repetitive binding of some transmitter molecules to post-synaptic receptors (see Katz & Miledi, 1973*a*; Colquhoun, Large & Rang, 1977; Gage & Van Helden, 1979; Cull-Candy *et al.* 1979). We find that at glutamate receptors in locust muscle $\tau_{m.e.j.c.}$ is generally longer than $\tau_{e.j.c.}$ when compared in the same fibre and also exceeds the mean value previously obtained for glutamate-operated channels in these cells (Anderson *et al.* 1978; Cull-Candy, Miledi & Parker, 1981). This results from the presence of a population of m.e.j.c.s of slightly prolonged time course, which are apparently not evoked by nerve impulses or are released only with very low probability. Assuming that these m.e.j.c.s do originate from nerve terminals their slow time course may be caused by abnormal release of some transmitter packets or by delayed diffusion of transmitter due to repetitive binding or by transmitter-activating receptors with slower channel kinetics. Why this should be more marked for certain spontaneous than for evoked transmitter packets is unknown. However, if the small percentage of slow time course m.e.j.c.s are omitted from the analysis then the time constants underlying m.e.j.c., e.j.c. and glutamate noise all approach 2·5 msec at $V_m = -80$ mV and $T = 22$ °C. In view of the finding that different agonists produce channels with different life-times at acetylcholine (ACh) and glutamate receptors (Katz & Miledi, 1973*b*; Colquhoun, Dionne, Steinbach & Stevens, 1975; Anderson *et al.* 1976; Crawford & McBurney, 1976*a*), agreement between the time constants underlying glutamate noise, m.e.j.c.s and e.j.c.s is consistent with the idea of glutamate being the natural transmitter (Usherwood & Machili, 1968; Beránek & Miller, 1968). Furthermore, the life-time of transmitter-induced channels has a voltage dependence which is of similar magnitude to glutamate-operated channels at these junctions.

M.e.j.c.s in isotonic Ca^{2+}

It has previously been shown that the inward synaptic current produced by acetylcholine can be entirely carried by Ca^{2+} at vertebrate end-plates (Katz & Miledi, 1969*a*) and in *Aplysia* neurones (Ascher, Marty & Neild, 1978; Marchais & Marty, 1979). Furthermore, a Ca^{2+} influx can occur at glutamate-operated channels at invertebrate nerve–muscle junctions (Onodera & Takeuchi, 1976; Dudel, 1974; Barker, 1975; Anwyl 1977; Cull-Candy & Miledi, 1980*a*) and at the squid giant synapse (Katz & Miledi, 1969*b*; Kusano, Miledi & Stinnakre, 1975). Our experiments

indicate that the synaptic currents at glutamate-sensitive receptors in locust can be entirely carried by Ca^{2+}, and that in these conditions the kinetics of the junctional channels are markedly altered.

The reduced amplitude of the mean m.e.j.c. may well reflect a decrease in the single channel conductance to about one-third normal size. However, we cannot exclude that some reduction in m.e.j.c. size may be due either to a changed receptor affinity (see Thieffry *et al.* 1980) or to a reduced efficacy of transmitter caused by the weak interaction known to occur between glutamate and Ca^{2+} (Curtis, Perrin & Watkins, 1960). Other possible effects of high Ca^{2+} such as impairment of the glutamate uptake mechanisms or a shift in the equilibrium potential for transmitter action would, if anything, tend to increase the m.e.j.c. amplitude.

At -60 mV clamp potential $\tau_{m.e.j.c.}$ in Ca^{2+} is about one-third of that in normal medium. Thus the duration of the channel life-time is markedly briefer in isotonic Ca^{2+} as described for ACh receptors in frog muscle (Bregestovski, Miledi & Parker, 1979). If Na-dependent glutamate uptake is important in the removal of transmitter from the synaptic cleft then in the presence of isotonic Ca^{2+}, $\tau_{m.e.j.c.}$ might in fact exceed the channel life-time due to delayed clearance of transmitter (Katz & Miledi, 1973*a*). It will be necessary to compare τ_{noise}, obtained in isotonic Ca^{2+} with $\tau_{m.e.j.c.}$ to decide this point.

Interpretation of channel kinetics in isotonic Ca^{2+}

When the main inward current is Ca^{2+} rather than Na^+ then the mean life-time of the channel is shortened as previously demonstrated for synaptic channels at vertebrate end-plates (Bregestovski *et al.* 1979; Lewis & Stevens, 1979). $\tau_{m.e.j.c.}$ obtained from spectral analysis in our experiments is prolonged by hyperpolarization as previously reported (Cull-Candy & Miledi, 1980*a*). However, caution is necessary in the interpretation of this observation since membrane hyperpolarization also increases the percentage of abnormally prolonged m.e.j.c.s. This results, at least partly, from an influence of the post-synaptic potential on presynaptic transmitter release. In such circumstance the mean estimate of $\tau_{m.e.j.c.}$ obtained from spectral analysis would not be expected to reflect channel properties since transmitter concentration in the cleft is not subsiding quickly (Katz & Miledi, 1973*a*). It was therefore necessary to analyse the decay of individual m.e.j.c.s to exclude the population of abnormal events which become increasingly prominent at hyperpolarized potentials. A reversed voltage sensitivity was still seen in isotonic Ca^{2+}, although weak or even absent in some fibres.

It is possible that Ca^{2+} passes through a separate channel which differs in kinetic properties from the other synaptic channels. If, as seems more likely, Na^+ and Ca^{2+} are passing through the same channels, then the kinetics of the channels and their voltage dependence appear to be influenced by the species of permeable cation as previously suggested for ACh-induced channels (Gage & Van Helden, 1979; Bregestovski *et al.* 1979; Lewis, 1979; Magleby & Weinstock, 1980).

A wide variety of schemes could explain a change in kinetic properties. It is possible that the effects of Ca^{2+} on the properties of transmitter-operated channels are caused by direct effects of Ca^{2+} on the receptor channel protein or on the phospholipid associated with the receptor channel. Ca^{2+} ions may also affect the interaction of

glutamate with receptor perhaps by affecting the charge distribution on transmitter or receptor. In this respect it is of interest that glutamate forms a weak bond with Ca^{2+} by acting as a Ca^{2+} chelator (see Curtis *et al.* 1960). Channel properties could also be altered by a direct effect of permeating Ca^{2+} ions on the channel. For example, Ca^{2+} ions may partially block channels by binding to a site which is either part of the pathway of permeating ions in the channel, or separate from this pathway (i.e. a 'regulatory' site). If Ca^{2+} binds to a regulatory site which is near the membrane–solution interface then the binding site could be relatively unaffected by applied voltage (see Bamberg & Läuger, 1979). Alternatively, the ion could bind specifically to a site located in the channel, before passing into the intracellular medium. If the open channel is blocked by this ion it would account for the reduction in $\tau_{m.e.j.c.}$. However, to account for the slight increase in channel life-time with hyperpolarization seen in some fibres it is necessary to assume that channel block is reduced by hyperpolarization.

Number of channels opened by a transmitter packet

From our estimates of the single channel current of about 8·5–12·5 pA (Anderson *et al.* 1978) for glutamate-operated junctional channels, and for a mean m.e.j.c. amplitude of 2·34 nA at −80 mV, we estimate that a single packet of transmitter opens about 190–280 ion channels. This is considerably less than the estimates of 1000–2000 channels opened by a packet of ACh at vertebrate end-plates (Katz & Miledi, 1972; Anderson & Stevens, 1973) and it could suggest that the size of the transmitter packet is small at locust junctions. On the other hand, recent experiments have indicated that extrajunctional glutamate receptors have a relatively low affinity compared with extrajunctional ACh-receptors in vertebrate muscle fibres (Cull-Candy *et al.* 1981) which may mean that only a small fraction of the transmitter packet is expected to bind or activate successfully the junctional glutamate receptors.

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