

TEMPERATURE AND SYNAPTIC EFFICACY IN FROG SKELETAL MUSCLE

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SUMMARY

1. Intracellular recording and voltage-clamp techniques were used to measure synaptic efficacy and the safety factor for neuromuscular transmission in frog skeletal muscle. All measurements were made in normal Ringer solution, in the absence of presynaptic or postsynaptic blocking agents.

2. Over a broad temperature range (10–30 °C), a small percentage of sartorius fibres (about 6%) could be found which produced only subthreshold end-plate potentials and no action potential in response to single, supramaximal nerve shock. At lower temperatures the proportion of such fibres increased; 42% of the fibres had subthreshold transmission at 5 °C, and 59% were subthreshold at 2.5 °C.

3. Threshold current, measured by intracellularly injecting short pulses of depolarizing current at end-plate regions, was independent of temperature between 2.5 and 20 °C. Thus, the reduced synaptic efficacy observed at low temperatures was not due to decreased electrical excitability of the postsynaptic membrane.

4. The amplitude of evoked end-plate currents (EPCs) decreased with cooling. At temperatures below 10 °C, the evoked EPCs at many end-plates were too small to initiate action potentials. The decline in EPC amplitude was due to three factors: a decrease in the amplitude of single quantum currents (MEPCs), an increase in the temporal dispersion of transmitter release, and (below 5 °C) a decrease in quantal content.

5. The safety factor for neuromuscular transmission decreased dramatically as temperature was lowered. At 30 °C average safety factor was large and positive (540 nA), but at 2.5 °C it was negative (–78 nA).

6. The quantal content of evoked transmitter release was independent of temperature change between 5 and 30 °C, the average value over this range being 180. However, at temperatures below 5 °C, quantal content fell off sharply (average value = 37).

7. The thermal independence of transmitter release may be an important mechanism in allowing poikilothermic animals to maintain physiological function over a wide range of body temperatures.

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INTRODUCTION

Poikilothermic animals are often capable of locomotion over a wide range of body temperatures. For example, the frog *Xenopus laevis* can swim strongly at both 10 and 30 °C (Miller, 1982). This remarkable ability depends upon the maintenance, at both temperatures, of effective neuromuscular transmission. It is interesting to note that most previous studies have shown transmitter release at the neuromuscular junction to be strongly temperature dependent (Q_{10} of 3–6; Steinbach & Stevens, 1979). Thus, the question arises of how synaptic efficacy is maintained in skeletal muscle during acute changes in body temperature.

The aim of the present study was to measure synaptic efficacy and the safety factor for neuromuscular transmission in frog skeletal muscle at different temperatures. Synaptic efficacy was measured by determining the proportion of subthreshold fibres (i.e. those which do not generate an action potential in response to single, supramaximal nerve stimuli). Safety factor was estimated by comparing the amplitude of end-plate current, evoked in normal Ringer solution, with the threshold current required to electrically excite muscle fibres. It was found that both synaptic efficacy and safety factor decrease when temperature is lowered. These changes are due to reduced amplitude of the evoked end-plate current, which in turn is caused by reduced amplitude of single quantum currents and reduced synchrony of transmitter release. Surprisingly, the quantal content of transmitter release did not change over a wide temperature range (5–30 °C). Changes in quantal content were only important in determining the amplitude of the evoked end-plate current at very low temperatures (2.5–5 °C).

METHODS

Xenopus laevis of both sexes ($n = 39$; mean mass \pm s.e.m. = 9.4 ± 0.80 g; range of snout-vent lengths 4–6 cm), obtained from a healthy laboratory population, were housed in aquaria containing dechlorinated, aerated tap water at 20 °C and were fed chopped beef. The sartorius muscle–nerve preparation was used for all experiments. During experiments the preparations were bathed exclusively in normal frog Ringer solution (120 mM-NaCl; 2 mM-KCl; 1.8 mM-CaCl₂; 4 mM-HEPES, pH adjusted to 7.60 at 23 °C with NaOH); no presynaptic or postsynaptic blocking agents or anticholinesterases were used. In all experiments the muscle was pinned at its *in situ* length (measured with the knee fully extended and the leg held at a 45 deg angle to the vertebral column). Ringer solution was perfused over the preparation continuously and was removed by aspiration. Temperature was varied by heating or cooling the Ringer before it entered the experimental chamber, and was recorded with a small glass-covered thermocouple placed within several millimetres of the muscle. The order of temperature exposures was random, and the preparation was allowed to equilibrate at each temperature for at least 5 min before measurements were taken.

Intracellular recording techniques

Micropipettes having resistances of 5–10 M Ω (3 M-KCl) were used for recording intracellular potentials and for injecting current. Membrane potentials were amplified using Getting Model 5 preamplifiers set on wide bandpass (DC to 10 kHz). Reference electrodes were chlorided silver wires connected to the bath via a Ringer solution–agar bridge. The time constant of the recording system measured with the recording electrode just barely submerged in the bath solution was about 50 μ s.

The muscle was pinned with its deep surface uppermost in a Sylgard-lined chamber. The sartorius nerve was placed in a separate chamber and stimulated by a pair of silver wire electrodes. During the course of an experiment, the electrodes were moved unidirectionally from one edge of

the muscle to the other, so as to sample a given fibre only once. All measurements were made at end-plate regions, visually identified by locating terminal nerve branches, and confirmed upon impalement by the presence of miniature end-plate potentials (MEPPs) having a fast rising phase (< 1 ms at 20°C). Fibres from all parts of the deep surface of the sartorius were examined.

To measure the percentage of subthreshold fibres, a single microelectrode was inserted at end-plate regions and the sartorius nerve given a single shock. The resultant electrical events (subthreshold end-plate potential (EPP) and/or propagated action potential) were recorded. The intensity of the nerve stimulus (0.1 ms, 30 V) was 3 times that eliciting full contractile activation of the muscle (assessed visually).

To measure the electrical excitability of muscle fibres, two electrodes were inserted into an end-plate region, separated by 50–100 μm . Usually the resting potential depolarized by a few millivolts upon insertion of the second electrode; only data from fibres where initial resting potential was at least -85 mV and did not depolarize by more than 5 mV following insertion of the second electrode are reported. The membrane potential was held at the initial resting level (-90 to -100 mV) by passing steady current. Square pulses of depolarizing current (2 ms duration, 10–50 μs rise time) were injected using a current-clamping circuit. Injected currents were monitored with a virtual ground circuit. The current magnitude was gradually increased until an action potential was initiated just at the end of the 2 ms pulse. Threshold current was defined as the minimum amplitude current pulse necessary to generate an action potential. Threshold potential was taken as the point at which the membrane potential inflected and became regenerative, leading to an action potential. Muscle fibre input impedance was calculated from the change in membrane potential in response to a 2 ms, subthreshold depolarizing current pulse. The change in membrane potential used in determining input impedance was kept 10–15 mV below that eliciting an active membrane response.

End-plate currents were measured using a conventional two-electrode voltage clamp. Separation of the voltage and current electrodes was 50–100 μm . The membrane potential was clamped at -90 mV. Focal placement of the electrodes was confirmed by the fast rise time of MEPCs (about 0.4 ms at 20°C). The clamping current was monitored by a virtual ground circuit, and was low-pass, actively filtered with a time constant of 56 μs . The voltage clamp gain was initially adjusted to give a high signal-to-noise ratio for MEPCs, and to reduce the residual voltage change during MEPCs to undetectable levels (viewed at 1 mV/cm). At each end-plate, ten to twenty well-clamped MEPCs (Gage & McBurney, 1972) were measured directly from the screen of a Nicolet digital storage oscilloscope; obvious 'giant' MEPCs (Liley, 1957) were excluded. The voltage clamp gain was then increased to near the point of oscillation and the motor nerve given a single, supramaximal shock. Often an evoked EPC could be recorded with only a small (0–4 mV) change in membrane potential. Typically, the unclamped change in membrane potential during the evoked EPC was 1–10% of the difference between the equilibrium potential (taken as 0 mV; Mallart, Dreyer & Peper, 1976) and the holding potential. The degree of membrane potential 'escape' varied with electrode placement and the size of the EPC. Only EPCs in which the unclamped change in membrane potential was less than 5% of the driving potential were used in subsequent analyses. Because the muscle twitch invariably dislodged the electrodes and usually damaged the fibre, only one measurement of the EPC was obtained at any given end-plate.

At some end-plates, charge entry during MEPCs was measured using an on-line analogue integrating circuit. The integrator's signal was linearly related to MEPC charge (Q_{MEPC}). Charge entry during the EPC (Q_{EPC}) was measured by integrating the area under photographed records of complete and reconstructed EPCs. Reconstructed EPCs were those in which the latter portion of the decay phase was interrupted by a propagated action potential (initiated at a distant end-plate); for such records the late decay was reconstructed by assuming that EPCs decay according to a single exponential (Magleby & Stevens, 1972*a*; Beam, 1976) and extrapolating from the early, uninterrupted portion of the decay. Quantal content of EPCs was estimated using the ratio EPC/MEPC or the ratio $Q_{\text{EPC}}/Q_{\text{MEPC}}$ for individual end-plates in which both MEPCs and a satisfactorily clamped EPC were recorded. The two methods of estimating quantal content were applied to data collected from different end-plates.

Values in the text are reported as mean \pm s.e.m., with the number of observations in parentheses.

RESULTS

The percentage of subthreshold fibres

At temperatures between 10 and 30 °C, a few fibres (5–7%) were found that produced only end-plate potentials (EPPs) and no action potential in response to single, supramaximal nerve stimuli. In contrast, at lower temperature many more 'subthreshold' fibres were observed (Fig. 1). At 5 °C approximately 42% of the fibres failed to produce action potentials, and at 2.5 °C the proportion increased to 59%. The absence of a muscle action potential implies that all end-plates on these multiply innervated fibres (Fatt & Katz, 1951) were below threshold.

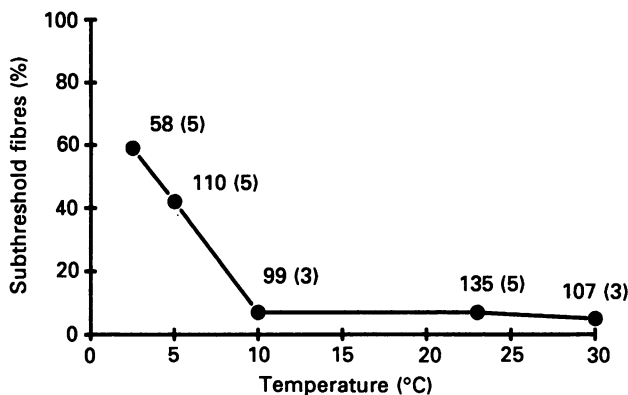


Fig. 1. The percentage of *Xenopus sartorius* fibres which produced only a subthreshold end-plate potential and no action potential in response to a single, supramaximal nerve shock. The number of fibres and the number of muscles (in parentheses) examined are shown for each temperature.

The size of EPPs recorded from these subthreshold fibres was also affected by temperature. The average height of EPPs, focally recorded from one end-plate on subthreshold fibres was 19 ± 2 mV ($n = 19$; 10, 23 and 30 °C combined). At lower temperatures (2.5 and 5 °C combined), the EPPs were smaller (9.1 ± 0.8 mV; $n = 77$) and often had distinct multiple peaks (see Fig. 3C).

It was frequently possible to record EPPs from suprathreshold fibres, and the size of these EPPs was also temperature dependent. In these instances, the recording electrode was inserted by chance at a subthreshold junction on a muscle fibre that also had at least one suprathreshold junction. The average height of these EPPs, recorded at 10, 23 and 30 °C combined was 18 ± 1 mV ($n = 77$). At 2.5 and 5 °C combined, the average height of such EPPs was only 10 ± 1 mV ($n = 54$).

The effects of temperature upon the proportion of subthreshold fibres and the size of the EPPs indicate that synaptic efficacy is constant between 10 and 30 °C, but decreases at lower temperatures. The fall in synaptic efficacy at low temperatures could be due to (1) reduced electrical excitability of the postsynaptic membrane, and/or (2) reduced amplitude of the evoked end-plate current. These two possibilities were further investigated in the experiments presented below.

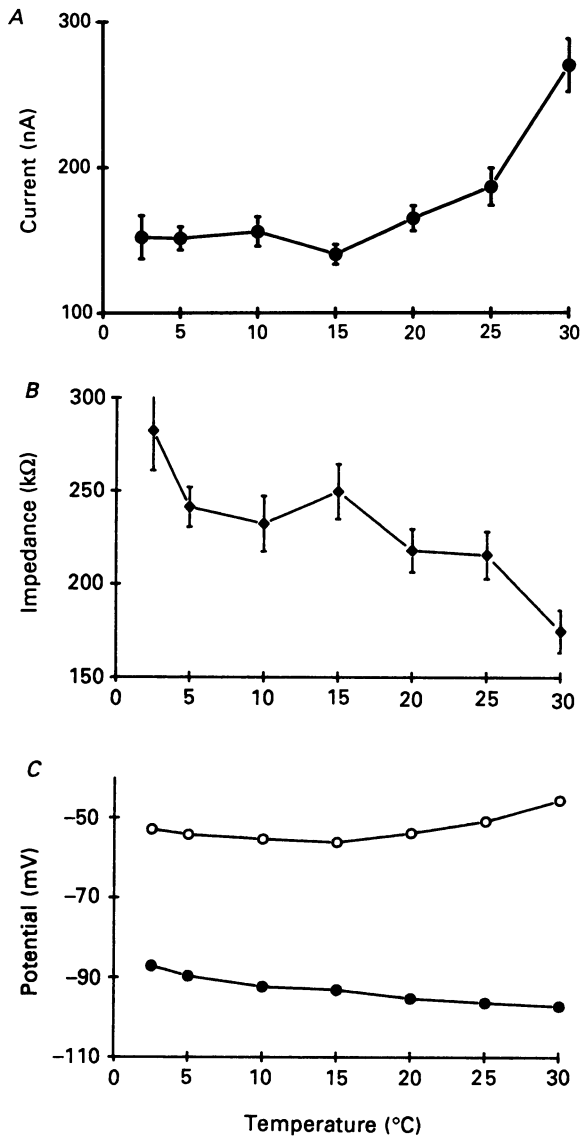


Fig. 2. Effect of temperature on the electrical excitability of *Xenopus sartorius* fibres. All measurements were made at end-plate regions. *A*, threshold current. *B*, input impedance. *C*, critical depolarization, shown as the difference between resting potential (filled symbols) and threshold potential (open symbols). In *A*, *B* and *C* each point represents the mean \pm s.e.m. of thirty to forty sartorius fibres sampled equally from eight different frogs. In *C* error bars do not extend beyond the boundary of the symbols.

Postsynaptic electrical excitability

The effect of temperature on the electrical excitability of sartorius fibres was assessed by measuring threshold current at end-plate regions (see Methods). End-plate regions were examined because (1) they are the normal site of action potential

initiation during neuromuscular transmission, and (2) previous studies have demonstrated that Na^+ channel density (Beam, Caldwell & Campbell, 1985; Caldwell, Campbell & Beam, 1986; Roberts, 1987) and electrical excitability (Nastuk & Alexander, 1973; Thesleff, Vyskocil & Ward, 1974) are both greater at end-plate than at extrajunctional regions. The present results demonstrate that regional differences exist in the electrical excitability of *Xenopus* sartorius fibres. At 23 °C, threshold current was 146 ± 5 nA ($n = 88$) at end-plate regions, and 166 ± 7 nA ($n = 89$) at extrajunctional regions. Threshold potential was -53 ± 0.4 mV ($n = 88$) at end-plate regions compared with -47 ± 0.4 mV at extrajunctional regions. Resting potential did not differ between the two regions (end-plate: -93 ± 0.5 mV ($n = 88$); extrajunctional: -93 ± 0.4 mV ($n = 89$)).

Figure 2 shows the effect of temperature on electrical excitability. Threshold current (Fig. 2A) increased between 20 and 30 °C ($Q_{10} = 1.64$), but did not change between 2.5 and 20 °C. The change in threshold current was correlated with a change in input impedance of the muscle fibres (Fig. 2B). Input impedance was inversely related to temperature ($Q_{10} = 0.84$, 2.5–30 °C). In addition, critical depolarization, shown as the difference between resting and threshold potentials (Fig. 2C), decreased with cooling between 30 and 2.5 °C ($Q_{10} = 1.16$). The temperature-dependent changes in threshold current, input impedance, and critical depolarization were all highly significant ($P < 0.001$, ANOVA). Thus, although electrical excitability of the fibres changed between 20 and 30 °C, it did not change between 2.5 and 20 °C. Therefore, alterations in postsynaptic electrical excitability could not account for the decreased synaptic efficacy observed at low temperatures (Fig. 1).

Evoked end-plate currents

Examples of evoked EPCs recorded in normal Ringer solution are shown in Fig. 3. In most records the decay phase of the EPC was interrupted by a propagated action potential originating from a distant junction on the same fibre. Figure 3A shows a typical record with interrupted decay. Occasionally a complete, uninterrupted EPC was recorded (Fig. 3B). Uninterrupted EPCs were rare and were only observed at temperatures below 10 °C. Figure 3C shows a small EPC with multiple peaks recorded at 2.5 °C.

Figure 4A shows the effect of temperature on EPC amplitude. At any given temperature, there was at least a tenfold range in the amplitude of evoked EPCs. Despite this variation, the average height of the EPCs was clearly temperature dependent, and decreased significantly with cooling. Average EPC heights were 810 ± 117 nA at 30 °C ($n = 21$), 453 ± 42 nA at 20 °C ($n = 28$), 264 ± 20 nA at 10 °C ($n = 53$), and 68 ± 13 nA at 2.5 °C ($n = 51$). Thus, the Q_{10} for EPC amplitude was approximately 1.7 between 10 and 30 °C, and approximately 6.0 between 2.5 and 10 °C. These results clearly indicate that reduced height of EPCs is the primary factor responsible for the decreased synaptic efficacy observed at low temperature.

Safety factor

The horizontal line in Fig. 4A represents the average threshold current obtained from the experiments presented in Fig. 2A. The vertical distance between this line and the points representing EPC amplitude provides an estimate of the safety factor for neuromuscular transmission at each end-plate. Safety factor was estimated for

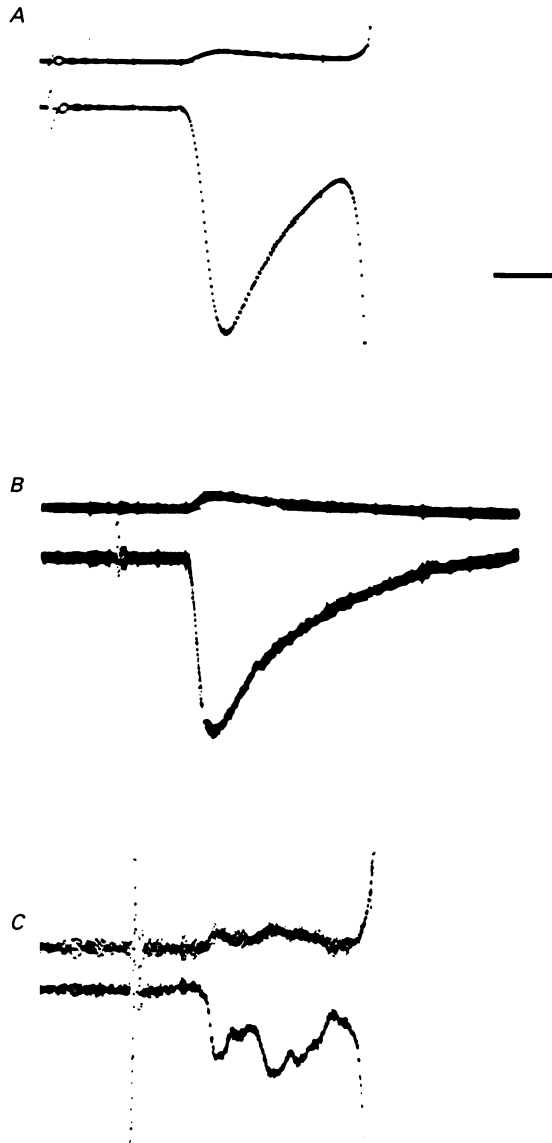


Fig. 3. Examples of evoked end-plate currents recorded in normal Ringer solution. In *A*, *B* and *C* the upper trace is membrane potential and the lower trace is clamping current. In all records the holding potential was -90 mV. *A*, typical evoked EPC showing interruption of the late decay phase by a propagated action potential. Temperature = 15 °C. *B*, an uninterrupted EPC recorded at 4 °C. *C*, an EPC recorded at 2.5 °C, showing distinct multiple peaks. Calibration bars = 25 mV, 250 nA, 1 ms (*A*); 5 mV, 50 nA, 5 ms (*B*); and 1 mV, 10 nA, 5 ms (*C*).

individual end-plates using the measured amplitude of the evoked EPC and the average threshold current determined for sartorius fibres at that temperature. Average safety factor was 540 ± 117 nA at 30 °C ($n = 21$), 288 ± 42 nA at 20 °C ($n = 28$), 108 ± 20 nA at 10 °C ($n = 53$), and -78 ± 13 nA at 2.5 °C ($n = 51$). From these numbers and from Fig. 4*A*, it is apparent that at temperatures above 10 °C

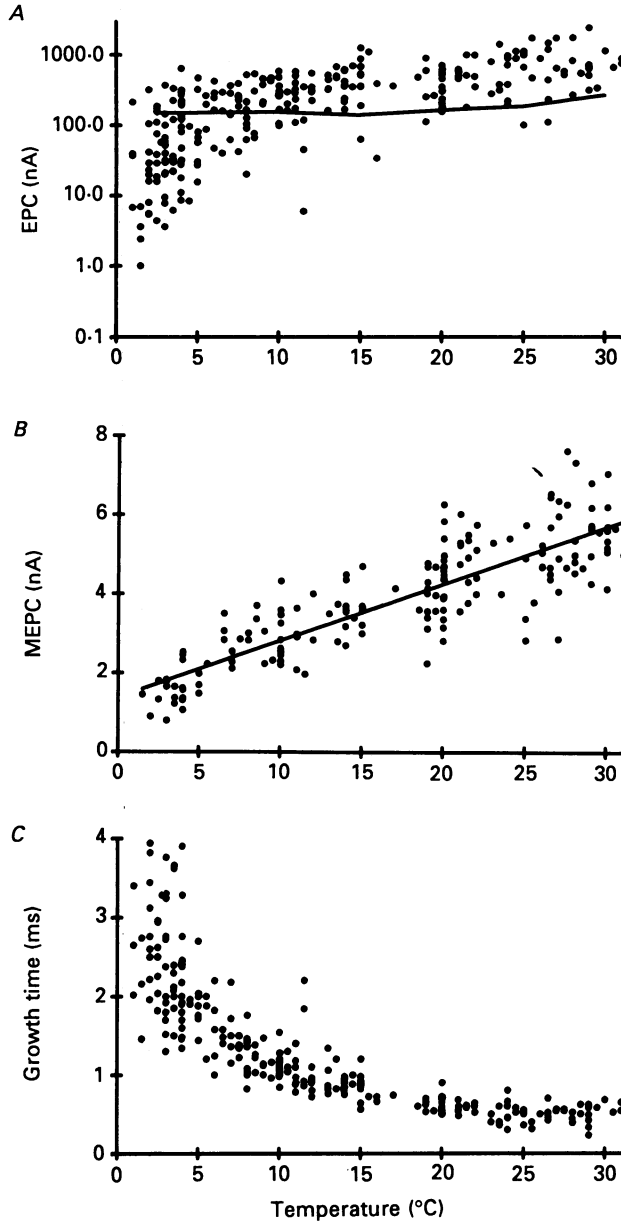


Fig. 4. Effect of temperature on the amplitude of evoked EPCs (*A*), spontaneous MEPCs (*B*), and the growth phase of evoked EPCs (*C*). In *A* and *C*, each point represents a single determination of the evoked EPC at one end-plate on a given muscle fibre. The horizontal line in *A* represents the average threshold current as presented in Fig. 2*A*. In *B* each point represents the average of ten to twenty well-clamped MEPCs recorded from a single end-plate. The continuous line represents the least-squares linear regression; slope = $0.14 \text{ nA}/^\circ\text{C}$ (95% confidence interval = $0.130\text{--}0.157$). Data presented in *A*, *B*, and *C* were collected from twenty different sartorius muscles.

most EPCs were larger than was required to excite the 'average' muscle fibre. However, at temperatures below 10 °C many EPCs were clearly subthreshold.

Several mechanisms, acting singly or in combination, could contribute to the reduction in EPC amplitude and safety factor with cooling: (1) decreased amplitude of single quantum currents; (2) decreased synchrony of transmitter release; and (3) decreased quantal content of evoked EPCs. Each of these possibilities is discussed below.

Spontaneous MEPCs

The amplitude of spontaneous MEPCs varied considerably among different end-plates. Despite this variation, average MEPC amplitude decreased linearly with cooling (Fig. 4*B*). At 30 °C average MEPC amplitude was 5.5 ± 0.2 nA ($n = 18$ fibres), decreasing to 1.4 ± 0.1 nA at 2.5 °C ($n = 11$ fibres; $Q_{10} = 1.63$). This result demonstrates that changes in MEPC amplitude are partly responsible for the observed temperature-dependent changes in evoked EPC amplitude.

Synchrony of transmitter release

The synchrony with which quanta of transmitter are released following nerve stimulation is reflected by the growth phase of the evoked end-plate current. Figure 4*C* illustrates the effect of temperature on the growth time of evoked EPCs. At 30 °C average growth time was 0.52 ± 0.03 ms ($n = 18$), increasing to 2.82 ± 0.16 ms at 2.5 °C ($n = 50$; $Q_{10} = 0.54$). The slowing of the growth phase with cooling primarily reflects an increase in the temporal dispersion of transmitter release (Katz & Miledi, 1965*a, b*) as well as an increase in the time course of individual MEPCs (Gage & McBurney, 1975). It may also reflect additional processes, such as increased repetitive binding of unhydrolyzed ACh to receptors at temperatures where acetylcholinesterase is inhibited (Katz & Miledi, 1973; Miledi & Parker, 1980; Magazanik, Nikolsky & Giniatullin, 1984). As transmitter release becomes more dispersed in time, and the time course of individual MEPCs becomes more prolonged, the single-quantum currents that compose the evoked EPC sum less effectively, and the amplitude of the EPC declines. The results presented in Fig. 4*C* indicate that decreased synchrony of transmitter release contributes to the decline in evoked EPC amplitude with cooling.

Charge entry during EPCs and MEPCs

The net charge entry during evoked EPCs (Q_{EPC}) and spontaneous MEPCs (Q_{MEPC}) was measured to allow estimation of quantal content using the ratio $Q_{\text{EPC}}/Q_{\text{MEPC}}$. Analysis of variance revealed that Q_{EPC} did not change between 5 and 30 °C ($P > 0.5$), but decreased at temperatures below 5 °C ($P = 0.02$; data not shown). The average value of Q_{EPC} at temperatures between 5 and 30 °C was 876 ± 44 pC ($n = 146$). At 2.5 °C, average Q_{EPC} was reduced to 562 ± 92 pC ($n = 35$). As temperature is lowered, EPC amplitude decreases (Fig. 4*A*), but EPC duration increases (Takeuchi & Takeuchi, 1959; Magleby & Stevens, 1972*b*). These combined effects explain the relative thermal independence of Q_{EPC} over the range 5–30 °C.

In contrast to Q_{EPC} , charge entry during MEPCs (Q_{MEPC}) increased linearly as temperature was lowered (regression slope = -0.009 pC/°C; 95% confidence

interval = -0.111 to -0.067). At $30\text{ }^{\circ}\text{C}$ average Q_{MEPC} was 3.9 ± 0.3 pC ($n = 15$ end-plates), increasing to 5.6 ± 0.3 pC at $2.5\text{ }^{\circ}\text{C}$ ($n = 19$ end-plates). The increase in Q_{MEPC} with cooling is probably due to temperature effects on the time course of MEPCs. Although MEPC amplitude declines ($Q_{10} = 1.5$, Gage & McBurney, 1975; $Q_{10} = 1.63$, this study), MEPC growth time and decay time constant both increase with cooling ($Q_{10} = 1.2$ and 3.1 , respectively; Gage & McBurney, 1975). The prolonged time course of MEPCs at the lower temperatures allows more charge to enter.

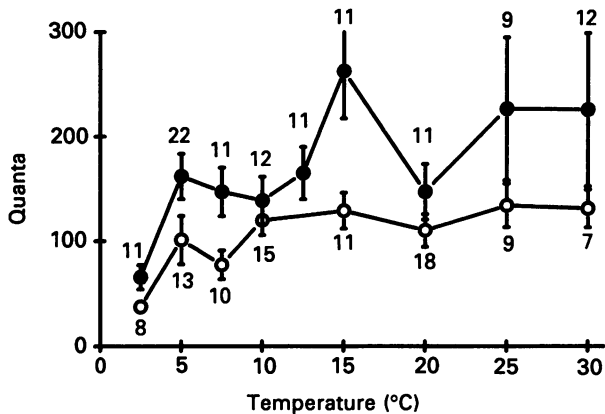


Fig. 5. Effect of temperature on the quantal content of evoked end-plate currents recorded in normal Ringer solution. O, quantal content calculated from the ratio EPC/MEPC; ●, quantal content calculated from the ratio $Q_{\text{EPC}}/Q_{\text{MEPC}}$. Bars indicate \pm S.E.M., with the number of end-plates examined shown for each temperature.

Quantal content

The quantal content of evoked EPCs can be estimated from the ratio of peak current during evoked EPCs and spontaneous MEPCs (EPC/MEPC). However, because the synchrony with which transmitter is released depends upon temperature, using the ratio EPC/MEPC gives an estimate of quantal content that is temperature dependent (Katz & Miledi, 1979). A more accurate and temperature-independent estimate of quantal content can be obtained from the ratio $Q_{\text{EPC}}/Q_{\text{MEPC}}$. Estimates of quantal content for individual end-plates, calculated using both peak current and charge entry ratios, are shown in Fig. 5. Different end-plates were analysed using each method. The two ratios gave qualitatively similar results; however, as expected, using the ratio EPC/MEPC gave lower values for quantal content. Quantal content varied tremendously among end-plates; at $20\text{ }^{\circ}\text{C}$ the range of estimates was 31–232 using EPC/MEPC and 31–300 using $Q_{\text{EPC}}/Q_{\text{MEPC}}$. Analysis of variance of the results shown in Fig. 5 revealed that quantal content, estimated using either ratio, did not change significantly between 5 and $30\text{ }^{\circ}\text{C}$, but decreased between 5 and $2.5\text{ }^{\circ}\text{C}$ ($P < 0.01$). The average value of quantal content over the temperature range 5– $30\text{ }^{\circ}\text{C}$ was 121 ± 7 using EPC/MEPC ($n = 76$ end-plates) and 181 ± 14 using $Q_{\text{EPC}}/Q_{\text{MEPC}}$ ($n = 99$ different end-plates). At $2.5\text{ }^{\circ}\text{C}$ the average values were 37 ± 8 using EPC/

MEPC ($n = 8$) and 66 ± 12 using $Q_{\text{EPC}}/Q_{\text{MEPC}}$ ($n = 11$). These results indicate that temperature-dependent changes in evoked EPC amplitude between 5 and 30 °C do not result from alterations in quantal content, but instead from temperature-dependent changes in MEPC amplitude and the synchrony of transmitter release. However, below 5 °C temperature-dependent changes in quantal content do become important in determining EPC amplitude.

DISCUSSION

The results presented here demonstrate that synaptic efficacy at the frog neuromuscular junction is constant over a wide range of temperatures, but then decreases as temperature is lowered beyond a certain point (10 °C). The change in synaptic efficacy is caused by a fall in the safety factor for neuromuscular transmission. Safety factor decreases as temperature is lowered, due to a temperature-dependent decrease in the amplitude of evoked end-plate currents. At temperatures below 10 °C, the EPCs in many muscle fibres are too small to initiate action potentials.

It should be mentioned that the present findings regarding safety factor, synaptic efficacy, and evoked end-plate currents apply only to single nerve stimuli. During repetitive nerve stimulation, the normal end-plate current is facilitated (Katz & Miledi, 1979), and it is therefore possible that tetanic stimulation would elicit action potentials in all muscle fibres, even at low temperatures. However, Marsh & Bennett (1985) have provided evidence that skeletal muscles are activated by twitch stimuli during locomotion at low temperatures. Thus, the observed decrease in safety factor and synaptic efficacy may also occur *in vivo*, and may be of great physiological significance in the cold.

It should also be mentioned that the quantitative results of this study would probably have been different if a different species had been used, or if the *Xenopus* used for these experiments had been acclimatized to a different temperature.

The finding that quantal content is constant over a wide temperature range and only decreases at very low temperatures (Fig. 5) is very surprising, and stands in sharp contrast to the large temperature coefficients reported for quantal content in curarized or Mg^{2+} -blocked preparations (Steinbach & Stevens, 1979). Katz & Miledi (1979), who also studied neuromuscular transmission under physiological conditions (i.e. in normal Ringer solution), reported that quantal content did not change between 5 and 23 °C. The different results obtained using curare- or Mg^{2+} -blocked preparations and those obtained using unblocked preparations may be due to the complicated effects of temperature on the actions of these blocking agents.

The thermal independence of transmitter release may have important biological implications for frogs and other poikilothermic animals. Since effective neuromuscular transmission is a prerequisite to muscle contraction, locomotion, and other behaviours, the relative constancy of transmitter release should help poikilotherms maintain functional competence despite large variations in body temperature.

The sharp reduction in quantal content as temperature is lowered below 5 °C could be due to several factors. One possibility is that the nerve action potential fails to

propagate into all branches of the nerve terminal; such failure of propagation is known to be highly temperature sensitive (Joyner, 1981; Eusebi & Miledi, 1983). Another possibility is that low temperature reduces Ca^{2+} entry into the nerve terminal, as has been observed at squid giant synapse (Charlton & Atwood, 1979). Alternatively, the biochemical mechanisms that couple Ca^{2+} entry to transmitter release in the nerve terminal may become inhibited at low temperatures.

The number of subthreshold fibres observed in the sartorius of *Xenopus laevis* (7% at 10–30 °C) is similar to that reported by Ridge & Thomson (1980) for the EDL IV of *Xenopus* (9% at 22 °C). However, it is much lower than the values reported by Grinnell & Herrera (1980) for sartorii of *Rana catesbiana* and *Rana pipiens* (20–30% at 15 °C). The discrepancy may be due to a difference in methods; Grinnell & Herrera (1980) measured twitch tensions in Ringer solution of various $[\text{Ca}^{2+}]$, but did not record intracellular responses to nerve stimulation. While intracellular recording has the disadvantage that only surface fibres can be examined, it is the most direct method of assaying the number of subthreshold fibres.

No previous studies appear to have determined the effects of temperature on the number of subthreshold fibres or on safety factor. However, the present results are qualitatively similar to those of Li & Gouras (1958), who found that subthreshold EPPs replaced action potentials at about 5 °C in the sartorius of *Rana pipiens*. In future studies, it would be interesting to repeat the present experiments using a mammalian neuromuscular preparation, in order to determine whether constancy of evoked transmitter release also occurs in homeothermic muscle or is a peculiarity of poikilothermic systems. Additionally, it would be of great interest to examine the temperature dependence of quantal transmitter release, under physiological conditions, at central synapses.

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