*J. Physiol.* (1978), **276**, *pp.* 183–192
*With 4 text-figures*
*Printed in Great Britain*



# EFFECTS OF MEMBRANE POTENTIAL AND TEMPERATURE ON THE EXCITATORY POST-SYNAPTIC CURRENT IN THE CRAYFISH MUSCLE

BY K. ONODERA AND A. TAKEUCHI

*From the Department of Physiology, School of Medicine Juntendo University, Hongo, Tokyo 113, Japan*

(*Received 30 June 1977*)

SUMMARY

1. Effects of membrane potential and temperature on the excitatory post-synaptic current (e.p.s.c.) were studied in the voltage-clamped crayfish muscle. E.p.s.c. was recorded either by measuring the feedback current through an intracellular wire electrode or by focal recording with an extracellular micro-electrode.

2. The amplitude of the e.p.s.c. obtained by the voltage clamp method varied almost linearly with membrane potential between $-100$ mV and $+70$ mV, whilst the reversal potential was $+23{\cdot}8 \pm 3{\cdot}9$ mV (S.E. of mean).

3. The declining phase of the extracellular e.p.s.c. was slightly prolonged by depolarization and shortened by hyperpolarization. Potential dependence of the decay time constant was expressed by $\tau = a \exp(AV)$, with $a = 2{\cdot}78$ msec and $A = 0{\cdot}0037$ mV$^{-1}$.

4. The decay time constant had a $Q_{10}$ of 2·3 and the growth time had a $Q_{10}$ of 1·5.

5. The voltage dependence of the decay phase of the e.p.s.c. was the reverse of that found in frog end-plate. It is concluded that the voltage dependence of the time course is not related either to the charge of ions which carry the synaptic current or to the charge of the transmitter.

## INTRODUCTION

In a previous study it was shown that the reversal potential of the excitatory post-synaptic current (e.p.s.c.) of the crayfish muscle was positive with respect to the bath solution, and the permeability change produced by the transmitter was predominantly due to an increase in the synaptic membrane conductance to $Na^+$ (Onodera & Takeuchi, 1975). It was assumed that the relationship between the membrane potential and the amplitude of the e.p.s.c. was linear and therefore the reversal potential was derived by extrapolating the line to the null potential. Recently, however, a non-linear relationship has been reported in the e.p.s.c. of the frog and crayfish muscles (Kordaš, 1969; Magleby & Stevens, 1972*b*; Dudel, 1974; Scuka, 1975). The membrane potential–e.p.s.c. relationship was, therefore, reinvestigated over a wider range of the membrane potential.

Non-linearity has been explained by a model which assumes that the duration of the effective acetylcholine (ACh) concentration at receptors is much shorter than the

mean lifetime of the open channel, and that the conductance change is proportional to the opening rate constant of the channels. Accordingly the non-linearity of the e.p.s.c.–membrane potential relation would be due to the decrease of the opening rate constant with hyperpolarization (Magleby & Stevens, 1972*b*; Dionne & Stevens, 1975). There are conflicting reports on the voltage dependence of the time course of the e.p.s.c. in crustacean and insect muscles (Dudel, 1974; Onodera & Takeuchi, 1975; Anderson, Cull-Candy & Miledi, 1976; Anwyl & Usherwood, 1976). Therefore, the effects of membrane potential and temperature on the time course of the e.p.s.c. was also investigated. It will be shown that the relationship between the membrane potential and the amplitude of the e.p.s.c. was almost linear between $-100$ and $+70$ mV and the e.p.s.c. reversed its sign at about $+20$ to $+30$ mV. The decay phase of e.p.s.c. was slightly prolonged by depolarization and became shorter by hyperpolarization of the membrane.

## METHODS

The opener muscle of the dactylpodite of the claw in the crayfish (*Cambarus clarkii*) was cannulated longitudinally with a stainless steel wire (70 $\mu$m in diameter) and the membrane potential was clamped as described in the previous report (Onodera & Takeuchi, 1975). The membrane resistance of the crayfish muscle is low and when the membrane was clamped at membrane potentials different from the resting level a large holding current was necessary. Since the holding current was carried mainly by chloride ions (Ozeki, Freeman & Grundfest, 1966), the chloride concentration of the bath solution was decreased by replacing it with propionate. Composition of the modified Van Harreveld's solution was (mM): $Na^+$, 207·5; $K^+$, 5·4; $Ca^{2+}$, 18·8; $Cl^-$, 43; propionate, 207·5. The pH was adjusted to 7·2 by Tris maleate buffer. Depolarization of the membrane potential to less than about $-50$ mV frequently resulted in muscle contraction. In order to decrease the movement artifact, Dantrolene sodium (1-[5-(*p*-nitrophenyl)furfuryl-ideneamino]hydantoin, 6 $\mu$g/ml.) (e.g. Snyder, David, Bickerton & Halliday, 1967; Odette & Atwood, 1974; Putney & Bianchi, 1974) was added to the solution. This drug reduced the twitch contraction without interfering with synaptic transmission. Its effect, however, was small in reducing contracture i.e. localized shortening (Takauji, Takahashi & Nagai, 1975).

When a longitudinal metal electrode is used to measure the e.p.s.c., the records represent the sum of the synaptic currents produced by all the synapses distributed over the muscle fibre. The e.p.s.c. at single synapses was, therefore, measured with a focal extracellular micro-electrode placed close to individual synapses (Dudel & Kuffler, 1961). For such measurements the micro-capillaries (about 1–2 $\mu$m in tip diameter) were filled with 1 M-NaCl or Van Harreveld's solution. In some cases large capillaries with a tip diameter of about 10 $\mu$m were used. However, the large capillaries were not suitable for accurately locating individual synapses. Most experiments were therefore performed with relatively small tipped capillaries. Since the extracellular e.p.s.c. at a single synapse is usually small, care was taken to avoid contamination of the synaptic current by the contribution from nearby synapses (Zucker, 1974). This was achieved by using a second micro-electrode as an indifferent reference lead which had to be placed close to the recording electrode vertically above the muscle surface. This provided a differential recording system. When the indifferent micro-electrode was moved to a distant point from the recording electrode or when it was shifted sideways from the vertical line, it picked up the field potential produced by other synapses in many cases. When the extracellular e.p.s.c. was large, no contamination by the field potential was usually seen. This, however, became manifest when the membrane potential was brought to the reversal potential or when the presynaptic impulse failed to produce an e.p.s.c. Therefore, the stimulation frequency was kept low and it was confirmed that little or no potential change was observed on the base line when the nerve spike evoked no transmitter release (see Fig. 3). Potential changes were displayed on the oscilloscope and photographed. They were also stored on an FM tape recorder (with 5 kHz band width) for later analyses.

The resting potential of the cannulated muscle fibres was usually lower in the chloride-deficient solution than that in the normal solution. The relatively low resting potential is not attributed to the toxic effect of propionate or the low chloride concentration in the solution, because the

transmission was well maintained for hours, and when the muscle was not cannulated the resting potential was in the normal range in the chloride-deficient solution. The low resting potential, therefore, may be attributed to the shunting resistance at the site of insertion of the longitudinal electrode. If it is assumed that the shunting resistance at the insertion point is 42·9 kΩ, in series with a longitudinal resistance of 22·7 kΩ and a lumped membrane resistance of 14 kΩ, then the resting membrane potential would be 67 mV, assuming the resting potential without cannulation to be 81·5 mV (Onodera & Takeuchi, 1975). When the lumped membrane resistance is increased in the low chloride solution (say doubled) the resting potential would be decreased to

$$81{\cdot}5 \times \left(1 - \frac{14 \times 2}{42{\cdot}9 + 22{\cdot}7 + (14 \times 2)}\right) = 57 \text{ mV}.$$

When the bath temperature was changed, the saline solution was circulated through a heat exchanger which was cooled with ice and the muscle was perfused rapidly with this solution. The bath temperature was measured with a small thermistor placed near the muscle fibre. When the bath temperature was lowered, in many cases the resting membrane potential was reversibly decreased by a few mV. The lowered resting potential may be due to a reduced activity of an electrogenic sodium pump (Adrian & Slayman, 1966). When the effect of temperature was measured, the membrane potential was clamped at the resting potential at room temperature (22–23 °C).

## RESULTS

### *Relationship between the amplitude of the e.p.s.c. and membrane potential*

In Fig. 1*A* are shown e.p.s.c.s produced at a stimulation frequency of 10 Hz, while the membrane potential was clamped at various membrane potentials. The inward current is indicated as a downward deflexion. When the membrane potential was depolarized, the amplitude of the e.p.s.c. became smaller and at about +30 mV it reversed its sign. Fig. 1*B* shows that the relationship between the amplitude of the e.p.s.c. and the clamped membrane potential was approximately linear between −100 mV and +70 mV. The reversal potential measured by interpolation ranged from +14 to +43 mV, with a mean value of +23·8 ± 3·9 mV (S.E. of the mean of eight experiments). This value is close to that previously obtained by extrapolation (Onodera & Takeuchi, 1975). Fig. 1*B* also shows that when the membrane was hyperpolarized beyond −100 mV, the amplitude of e.p.s.c. tended to decrease. However, such a deviation from linearity was not regular and varied from preparation to preparation. In many cases the relation was linear up to −120 mV.

### *Effect of membrane potential on the time course of the e.p.s.c.*

The decay phase of e.p.s.c. was plotted on a semilogarithmic scale and the time constant was measured from its linear portion (Onodera & Takeuchi, 1975). Time constants ($\tau$) recorded at various membrane potentials are plotted in Fig. 1*C*. As the membrane was depolarized from about −80 mV to +70 mV the time constant was increased. However, the time constant also increased as the membrane potential was hyperpolarized to about −100 mV or beyond. In the previous study (Onodera & Takeuchi, 1975) it was suggested that a large hyperpolarizing current might have some effect on the nerve terminal and change the release process of the transmitter. In order to test this possibility the e.p.s.c.s were recorded from single synapses with an extracellular micro-electrode, whilst the membrane potential was controlled by the voltage clamp method. In Fig. 2 the nerve was stimulated at 8 Hz and about three traces were superimposed. In this case the resting potential was −46 mV. When

the membrane was hyperpolarized to about −130 mV, repetitive e.p.s.c.s appeared after the nerve stimulation, whilst no such effect was observed when the membrane was depolarized. During a large depolarization, however, there was a considerable increase in the synaptic delay. These results suggest that the polarizing current has an effect on the nerve terminal.

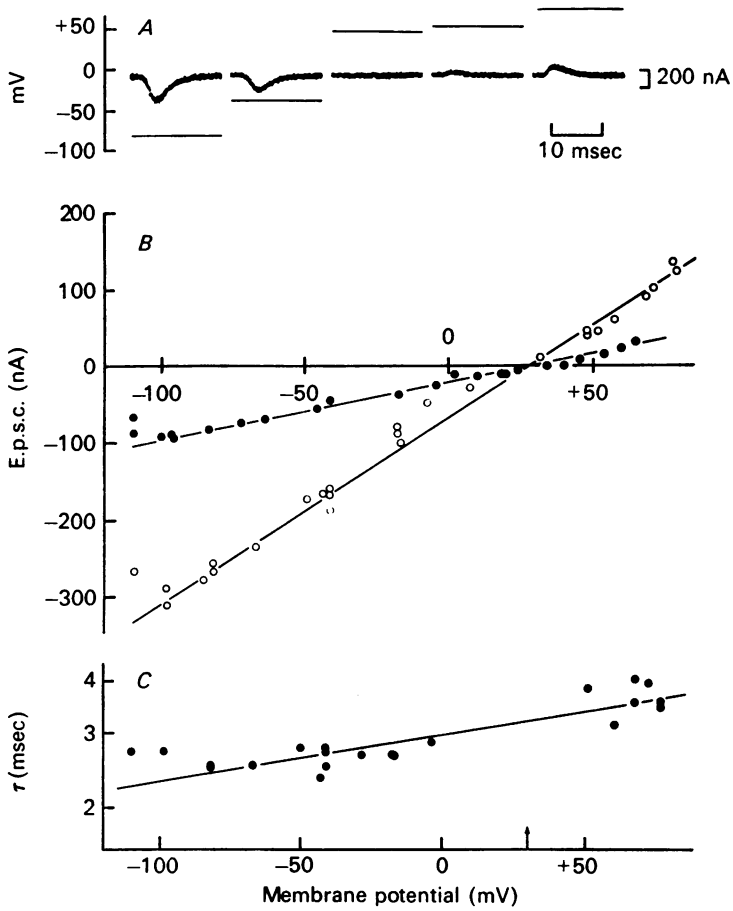


Fig. 1. Effect of changing the membrane potential on the excitatory post-synaptic current (e.p.s.c.). *A*, e.p.s.c.s and clamped membrane potentials recorded from a muscle fibre with a longitudinal intracellular metal electrode; stimulus was applied at 10 Hz and about three traces were superimposed; second from the left indicates the e.p.s.c.s at the resting potential; inward current is shown as a downward deflexion. *B*, relationship between membrane potential and amplitude of the intracellular e.p.s.c. Ordinate: peak amplitude of e.p.s.c.; negative sign indicates inward current. Absoissa: clamp potential. ○, stimulated at 10 Hz. ●, at 5 Hz. *C*, relationship between the clamp potential and the time constant of decay phase (the logarithmic ordinate); arrow indicates the reversal potential; the line is drawn through points less than −70 mV.

Repetitive appearance of e.p.s.c.s at a single synapse during a large hyperpolarization may be due either to an increase in the probability of the quantal release or to repetitive firing of the nerve terminal. Although the reason for these phenomena is not clear, it is likely that the hyperpolarizing current through the muscle membrane

may depolarize the nerve terminal, causing prolonged release of the transmitter. It was concluded, therefore, that a large hyperpolarizing current causes a repetitive appearance of e.p.s.c.s at individual synapses and that the intracellularly recorded e.p.s.c. is prolonged because such recording represents the sum of e.p.s.c.s from synapses distributed over the whole surface of the muscle fibre.

If a regression line is drawn between about $-70$ mV and $+70$ mV in Fig. 1*C*, it is fitted by the equation of $\tau = a \exp(AV)$, where $a$ and $A$ are constant and $V$ is the clamped membrane potential, with $A = 0{\cdot}0025\ \text{mV}^{-1}$ and $a = 2{\cdot}9$ msec. These values are similar to those obtained with the extracellularly recorded e.p.s.c.s (see Fig. 3).

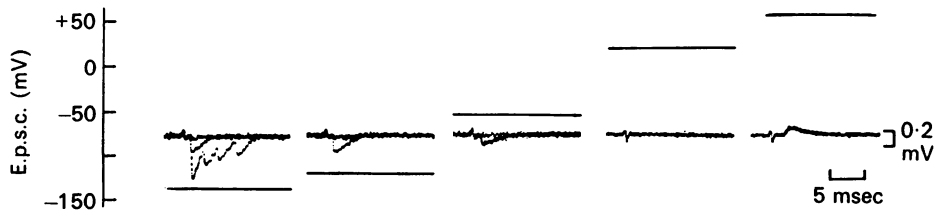


Fig. 2. Focal records of extracellular e.p.s.c.s in voltage clamped muscle fibre at various membrane potentials. Stimulation frequency was 8 Hz and three traces were superimposed.

*Effect of membrane potential on the time course of the extracellular e.p.s.c.*

The membrane potential was clamped at various levels and the extracellular e.p.s.c.s were recorded from individual synapses. When the membrane was depolarized to less than $-50$ mV, contractures were frequent and in many cases the extracellular electrode was dislodged from the synaptic region. When the extracellular e.p.s.c. was successfully recorded over a wider range of the membrane potential, the extracellular e.p.s.c. reversed its sign at about $+20$ to $+30$ mV (Fig. 3*A*).

When the time constant of the decay phase of the extracellular e.p.s.c. was plotted against the clamped membrane potential, the time constant became slightly smaller as the membrane was hyperpolarized and became larger during depolarization (Fig. 3*B*). If the time constant ($\tau$) is expressed as $\tau = a \exp(AV)$, the regression line is fitted with $a = 2{\cdot}7$ msec and $A = 0{\cdot}0021\ \text{mV}^{-1}$ (correlation coefficient $r = 0{\cdot}70$, $P < 0{\cdot}01$, Student's $t$ test). The value of $A$ ranged from 0·0067 to 0·0017 $\text{mV}^{-1}$ with the mean value of $0{\cdot}0037 \pm 0{\cdot}0010\ \text{mV}^{-1}$ (S.E. of mean in six experiments). The mean value of $a$ was $2{\cdot}78 \pm 0{\cdot}32$ msec (S.E. of mean in six experiments).

When the effect of membrane potential on the growth phase was investigated (Fig. 3*C*), the e.p.s.c. was displayed on the oscilloscope on a fast time base (five times that in Fig. 3*A*). Because of a large background noise, the time course of the growth phase was expressed by the time from 20 to 80% of the peak amplitude (growth time) (Gage & McBurney, 1975). The growth time tended to increase as the membrane was depolarized. The regression line (growth time $= b \exp(BV)$) was fitted with $b = 0{\cdot}24$ msec and $B = 0{\cdot}0053\ \text{mV}^{-1}$ ($r = 0{\cdot}61$, $P < 0{\cdot}01$).

The effect of membrane potential on the time course of spontaneous e.p.s.c.s was also investigated. Since the frequency of spontaneous e.p.s.c.s was very low, the

membrane potential was repeatedly clamped near −60 mV (from −57 mV to −63 mV) and −110 mV (from −107 to −112 mV) for a relatively short duration, such as 10 sec, and the spontaneous e.p.s.c.s were pooled on magnetic tape. The time constant for the decay phase when clamped near −60 mV was 1·5 ± 0·03 msec (S.E. of mean for thirty-nine samples) and when near −110 mV was 1·03 ± 0·036 msec (S.E. of mean for twenty-three samples). The growth time near −60 mV was 136 ± 6·3 μsec

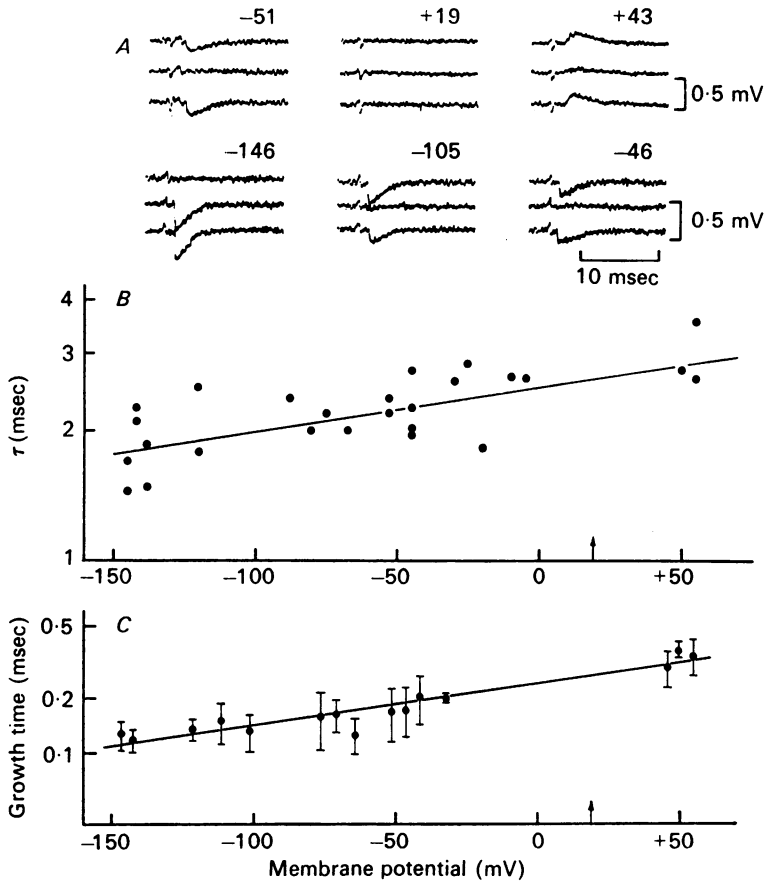


Fig. 3. Effect of membrane potential on the extracellular e.p.s.c. in a voltage-clamped muscle fibre. *A*, e.p.s.c.s recorded from a single synapse. *B*, the time constant of decay phase, and *C*, the growth time. Note the logarithmic ordinate. Arrows indicate the reversal potential. Bars indicate S.D.

($n$ = 45) and near −110 mV it was 123 ± 3·8 μsec ($n$ = 59). Prolongation of the decay time constant was highly significant ($P < 0·01$). An increase in the growth time was small and slightly significant ($0·05 < P < 0·10$).

*Effect of temperature on the time course of the extracellular e.p.s.c.*

The spontaneous e.p.s.c.s were recorded with an extracellular micro-electrode at a single synapse in the voltage-clamped muscle fibre. Fig. 4*A* indicates spontaneous e.p.s.c.s recorded at a membrane potential of 57 mV at three different temperatures.

In many cases the spontaneous e.p.s.c. at 22 °C reached its peak in 200–300 μsec and decayed exponentially with a short initial non-exponential phase. The time course of e.p.s.c.s was variable even in records from an individual synaptic region. The time for peak e.p.s.c. was sometimes as long as 500–800 μsec and there also was a large variation in the duration of the non-exponential phase. This phase ranged from less than 0·3 msec to several msec and in some e.p.s.c.s the decay phase was not exponential but decayed almost linearly. Variability in the time course of spontaneous e.p.s.c. was similar to those reported in frog and crab muscles (Gage & McBurney, 1975; Crawford & McBurney, 1976). Further studies are needed to decide whether the

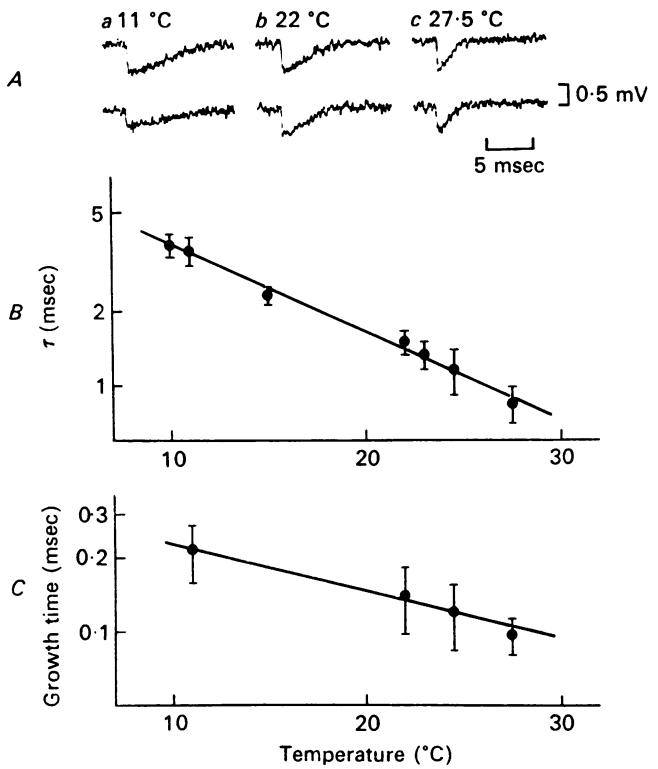


Fig. 4. Effect of temperature on the spontaneous e.p.s.c. *A*, sample records of spontaneous e.p.s.c.s recorded with an extracellular micro-electrode at a single synapse; membrane potential was clamped at −57 mV. *B*, time constant of decay phase (mean of six to seventeen samples). *C*, growth time (mean of seventeen to sixty-eight samples). Bars indicate S.D. Note the logarithmic ordinate.

variability is due to the release process of a quantum or to post-synaptic events, such as retention of the transmitter in the synaptic cleft or a heterogeneous distribution of receptors which have different kinetics. At lower temperatures the peak of e.p.s.c.s became round.

When the time constant of the decay phase was measured, those e.p.s.c.s were selected which had a non-exponential phase of less than about 1·5 msec. The decay time constant was 1·49 ± 0·17 msec (mean ± S.D.) at 22 °C. Lowering the temperature

to 11 °C increased the time constant to 3·49 ± 0·45 msec. In Fig. 4*B* are plotted the decay time constants at various temperatures. The regression line was drawn by the method of least squares ($r = 0{\cdot}74$; $P < 0{\cdot}01$) and the $Q_{10}$ was 2·3. The growth time was 139 ± 42 μsec (mean ± S.D.) at 22 °C and it increased to 214 ± 57 μsec at 11 °C. The growth time at various temperatures are plotted in Fig. 4*C* and the regression line ($r = 0{\cdot}73$, $P < 0{\cdot}01$) gave a $Q_{10}$ of 1·5. A relatively small $Q_{10}$ for the growth time compared to that for the decay phase has been observed in the toad end-plate (Gage & McBurney, 1975).

The time course of the nerve-evoked e.p.s.c. recorded extracellularly was essentially the same as that of spontaneous e.p.s.c. The mean value of the decay time constant was 1·59 ± 0·18 msec (mean ± S.D.) at 23 °C and 3·78 ± 0·29 msec, at 13 °C. The $Q_{10}$ for the decay phase was 2·4.

## DISCUSSION

The present experiments confirmed the previous observation that the reversal potential of the e.p.s.c. was positive with respect to the bath solution (Onodera & Takeuchi, 1975). It varied from preparation to preparation and ranged from +14 to +43 mV with a mean value of +23·8 mV. The variability of the reversal potential might be due to an increase in the inside concentration of sodium, when the muscle was cannulated with a longitudinal metal electrode (Bittar, Chen, Danielson, Hartmann & Tong, 1972).

Peak amplitude of e.p.s.c. was almost linear between −100 mV and +70 mV. In the frog end-plate, it has been reported that the peak amplitude tended to decrease and deviate from linearity as the membrane was hyperpolarized (Kordaš, 1969; Magleby & Stevens, 1972*b*; see also Dudel, 1974, for the crayfish e.p.s.c.). The voltage-dependent decrease in the peak amplitude is explained by the hypothesis that the opening rate constant of the ionic channel is decreased as the membrane is hyperpolarized (Magleby & Stevens, 1972*b*; Dionne & Stevens, 1975). Recently, however, it was found in the frog and toad muscles that the voltage sensitivity of the opening rate constant is negligible (Neher & Sakmann, 1975) and the growth time is insensitive to the membrane potential (Gage & McBurney, 1975), which seems to agree with the linear relation between the membrane potential and the e.p.s.c. in frog neuromuscular junction (Trautman & Zilber-Gachelin, 1976).

In crayfish muscle the growth time was slightly decreased as the membrane was hyperpolarized. The factors controlling the growth rate are not well understood; if one assumes that the opening rate constant is increased by hyperpolarization, according to the above hypothesis one expects that the peak amplitude of the e.p.s.c. should increase as it deviates from linearity with the hyperpolarization. No such effect, however, was observed in the crayfish e.p.s.c. Another possibility would be that if the presence of the transmitter in the synaptic cleft is relatively long, the peak amplitude would be less sensitive to the rate constant of the channel opening (Dionne & Stevens, 1975). The time constant calculated from the cut-off frequency of the glutamate noise was slightly smaller than the decay time constant of the e.p.s.c. in insect and crayfish muscles (Anderson *et al.* 1976; K. Onodera & A. Takeuchi, unpublished observation). The discrepancy between the time constant of e.p.s.c. and

ACh noise in frog end-plate is attributed to the persistence of transmitter in the synaptic cleft (Katz & Miledi, 1973).

The decay phase of the extracellular e.p.s.c. was prolonged as the membrane was depolarized and shortened during hyperpolarization (Fig. 3; see also Dudel, 1974 for the crayfish muscle and Anderson *et al.* (1976) for insect muscle). The potential dependence of the decay phase in the crayfish muscle was the reverse of that in the frog neuromuscular junction and the degree of dependence was smaller by a factor of about two (Kordaš, 1969; Magleby & Stevens, 1972*a*, *b*).

The direction and degree of voltage dependence of the decay time of synaptic currents apparently differs in various species. Prolongation by hyperpolarization was found in the frog end-plate (Takeuchi & Takeuchi, 1959; Kordaš, 1969; Magleby & Stevens, 1972*a*, *b*; Gage & McBurney, 1975; Colquhoun, Large & Rang, 1977) and the inhibitory synapse of *Aplysia* neurones (Adams, Gage & Hamill, 1976), while shortening by hyperpolarization was observed in the crayfish inhibitory synapse (Onodera & Takeuchi, 1976), crayfish e.p.s.c. (Dudel, 1974; present report) and insect e.p.s.c. (Anderson *et al.* 1976). No voltage dependence has been reported in the squid giant synapse (Llinás, Joyner & Nicholson, 1974) and the insect e.p.s.c. (Anwyl & Usherwood, 1976). These results indicate that the voltage dependence of the decay phase is related neither to the sign of ionic charges which carry the synaptic current, nor to the net charge of the transmitter substances at a physiological pH.

The $Q_{10}$s for the decay phase of the spontaneous and nerve-evoked e.p.s.c.s were similar to those reported for the frog and toad's e.p.s.c.s (Takeuchi & Takeuchi, 1959; Kordaš, 1972; Magleby & Stevens, 1972*b*; Gage & McBurney, 1975; Colquhoun *et al.* 1977; see, however, Anwyl & Usherwood (1976) for insect e.p.s.c.). Relatively large $Q_{10}$ values suggest that the rate-limiting process for the decay of e.p.s.c. is not the diffusional loss of transmitter from the synaptic region, but involves processes which require a high activation energy, such as the conformational change of the receptor molecule or the dissociation of a transmitter-receptor complex.

The authors wish to thank Professor S. W. Kuffler for valuable comments on the manuscript. This work was supported, in part, by grants from the Ministry of Education.

## REFERENCES

Adams, D. J., Gage, P. W. & Hamill, O. P. (1976). Voltage sensitivity of inhibitory postsynaptic currents in *Aplysia* buccal ganglia. *Brain Res.* **115**, 506–511.

Adrian, R. H. & Slayman, C. L. (1966). Membrane potential and conductance during transport of sodium, potassium and rubidium in frog muscle. *J. Physiol.* **184**, 970–1014.

Anderson, C. R., Cull-Candy, S. G. & Miledi, R. (1976). Glutamate and quisqualate noise in voltage-clamped locust muscle fibres. *Nature, Lond.* **261**, 151–153.

Anwyl, R. & Usherwood, P. N. R. (1976). Factors affecting the time course of decay of excitatory postsynaptic currents at a glutamate synapse. *J. Physiol.* **254**, 46–47*P*.

Bittar, E. E., Chen, S., Danielson, B. G., Hartmann, H. A. & Tong, E. Y. (1972). An investigation of sodium transport in barnacle muscle fibres by means of the microsyringe technique. *J. Physiol.* **221**, 389–414

Colquhoun, D., Large, W. A. & Rang, H. P. (1977). An analysis of the action of a false transmitter at the neuromuscular junction. *J. Physiol.* **266**, 361–395.

Crawford, A. C. & McBurney, R. N. (1976). On the elementary conductance event produced by L-glutamate and quanta of the natural transmitter at the neuromuscular junctions of *Maia squinads*. *J. Physiol.* **258**, 205–225.

DIONNE, V. E. & STEVENS, C. F. (1975). Voltage dependence of agonist effectiveness at the frog neuromuscular junction: resolution of a paradox. *J. Physiol.* **251**, 245–270.

DUDEL, J. (1974). Nonlinear voltage dependence of excitatory synaptic current in crayfish muscle. *Pflügers Arch.* **352**, 227–241.

DUDEL, J. & KUFFLER, S. W. (1961). The quantal nature of transmission and spontaneous miniature potentials at the crayfish neuromuscular junction. *J. Physiol.* **155**, 514–529.

GAGE, P. W. & MCBURNEY, R. N. (1975). Effects of membrane potential, temperature and neostigmine on the conductance change caused by a quantum of acetylcholine at the toad neuromuscular junction. *J. Physiol.* **244**, 385–407.

KATZ, B. & MILEDI, R. (1973). The binding of acetylcholine to receptors and its removal from the synaptic cleft. *J. Physiol.* **231**, 549–574.

KORDAŠ, M. (1969). The effect of membrane polarization on the time course of the end-plate current in frog sartorius muscle. *J. Physiol.* **204**, 493–502.

KORDAŠ, M. (1972). An attempt at an analysis of the factors determining the time course of the end-plate current. II. Temperature. *J. Physiol.* **224**, 333–348.

LLINÁS, R., JOYNER, R. W. & NICHOLSON, C. (1974). Equilibrium potential for the postsynaptic response in the squid giant synapse. *J. gen. Physiol.* **64**, 519–535.

MAGLEBY, K. L. & STEVENS, C. F. (1972*a*). The effect of voltage on the time course of end-plate currents. *J. Physiol.* **223**, 151–171.

MAGLEBY, K. L. & STEVENS, C. F. (1972*b*). A quantitative description of end-plate currents. *J. Physiol.* **223**, 173–197.

NEHER, E. & SAKMANN, B. (1975). Voltage-dependence of drug-induced conductance in frog neuromuscular junction. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2140–2144.

ODETTE, L. L. & ATWOOD, H. L. (1974). Dantrolene Sodium: effects on crustacean muscle. *Can. J. Physiol. Pharmac.* **52**, 887–890.

ONODERA, K. & TAKEUCHI, A. (1975). Ionic mechanism of the excitatory synaptic membrane of the crayfish neuromuscular junction. *J. Physiol.* **252**, 295–318.

ONODERA, K. & TAKEUCHI, A. (1976). Inhibitory post-synaptic current in the voltage clamped crayfish muscle. *Nature, Lond.* **263**, 153–154.

OZEKI, M., FREEMAN, A. R. & GRUNDFEST, H. (1966). The membrane components of crustacean neuromuscular systems. II. Analysis of interactions among the electrogenic components. *J. gen. Physiol.* **49**, 1335–1349.

PUTNEY, J. W., Jr. & BIANCHI, P. (1974). Site of action of dantrolene in frog sartorius muscle. *J. Pharmac. exp. Ther.* **189**, 202-212.

SCUKA, M. (1975). The amplitude and time course of end-plate current at various pH levels in the frog sartorius muscle. *J. Physiol.* **249**, 183–195.

SNYDER, H. R. Jr., DAVID, C. S., BICKERTON, R. K. & HALLIDAY, R. P. (1967). 1-[(5-arylfurfurylidene)amino] hydantoins. A new class of muscle relaxants. *J. med. Chem.*, **10**, 807–810.

TAKAUJI, M., TAKAHASHI, N. & NAGAI, T. (1975). Effect of dantrolene sodium on excitation–contraction coupling in frog skeletal muscle. *Jap. J. Physiol.* **25**, 747-758.

TAKEUCHI, A. & TAKEUCHI, N. (1959). Active phase of frog's end-plate potential. *J. Neurophysiol.* **22**, 395–411.

TRAUTMANN, A. & ZILBER-GACHELIN, N. F. (1976). Further investigations on the effect of denervation and pH on the conductance change at the neuromuscular junction of the frog. *Pflügers Arch.* **364**, 53–58.

ZUCKER, R. S. (1974). Crayfish neuromuscular facilitation activated by constant presynaptic action potentials and depolarizing pulses. *J. Physiol.* **241**, 69–80.