THE EFFECT OF TEMPERATURE ON THE NEUROMUSCULAR JUNCTION OF THE FROG

NORIKO TAKEUCHI*

Department of Physiology, School of Medicine, Juntendo University

In 1941 Eccles, Katz and Kuffler showed that lowering the temperature reduced the size of end-plate potential in the curarized frog muscle. On the other hand, it was observed recently by Boyd and Martin (1956*b*) that lowering the temperature caused an increase in the amplitude of the end-plate potential in the magnesium treated mammalian neuromuscular junction, and that the increase in amplitude was largely due to an increase in quantum content. They described, however, that in the curarized muscle the end-plate potential size decreased when the temperature was lowered and that it might be attributed to changes in a post-synaptic effect of curarine at low temperature.

In the present investigation a study has been made, using the intracellular recording method, of whether or not this discrepancy was also observed in the frog neuromuscular junction, and of the effect of temperature on the transmitter action. A preliminary report appeared in 1957 (Takeuchi and Miyagawa).

METHODS

All experiments were performed from October 1956 to March 1957, using well fed winter frogs (*Rana nigromaculata*). The sciatic nerve-sartorius muscle preparation was mounted in the Ringer bath illustrated in fig. 1. The muscle was stretched by 20% of the resting length throughout the present work, for it has been generally accepted that the muscle length has an influence on the output of transmitter from the nerve endings (Hutter and Trautwein, 1956; Takeuchi, A., 1957; Takeuchi, N., 1957). The chamber for the bathing fluid was surrounded by a jacket through which warm or cold water was circulated from two thermoses. The temperature of bathing fluid was measured by a thermister-thermometer (fig. 1). The experiments were performed mainly between 10° C. and 20° C.

The sciatic nerve was stimulated with silver electrodes in the second compartment which was filled with paraffin-oil. As recording electrode the intracellular microcapillary filled with 3 M KCl was used (Ling and Gerard, 1949; Nastuk and Hodgkin, 1950). The direct current resistance of the electrode was between 10 M Ω and 20 M Ω . The location of an end-plate focus was determined by successive insertion of the electrode at different points along a muscle fibre and finding the position of the maximum amplitude and the shortest time course of end-plate potential. The electrode was connected with a balanced direct

Received for publication May 15, 1958.

* 竹内宣子

FIG. 1. Diagram of experimental arrangement.

coupled amplifier through a high impedance matching cathode-follower stage. The recording was mainly made by a cathode-ray oscilloscope and an electromagnetic oscillograph was used in order to measure the frequency of the spontaneous miniature end-plate potentials. The frequency characteristics of the recording system including the cathode-follower stage were flat until 5 kc and the total input time constant was $200 \mu\text{sec.}$ for an electrode of resistance $30 \text{ M}\Omega$.

The Ringer solution used had the following composition: sodium chloride, 111.5 mm; potassium chloride, 2 mm; calcium chloride 2 mm and phosphate buffer (1.08 mm Na_2HPO_4 ; 0.42 mm NaH_2PO_4) was added to maintain the pH of the solution at 7.2. In many cases the Ringer solution was oxygenated in order to prevent the depolarization of the membrane potential at the higher temperature. In order to block the neuromuscular transmission *d*-tubocurarine chloride ($6\text{--}3 \times 10^{-6} \text{ g/cc.}$) was added to the normal Ringer solution. In some cases 16–20 mm magnesium or about 40 mm calcium or sucrose was added to replace the sodium and transmission was blocked without *d*-tubocurarine. When the high concentration of calcium was used, phosphate buffer was not added. In measuring the probability of appearance of the responses at the neuromuscular junction 10 mm magnesium was added to Ringer's solution and the concentration of calcium was decreased.

The temperature change had little effect on the resting potential of the muscle fibre between 10°C. and 20°C. , as described by Ling and Woodbury (1949) on the frog muscle and by Hodgkin and Katz (1949) on the giant axon of the squid. But at temperatures lower than 6°C. the resting potential was somewhat decreased and at temperatures above 25°C. was often decreased, so the present experiment was mainly performed between 10°C. and 20°C. If the resting potential was decreased and was not reversible during the investigation, the measurements were rejected.

RESULTS

1. Effect of temperature on the amplitude of end-plate potential

A) Amplitude of end-plate potential

The amplitude of the end-plate potential (e.p.p.) was, in general, reduced

by lowering the temperature. An example of the effect of temperature on the curarized e.p.p. recorded intracellularly is shown in fig. 2. From this it is clear that the amplitude of the e.p.p. is reduced and the time course is prolonged when the temperature is lowered. The amplitudes of e.p.p.'s obtained by four different blocking methods are plotted against temperatures in fig. 3. Each measurement was made on the same end-plate, first at about 20°C. and then cooling gradually to about 10°C. and completed by returning to the original temperature.

In fig. 3, *A* is an example of the results obtained by blocking the neuromuscular transmission by the addition of 3×10^{-6} (g/cc.) *d*-tubocurarine to the bathing solution, and *B* is one blocked by sodium deficient Ringer's solution, in which 88 mm sodium was replaced by sucrose. The results which were obtained from the preparation blocked by high (about 40 mm) calcium (Ca-block) and 16-20 mm magnesium Ringer's solution (Mg-block) are shown in *C* and *D* respectively. Comparing these results it is noteworthy that there are some interesting differences between them: the rate of change in the e.p.p. size against the temperature is almost the same in *A* and *B*, but in *D* the rate of change is far greater than that of *A* and *B*. On the contrary in *C*, Ca-block, the change in the temperature has little or no effect on the e.p.p. size. The mean ratio of the increase in the e.p.p. size by a 10°C. raise in temperature

FIG. 2

FIG. 3

FIG. 2. The end-plate potential recorded intracellularly from a curarized end-plate at various temperatures. Voltage scale: 5 mV. Time scale: 5 msec.

FIG. 3. Effect of temperature on the amplitude of end-plate potential obtained by four different blocking methods. *A*, blocked by *d*-tubocurarine; *B*, by sodium-deficiency (replaced by sucrose); *C*, by increasing the calcium concentration; *D*, by addition of magnesium. Ordinates: peak amplitudes of end-plate potentials, in mV. Abscissae: temperature.

in the same end-plate was about 2 in the tubocurarine-block and Na-deficiency, while about 3 in Mg-block. In the tubocurarine-block and Mg-block the change of amplitude was approximately linear against the temperature over the range of 10°–20° C.; in Na-deficiency, however, the rate of change was smaller at lower temperature. In Ca-block it may be noticed that when the temperature was raised up to about 19° C. the amplitude of e.p.p. tended to decrease and at 19.5° C. the amplitude showed large variation, which might be due to a partial block of the terminal twigs of the nerve, and then the response of e.p.p. failed in the vicinity of 20° C. So that the reduction in the e.p.p. size at higher temperature may be attributed to a conduction block at the nerve twigs.

B) Probability of appearance of responses

In order to investigate the cause of the change in the e.p.p. size by temperature change the following experiments were performed. By addition of magnesium and lowering the calcium concentration, the probability of appearance of e.p.p. was reduced (del Castillo and Katz, 1954 *b*), and an influence of temperature on this probability was studied.

An example recorded from the same end-plate at 20° C. and 14° C. is shown in fig. 4. From this it is clearly shown that at higher temperatures the number of appearance of the response is much more increased than at lower temperatures and the amplitudes are almost the same as those of spontaneous miniature potentials. In fig. 5 the probability of appearance of the responses obtained from four end-plates is plotted against temperature. In each case an increase in the probability is observed at higher temperatures. This result suggests that the increase in the amplitude of the e.p.p. at higher temperatures is largely due to an increase in the amount of the transmitter released from

FIG. 4

FIG. 5

FIG. 4. Influence of temperature on the probability of appearance of the end-plate potential by a nerve impulse. A and B are recorded from the same end-plate at 20° C. and 14° C. respectively. At 20° C. nerve impulse produced five responses out of seven, but at 14° C. only two out of seven. Each record shows also the random appearance of the spontaneous activity.

FIG. 5. Effect of temperature on the probability of appearance of the end-plate potential response, obtained from four end-plates. Ordinate: probability. Abscissa: temperature.

the nerve endings. But variation of the probability in each measurement was rather great, therefore, the rate of change in the probability was not calculated.

2. Effect of temperature on the time course of end-plate potential

A) Time course of e.p.p.

It is well known that lowering the temperature prolonged the time course of e.p.p. (see fig. 2; Eccles *et al.*, 1941; Boyd and Martin, 1956 *b*). In this experiment details of the change in the time course of e.p.p. was investigated. The results are illustrated in fig. 6, where the duration of the rising phase of e.p.p. (rise time), and half decay time from peak are plotted against the temperature. As clearly shown in fig. 6, the time course of the e.p.p. has about linear relation to the temperature, although in sodium-deficiency the rate of change in the time course is somewhat larger at lower temperatures than at higher temperatures.

FIG. 6. Effect of temperature on the time course of the end-plate potential. Full circles: the duration of rise time. Hollow circles: from peak to half decay time. A, blocked by *d*-tubocurarine; B, by sodium-deficiency (replaced by sucrose); C, by increasing the calcium concentration; D, by adding magnesium. Ordinates: duration of the rise time and the half decay time of the end-plate potential, in msec. Abscissae: temperature.

Q_{10} of rise time was in average 2.7. This result is identical with that of Eccles *et al.* (1941). But on the contrary to the authors the time course of decay of the e.p.p. was appreciably affected by the temperature change ($Q_{10} = 2.2$).

B) The electrical characteristics of muscle membrane

It has been generally accepted that the falling phase of the e.p.p. is a passive process which is mainly due to dissipation of electric charge along and across the muscle fibre membrane (Eccles *et al.*, 1941; Fatt and Katz, 1951). Then the reason why the temperature coefficient for the time course of falling phase was relatively large in the present experiment may be based on 1) the

change in the time constant of the muscle membrane, or 2) the change in the duration of the transmitter activity at the end-plate. In order to investigate whether the time constant changes or not by temperature change, the polarizing microelectrode was inserted into the same muscle fibre within a distance of $50\ \mu$ of recording one under the binocular microscope, and the polarizing current was passed through a series resistance of $100\ \text{M}\Omega$.

In fig. 7, the effects of temperature on the e.p.p. and the membrane potential change caused by the polarizing current through the same muscle fibre membrane are compared. The half decay times of e.p.p. and polarized membrane potential are plotted against the temperature in fig. 8, where full circles denote the half decay time of polarized membrane potential and hollow circles that of the e.p.p. They reveal that the time course of decay of the polarized membrane potential is a little affected by temperature change, although it tends to increase at the lower temperature, while the half decay time of the e.p.p. is lengthened appreciably as cited above. It is clear from this result that the most part of the large Q_{10} of the half decay time of e.p.p. does not depend on the change in the membrane time constant.

FIG. 7

FIG. 8

FIG. 7. Comparison between the effects of temperature on the time course of the end-plate potential and the membrane potential change caused by the polarizing current. *A*, records of end-plate potentials at three temperatures. *B*, polarized membrane potentials obtained from the same muscle fibre at same temperatures, and the bottom record is the polarizing current through the membrane. The amplification was the same in all records and the voltage scale is 5 mV; time scale, 10 msec.

FIG. 8. Effect of temperature on the time course of the decay of the end-plate potential and that of polarized membrane potential. Hollow circles: end-plate potential. Full circles: polarized potential. Ordinate: half decay time of both end-plate potential and polarized potential, in msec. Absciss: temperature.

C) Time course of displacement of charge along and across the muscle fibre membrane during the e.p.p.

To clarify this problem further, displacement of electric charge during the e.p.p. was measured, using the method described by Fatt and Katz (1951). An

example is presented in fig. 9. The upper figures show the superposition of several tracings which indicate characteristic decline and temporal spreading of the wave form as the electrode is moved outward from the end-plate focus. From these figures the spread of the e.p.p. along the muscle fibre at various moments is measured. In the lower figures, the total charge on the fibre surface at various moments was plotted on a logarithmic scale. At 20°C., the charge rises to a maximum at about 2 msec. as observed by Fatt and Katz (1951), and from then on decays exponentially with nearly the same time constant of muscle fibre. At 10°C., however, the charge reaches a maximum at about 5-6 msec. This is much more longer than that at 20°C. These results show that at the lower temperature the e.p.p. is maintained actively for long time, *i.e.* the duration of the transmitter action is prolonged.

FIG. 9. Upper figures: tracings of end-plate potentials obtained from two muscle fibres at 20°C. (left) and 10°C. (right), at different distances from end-plate focus. In superimposing the records, the stimulus artefact was taken at the common point. Numbers attached to the curves indicate distances from end-plate focus in mm. Lower figures: the time courses of displacement of the total charges calculated from upper figures at various moments. Left: obtained from the tracings at 20°C. Right: from at 10°C. Ordinates: area of the potential space curves *i.e.* total charge at various moments in $V \times \text{cm}$, on a logarithmic scale. Abscissae: time in msec.

D) Cholinesterase activity

In order to investigate a cause for the prolongation of the transmitter activity, the effect of the temperature on the eserized muscle was studied. In fig. 10, the e.p.p.'s recorded from a curarized preparation (*d*-tubocurarine, 3×10^{-6} g/cc.) and a preparation in curarine-eserine Ringer's solution (*d*-tubocurarine, 5×10^{-6} g/cc. and eserine, 1×10^{-5} g/cc.) at various temperatures are compared. The relation between the half decay times in both conditions and the temperature is illustrated in fig. 11, A, where hollow circles represent those in the curarine-eserine Ringer's solution and full circles in curarine Ringer's solution. Although in curarine Ringer's solution, temperature change influenced the half decay time of the e.p.p. considerably as described above, in eserized

FIG. 10

FIG. 11

FIG. 10. Comparison between the effects of temperature on the curarized end-plate potential and eserized end-plate potential. *A*, records of end-plate potential obtained from curarized preparation (3×10^{-6} *d*-tubocurarine) at three temperatures. *B*, records obtained from a preparation treated with *d*-tubocurarine and eserine (5×10^{-6} *d*-tubocurarine + 1×10^{-5} eserine) at same temperatures. Voltage scales: 5 mV. Time scales: 5 msec.

FIG. 11. Effects of temperature on the curarized end-plate potential and eserized end-plate potential. Full circles: curarized end-plate potential. Hollow circles: eserized end-plate potential. *A*, changes in the time course of the end-plate potential in the curarized and eserized preparation, ordinate: half decay time of end-plate potential, in msec., abscissa: temperature. *B*, changes in the amplitude of the end-plate potentials, ordinate: peak potential of the end-plate potential, in mV, abscissa: temperature.

muscle the temperature change had a little effect on the decay time. These results support the possibility that the large temperature coefficient for the decay time of the e.p.p. is mostly due to inactivation of cholinesterase and the duration of the transmitter activity at the end-plate is prolonged at the lower temperature.

On the other hand in the eserized muscle the rate of increase in the magnitude of e.p.p. caused by raising the temperature was greater than that in the curarine Ringer's solution as seen in *B*, in fig. 11. This result is to be expected since in the eserized preparation counter-effect of the inactivation of cholinesterase against the temperature effect on the amplitude of e.p.p. is lost.

3. Effect of temperature on the spontaneous miniature e.p.p.

It has been observed by some authors that the frequency of the spontaneous miniature e.p.p. was reduced by lowering the temperature (Fatt and Katz, 1952; Boyd and Martin 1956 *a*; Liley, 1956 *a*). The effect of temperature on the spontaneous miniature e.p.p. is presented in fig. 12, in which each record was obtained from the same muscle fibre in a hypertonic Ringer's solution (1.5 times of normal Ringer's solution by addition of sucrose), because in normal Ringer's solution the frequency of the miniature e.p.p. was so small that was difficult to photograph when the temperature was lowered to 17°–15° C. and both in the normal Ringer and hypertonic Ringer solution the effect of the temperature change on the frequency of the spontaneous miniature e.p.p.'s was almost the

same. The frequency (sec^{-1}) which was measured from two muscle fibres in normal Ringer's solution at the several temperatures was plotted in fig. 13. These curves were approximately linear, although the frequency differed from end-plate to end-plate. The temperature coefficient was 3.0-4.8 (mean of six experiments was 4.0) in the range of temperature 10° - 25°C . This is somewhat greater than that observed by Fatt and Katz (1952) on the frog muscle. It was not observed in the present experiment that the frequency-temperature curve had an upward concavity and minimum at 25°C . as described by Liley (1956 *a*) on the mammalian muscle.

FIG. 12

FIG. 12. Effect of temperature on the spontaneous miniature potentials. Records were obtained from the same muscle fibre in a hypertonic Ringer's solution (1.5 times of normal Ringer's concentration by addition of sucrose) at three temperatures.

FIG. 13

FIG. 13. Two examples of effects of temperature on the frequency of spontaneous miniature potentials obtained from two muscle fibres in normal Ringer's solution. Ordinate: frequency, sec^{-1} . Abscissa: temperature.

FIG. 14

FIG. 14. Effect of temperature on the amplitude of spontaneous miniature potentials. The bars indicate $2 \times \text{s.d.}$ Ordinate: mean amplitude, in mV. Abscissa: temperature.

A large increase in the frequency of miniature e.p.p. by raising the temperature was not changed by adding 6 mm magnesium to Ringer's solution. This means that an increase in the frequency of miniature e.p.p. at the higher temperature does not depend upon the depolarization of the membrane of the nerve terminals (Liley, 1956 *c*). It should be noticed that in fig. 13 the frequency-temperature curves represent the one which is obtained by lowering the temperature from the high point. When the temperature was raised from the lower point, the frequency temperature curve somewhat deviated to the right.

In the mammalian muscle Boyd and Martin (1956 *a*) reported that the mean amplitude of the miniature potentials at 20° C. was 50% greater than that at 37° C., but in some fibres no change in amplitude was observed by lowering the temperature, on the other hand Liley (1956 *a*) reported that no consistent relationship between peak amplitude and temperature was observed. The mean amplitude of spontaneous miniature e.p.p. obtained from the present investigation was plotted against the temperature in fig. 14, where the bars indicate $2 \times \text{s.d.}$ From the discussion above cited, it is to be expected that the amplitude of the miniature e.p.p. is greater at lower temperatures than at high temperatures. But the variation in amplitude is so great that it cannot be decided at present whether this is the case or not.

DISCUSSION

The factors which determine the amplitude of the e.p.p. are as follows: the resting potential and electrical characteristics of the muscle fibre membrane, the activity of cholinesterase, the sensitivity of end-plate to the transmitter and output of the transmitter from the nerve endings. The temperature had little influence on the resting potential of the muscle fibre between 10° C. and 20° C. (Ling and Woodbury, 1949; see Method), and the effect of temperature on the electrical properties of the muscle membrane was not sufficient to explain the change of the e.p.p. size (del Castillo and Machne, 1953; see Results). As described in 'Results,' the cholinesterase activity was reduced at the lower temperature. From this result it may be expected that the amplitude of e.p.p. tends to increase at the lower temperature. But this was not the case and the e.p.p. size was reduced at the lower temperature. As shown in fig. 4, the probability of appearance of the response at the neuromuscular junction was greater at the higher temperature. An increase in the probability means that the output of the transmitter from the nerve endings increases (del Castillo and Katz, 1954 *b*). So that it may be concluded that the increase in amplitude of the e.p.p. at the higher temperature depends mostly on the increase in the output of the transmitter. This conclusion may also be supported by following observations: a) The temperature effect on the amplitude of the e.p.p. was greater in Mg-treated muscle and was smaller in the preparation treated with a high calcium concentration as shown in fig. 3. It has been known that the output of transmitter was reduced in the Mg-treated muscle and increased in a high concentration of calcium (del Castillo and Stark, 1952; del Castillo and Engbaek, 1954; del Castillo and Katz, 1954 *a*), then it is possible to explain that the temperature change has a greater effect on the output of the transmitter when the output is reduced, but has a smaller effect when the output is great, as reported on the neuromuscular facilitation by muscle stretch (Hutter and Trautwein, 1956; Takeuchi, A., 1957; Takeuchi, N., 1957). b) The frequency of the spontaneous miniature potentials was remarkably increased at the higher temperature. It has been observed that in many cases an increase in the frequency of the miniature potentials is parallel with an increase in the output of the transmitter from the nerve endings (Liley, 1956 *b* and *c*; Hutter and Trautwein, 1956; Takeuchi, N., 1957). c) The depression of the e.p.p. after

stimulation was increased at the higher temperature and the depression was greater when the output of the transmitter was great (Takeuchi, 1958). d) The transmitter activity in the neuromuscular transmission, measured by a voltage clamp method, was markedly reduced when the temperature was lowered (Takeuchi and Takeuchi).

On the other hand the mean amplitude of the spontaneous miniature e.p.p. was little affected by the temperature change, and this suggests that the sensitivity of the end-plate to the transmitter is not altered appreciably by the temperature change. Although in the present investigation the sensitivity was not directly measured, it may be conceivable that at the lower temperature the sensitivity of end-plate for the transmitter is somewhat reduced and two antagonizing effects on the amplitude of the spontaneous miniature potentials, *i.e.* a decrease of the sensitivity and an inactivation of the cholinesterase, cancel each other.

In mammalian muscles Boyd and Martin (1956 *b*) reported that lowering the temperature decreased the e.p.p. size of curarized muscle but increased the size in the magnesium-treated preparation and also the quantum content of the transmitter. In the present investigation of the frog's e.p.p. the discrepancy of the temperature effect between the curarized and magnesium-treated preparations was not observed. The reason of the difference between the results in the present experiments and those obtained by Boyd and Martin (1956 *b*) cannot be decided at present, however, it should be noted that species and range of temperature observed, in both cases were different.

In the early experiments of Eccles, Katz and Kuffler (1941) it has been reported that the temperature coefficient for the duration of the rising phase was high ($Q_{10}=2.65$), but that for the time factor of decay of the e.p.p. was low ($Q_{10}=1.29$). In the present experiment the temperature coefficient for the rise time was high ($Q_{10}=2.7$) coinciding with their results, while that for the falling phase was not so low as reported by them but was considerably high ($Q_{10}=2.2$). In most cases, in order to increase the accuracy of measurement, the temperature coefficient for the falling phase was measured from the peak to the half decay time. And in some cases the temperature coefficient measured from the time of fall to $1/e$ was used, but remarkable difference was not observed between these two measurements. The difference between their results and the present results may be presumably due to a difference of the recording conditions. The large temperature coefficient for the rising phase indicates that active or chemical processes underlie the building up of the e.p.p. (Eccles *et al.*, 1941). The processes which are concerned in the time factor of the rising phase may be as follows, *i.e.* the nerve impulse and release mechanism of the transmitter at the nerve endings, the reaction of the transmitter with the receptor, the electrical change at the end-plate membrane and the action of the cholinesterase or diffusion which removes the transmitter from the receptor. Since it has been known that the temperature coefficient for the duration of the nerve impulse is large (Tasaki and Fujita, 1948; Hodgkin and Katz, 1948), at the lower temperature the release of the transmitter from the nerve endings may become slower. Furthermore a decrease in the reaction velocity of the

transmitter with the receptor and an inactivation of the cholinesterase may prolong the rising phase of the e.p.p. On the other hand it may be concluded that the high temperature coefficient for the time factor of decay of the e.p.p. is mostly due to prolongation of the transmitter action, and its main cause may be inactivation of the cholinesterase, because 1) the change in the electrical properties of the muscle membrane was not so large as to explain the high temperature coefficient for the decay time of the e.p.p. (del Castillo and Machne, 1953; see fig. 7), 2) the curve of displacement of electric charge on the surface membrane of the muscle fibre during the e.p.p. at low temperature rised slowly and did not show the exponential decay for about 8 msec. from the start, 3) the temperature coefficient for the falling phase of e.p.p. was made smaller by adding eserine to the bathing solution, 4) the eserinizaton augmented the rate of increase in the amplitude by raising the temperature, 5) in the e.p.p. obtained by sodium-deficiency, the rate of decrease in the amplitude by a fall of temperature was small and that of prolongation of the time course was large, this is to be expected since it has been known that the effect of anticholinesterase is remarkable in the e.p.p. blocked by lowering the external sodium concentration (Fatt and Katz, 1951), 6) it has been reported that the effect of eserine was reduced by the lowering of temperature (Eccles *et al.*, 1942). But it must be considered that del Castillo and Machne (1953) reported that the membrane resistance of the muscle fibre increased about 30% while the membrane capacitance decreased slightly by a 10° C. lowering of the temperature. Also in the present investigation the fact that the half decay time of the passive polarizing membrane potential tends to somewhat increase at the lower temperature coincides with their observation. This effect may also contribute to the increase in the decay time of e.p.p. by lowering the temperature.

From present results it may be concluded that the facilitation of the neuromuscular transmission by raising temperature is mainly due to an increase in output of the transmitter, but the mechanism of the increase in output is not decided from these results. In the sympathetic ganglion of cat, a decrease in the release of acetylcholine has been directly measured when the temperature is lowered (Brown, 1954; Kostial and Vouk, 1956), and since these results coincide in general with the present results, it seems that the temperature effect on the output of transmitter is not specific in the myoneural junction. It may be worth to notice that the resistance of nodal membrane in the medullated nerve fibre is increased by lowering temperature and the action current of nerve fibre is decreased at lower temperatures (Tasaki, 1955). But the change in the output of the transmitter is not only due to the impulse at the presynaptic nerve endings but also probably to amount of the available transmitter at the nerve endings or a release mechanism of the transmitter. On the other hand the parallelism between the frequency of the miniature e.p.p.'s and the magnitude of transmitter released by the nerve impulse was observed in the cases of lengthening of the muscle (Hutter and Trautwein, 1956; Takeuchi, N., 1957), post-tetanic potentiation (Liley, 1956 *b*), the polarization of nerve endings (del Castillo and Katz, 1954 *c*; Liley, 1956 *c*) and the temperature effect. These parallelisms may be a clue to clarify the mechanism of the release of th

transmitter from the nerve endings by the impulse.

SUMMARY

1. The effect of temperature on the neuromuscular transmission of the frog's sartorius muscle was studied, with the intracellular electrode, at temperatures between 10° C. and 20° C.
2. The end-plate potential diminished, in general, in amplitude as the temperature was lowered. The rate of change in the amplitude was large in the condition that the output of the transmitter from the nerve endings was reduced.
3. The probability of appearance of end-plate potential recorded from the preparation soaked in a solution of a low calcium and high magnesium concentration was smaller at lower temperature.
4. The effects of temperature on the time course of end-plate potential were considerably great, not only on the rising phase ($Q_{10}=2.7$), but also on the falling phase of end-plate potential ($Q_{10}=2.2$). The falling phase of polarized membrane potential was little influenced by temperature.
5. The time course of displacement of total charge during the end-plate potential was remarkably lengthened at lower temperature.
6. In the eserinated end-plate potential the temperature change had little effect on the falling phase, but the rate of change in the amplitude was greater than that of the non-eserinated end-plate potential.
7. The frequency of the spontaneous miniature end-plate potentials had a considerably high temperature coefficient, the mean Q_{10} being 4.0.
8. From these results it may be concluded that an increase in the amplitude of the end-plate potential at higher temperature is mainly due to an increase in output of the transmitter from the nerve endings, and the relatively high temperature coefficient of the falling phase of the end-plate potential is due to a prolongation of transmitter activity at lower temperature.

ACKNOWLEDGEMENT

I wish to express my thanks to Prof. S. Sakamoto and Prof. H. Mashima for their invaluable advice and encouragement. Thanks are also due to Prof. T. Wakabayashi of University of Tokyo for some valuable suggestions and to Dr. Ebashi for kindly supplying pure *d*-tubocurarine chloride for this study. I am also indebted to Mr. T. Miyagawa for his assistance in carrying out the experiments.

REFERENCES

1. BOYD, I. A. AND MARTIN, A. R. *J. Physiol.* 132: 61, 1956 *a*.
2. BOYD, I. A. AND MARTIN, A. R. *J. Physiol.* 132: 74, 1956 *b*.
3. BROWN, G. L. *J. Physiol.* 124: 26P, 1954.
4. DEL CASTILLO, J. AND STARK, L. *J. Physiol.* 116: 507, 1952.
5. DEL CASTILLO, J. AND MACHNE, X. *J. Physiol.* 120: 431, 1953.
6. DEL CASTILLO, J. AND ENGBAER, L. *J. Physiol.* 124: 370, 1954.
7. DEL CASTILLO, J. AND KATZ, B. *J. Physiol.* 124: 553, 1954 *a*.
8. DEL CASTILLO, J. AND KATZ, B. *J. Physiol.* 124: 560, 1954 *b*.

9. DEL CASTILLO, J. AND KATZ, B. *J. Physiol.* 124 : 586, 1954 c.
10. ECCLES, J. C., KATZ, B. AND KUFFLER, S. W. *J. Neurophysiol.* 4 : 362, 1941.
11. ECCLES, J. C., KATZ, B. AND KUFFLER, S. W. *J. Neurophysiol.* 5 : 211, 1942.
12. FATT, P. AND KATZ, B. *J. Physiol.* 115 : 320, 1951.
13. FATT, P. AND KATZ, B. *J. Physiol.* 117 : 109, 1952.
14. HODGKIN, A. L. AND KATZ, B. *J. Physiol.* 109 : 240, 1949.
15. HUTTER, O. F. AND TRAUTWEIN, W. *J. Physiol.* 133, 610, 1956.
16. JENKINSON, D. H. *J. Physiol.* 138 : 434, 1957.
17. KOSTIAL, K. AND VOUK, V. B. *J. Physiol.* 132 : 239, 1956.
18. LILEY, A. W. *J. Physiol.* 132 : 650, 1956 a.
19. LILEY, A. W. *J. Physiol.* 133 : 571, 1956 b.
20. LILEY, A. W. *J. Physiol.* 134 : 427, 1956 c.
21. LING, G. AND GERARD, R. W. *J. cell. comp. Physiol.* 34 : 383, 1949.
22. LING, G. AND WOODBURY, W. *J. cell. comp. Physiol.* 34 : 407, 1949.
23. NASTUK, W. L. AND HODGKIN, A. L. *J. cell. comp. Physiol.* 35 : 39, 1950.
24. TAKEUCHI, A. *Seitai no Kagaku* 8 : 133, 1957 (Japanese).
25. TAKEUCHI, A. *Jap. J. Physiol.* 8 : 102, 1958.
26. TAKEUCHI, A. AND TAKEUCHI, N. *J. Neurophysiol.* (in press).
27. TAKEUCHI, N. *Shinkei Shimpo* 2 : 166, 1957 (Japanese).
28. TAKEUCHI, N. AND MIYAGAWA, T. *J. Physiol. Soc. Jap.* 19 : 687, 1957 (Japanese).
29. TASAKI, I. *Amer. J. Physiol.* 181 : 639, 1955.
30. TASAKI, I. AND FUJITA, M. *J. Neurophysiol.* 11 : 311, 1948.