INFLUENCE OF TEMPERATURE ON THE TRANSMEMBRANE POTENTIAL OF ASCARIS MUSCLE CELLS

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INTRODUCTION

Since the initial work of Jarman (1959) on the electrophysiology of the somatic muscle cells of Ascaris lumbricoides, a considerable amount of information has been gathered on this subject. The available data indicate that the rhythmic action potentials which activate the contraction of those cells are of myogenic origin (see del Castillo, de Mello & Morales, 1967). In this paper, an attempt is made to correlate the transmembrane potential and the frequency of spike firing with temperature.

METHODS

Preparations. All the experiments have been performed on preparations of the body wall of Ascaris lumbricoides var. suum, obtained from a local slaughterhouse. A cylindrical fragment of the worm, about 2–3 cm. in length, was split open along one of the lateral lines. After removal of the intestine the resulting rectangular piece of tissue was pinned (cuticle side down) on a slab of Teflon inside a double-walled Perspex chamber.

Solutions. Ascaris tissue can be maintained in a functional condition immersed in an isotonic salt solution made by diluting 30 parts of sea water with 70 parts of distilled water (Hobson, Stephenson & Beadle, 1952). Although the ionic content of this solution differs from that of the perienteric fluid of Ascaris, it has been found suitable for electrophysiological work (Jarman, 1959; DeBell, del Castillo & Sanchez, 1963).

Temperature regulation. The temperature of the bathing solution was maintained constant by circulating water from a thermostatically controlled reservoir between the walls of the Perspex chamber, and it was measured with a thermistor placed close to the preparation. When the experiment required rapid thermal changes, 30 % sea water already at the desired temperature was passed directly through the bath until the temperature of the water circulating between the walls of the chamber reached the same value.

Electrical technique. Transmembrane and spike potentials were recorded from the muscle cell bellies with conventional intracellular microelectrodes filled with 3 M-KCl and connected via a high input impedance amplifier (Bioelectric Instruments, Inc.) to a Tektronix, type 502, cathode-ray oscilloscope.

Drugs. In some experiments D-tubocurarine was added to the 30 % sea water in a concentration of $10^{-4}$ (w/v).
RESULTS

Effect of temperature on the transmembrane potential

Figure 1 shows the results of a typical experiment. At the beginning the preparation was immersed in a bath kept between 22° and 20° C, and the resting potentials of twenty cells were measured three times at intervals of 20 min. Then the temperature was increased to 38° C, and two more series of twenty measurements were made.

Fig. 1. Relationship between transmembrane potential and temperature in one preparation. The centre of each circle is the average value of twenty measurements in separate cells; the standard error and the standard deviation of each set of measurements are represented, respectively, by the diameter of the circle and the length of the vertical lines.

Fig. 2. Distribution of the transmembrane potentials in twelve preparations. The distribution at 38° C represents about 500 measurements and that of 22° C represents about 300. Because of the different size of the two samples, the height of each bar is the percentage of measurements falling within each interval.
Finally, the temperature was lowered and two more groups of measurements were made. In this experiment the average transmembrane potentials recorded at 38 °C. were about 10 mV lower than those recorded at 20 °C.

Figure 2 shows the distribution of the transmembrane potentials of 800 cells; their average values at 22 °C. and 38 °C. were 33.61 ± 0.29 and 24.43 ± 0.37 mV. respectively. The spread of the values is partly due to a significant difference of about 4 mV. in the average transmembrane potential between preparations taken from males and females at all temperatures.

In another series of experiments, the transmembrane potentials of about 150 cells from a dozen preparations (males and females) were measured at 12.5 °C, 22 °C, 30 °C and 38 °C. The results (Fig. 3) show that between 38 °C. and 22 °C. there is an approximately linear increase in the transmembrane potential of about 0.53 mV/°C. as the temperature is lowered, but between 22 °C. and 12.5 °C. the average transmembrane potential remains unchanged.

![Fig. 3. Relationship between the average transmembrane potential and temperature in twelve preparations (open circles). The black circles represent a separate experiment performed in the presence of D-tubocurarine 10^{-4} (w/v). The diameter of the circles is the standard error of each set of measurements. The standard deviation is given by the length of the vertical lines.](image)

**Effect of D-tubocurarine on the transmembrane potential at different temperatures**

The addition of D-tubocurarine in a concentration of 10^{-4} (w/v) to the bathing solution, at 38 °C., results in an increase of the transmembrane potential of a few millivolts (del Castillo, de Mello & Morales, 1963). In our experiments the transmembrane potentials of 200 cells were measured at 20 °C. and 39 °C. in the presence of D-tubocurarine in the surrounding medium.

The results (Fig. 3) show first that the change in transmembrane potential per degree change in temperature is reduced by D-tubocurarine from 0.56 mV/°C. for the untreated cells to 0.31 mV/°C.; and second that around 20 °C. D-tubocurarine ceases to have an effect on the transmembrane potential.
Effect of temperature on the frequency of the spikes

Most preparations show a frequency of about 7 impulses/sec. at 40° C., although higher and lower frequencies are not unusual.

A variable reduction of the frequency of the spikes is seen as the temperature is reduced. Most preparations become silent as the temperature is lowered below 30° C. Occasionally, however, preparations are seen that remain active at lower temperatures. In one instance action potentials continued to fire at a rate of 0.25 impulses/sec. for some minutes after the temperature was lowered to 20° C.

![Fig. 4. Relationship between spike frequency and temperature in one muscle cell. The temperature, initially 39° C., was gradually lowered until the firing stopped. The average change in spike frequency as the temperature is lowered, represented by the broken line, is 0.28 spikes/sec. °C.](image)

A variable amount of accommodation in the increased firing rate is seen after an increase in temperature. Fresh preparations show a larger static component than those taken from worms collected 1 day or more before they are used. The static component is shown as a function of temperature in Fig. 4. This graph suggests a linear relationship between both parameters of about 0.3 impulses/sec. The dynamic response is probably much larger because frequencies up to 16 impulses/sec. have been observed after a rapid increase in temperature of 4° or 5° C. The $Q_{10}$ is about 2 for the static component.

**DISCUSSION**

**Effect of temperature on the transmembrane potential**

Del Castillo et al. (1963) have suggested that the membrane of cells showing spike activity is maintained in a partly depolarized state by a continuous liberation of acetylcholine from the nerve fibres of the dorsal and ventral nerve cords.

An increase in the values of the transmembrane potentials in presence of D-tubocurarine should be expected if, in effect, a continuous liberation of acetylcholine in the
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neuromuscular junctions takes place at a physiological temperature. Moreover, the observation that, at temperatures of 20° C. and below, d-tubocurarine ceases to have an effect on the transmembrane potentials suggests that the increase in membrane potential as the temperature is reduced is due to a gradual decrease in the liberation of depolarizing transmitter which would become exceedingly low at 20° C.

These results can be explained by two alternative mechanisms. Either there are cholinergic nerve cells that function as thermal receptors firing impulses at a rate proportional to the temperature, or there is a continuous liberation of acetylcholine from the motor nerve endings which is affected by the temperature in a manner similar to that described by Fatt & Katz (1952) in the amphibian motor terminals.

While we have no evidence in favour of the first suggestion, a low-amplitude noise, reminiscent of the synaptic noise caused by the spontaneous liberation of acetylcholine in the vertebrate myoneural junction, has been observed in Ascaris (del Castillo et al. 1967). Moreover, this noise decreases and breaks up into small depolarizations of about 1 mV. or less as the temperature is lowered. This phenomenon must be further investigated before any conclusions on its nature can be drawn, but it strongly supports the second possibility.

Effect of temperature on the frequency of the spikes

The decreased frequency of the spikes as the temperature is lowered is probably due to the increased transmembrane potential. Our data show a 0.6 impulse/sec./mV. decrease in the frequency as the transmembrane potential increases upon cooling. However, other factors may determine the generation of spike potentials since we have found that acetylcholine does cause a depolarization of the membrane at 20 °C without eliciting spike activity.

SUMMARY

1. It has been shown that there is an almost linear inverse relationship between the transmembrane potential of the somatic muscle cells of Ascaris lumbricoides and the bath temperature between 38° and 20° C.
2. It has also been shown that the spike frequency reflects these changes, decreasing at a rate of 0.6 impulse/sec./mV.
3. d-Tubocurarine interferes with the response of these cells to thermal changes between 38° and 20° C. but it is ineffective below 20° C.
4. It is proposed that the presynaptic nerve fibres are responsible for the thermal sensitivity of the muscle cells, since the amount of depolarizing neurohormone spontaneously released at the neuromuscular junctions would depend upon the temperature.

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REFERENCES


