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THE EFFECT OF TEMPERATURE ON NEUROMUSCULAR TRANSMISSION IN THE VAS DEFERENS OF THE GUINEA-PIG

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In the isolated hypogastric-nerve–vas-deferens preparation submaximal nerve stimulation produces a small depolarization of the smooth muscle cell membrane. This so-called 'junction potential' (Burnstock & Holman, 1961) is also produced by field stimulation of the muscle with stimuli too short to excite the muscle fibres directly (Kuriyama, 1963). Repetitive stimulation of the hypogastric nerve or repetitive field stimulation enhances the amplitude of these junction potentials. In this respect it resembles the end-plate potential of skeletal muscle. However, in contrast to the end-plate potential, the junction potential is also increased by increasing the strength or the duration of the stimulus. It is therefore likely that several nerve fibres innervate a single smooth-muscle cell and that more than one must be excited before the junction potential reaches the threshold for spike generation. This conclusion is supported by a detailed study of facilitation in this preparation (Burnstock, Holman & Kuriyama, 1964).

The experiments to be described in this paper were designed to observe the effect of temperature on neuromuscular transmission in smooth muscle, by studying separately, as far as possible, its effect on each of the different stages of the process of transmission of excitation from the nerve to the muscle cell.

METHODS

Experimental methods were the same as in the previous paper (Kuriyama, 1963). To elicit junction potentials a short pulse (0·01–0·1 msec) was applied to the nerve or muscle. To obtain spike potentials the duration was increased to 5 msec. All changes in temperature were made from an initial standard temperature of 36° C. The Q_{10} values in this paper were calculated from the ratio of measurements made at high to those at low temperature.

The normal Krebs's solution used in all experiments contained (mM): Na 137·4; K 5·9; Mg^{2+} 1·2; Ca^{2+} 2·5; Cl^- 134; $H_2PO_4^-$ 1·2; HCO_3^- 15·5; glucose 11·5; it was bubbled with 97 % O_2 and 3 % CO_2.

RESULTS

Spontaneous brief depolarizations

Spontaneous brief depolarizations of the muscle membrane, as described by Burnstock & Holman (1961, 1962*a*, *b*) have been observed in the present experiments. The amplitude varied from the noise level (less than 1 mV) to more than 10 mV. It remains uncertain whether they are comparable to 'miniature end-plate potentials', since their amplitude was sometimes twice that of the junction potentials triggered by nerve stimulation (Fig. 1*a*). The duration of the brief spontaneous potential

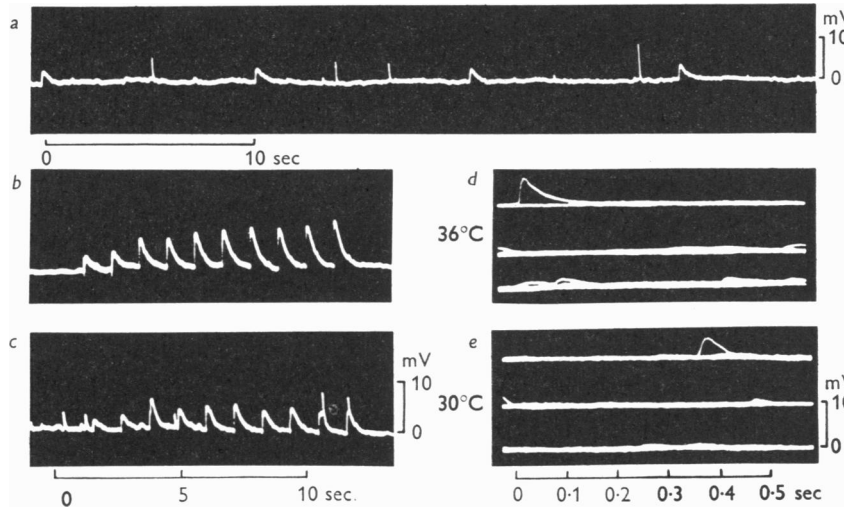

Fig. 1. Intracellular records from smooth-muscle cells in the guinea-pig's vas deferens. *a* Spontaneous brief depolarizations and junction potentials evoked by hypogastric nerve stimulation (0·1 c/s, 10 V, 0·01 msec). *b* Facilitation of junction potentials in the absence and *c* in the presence of frequent spontaneous depolarizations. *d* and *e* Spontaneous brief depolarizations recorded at 36 and 30° C. For explanation see text.

changes varied from 50 to 140 msec (mean value 75 msec). Their mean interval was 3·2 sec ($n = 130$) at 36°C.

The brief spontaneous depolarizations appeared not only during the resting state but also superimposed on the potential changes produced by nerve stimulation (Fig. 1*a*, *c*). There was an interaction between the spontaneous potentials and those produced by electrical stimulation. The frequency of the brief spontaneous depolarizations was increased during hypogastric nerve stimulation at low rates (from 0·1 to 0·5 sec) (cf. Kuriyama, 1963, Fig. 1), while nerve stimulation at higher rates (more than 2/sec) decreased the frequency of spontaneous depolarizations. The

amplitude and regularity of the junction potentials produced by repetitive electrical stimulation was influenced by the frequency of the brief spontaneous depolarizations. This is shown in Fig. 1*b* and *c*. Fig. 1*b* shows junction potentials elicited by 10 sec stimulation at 0·75/sec. The progress of facilitation was smooth when spontaneous depolarizations were rare (*b*), but, with the same conditions of stimulation, the junction potentials were smaller and irregular when spontaneous depolarizations were more frequent (*c*).

TABLE 1. Frequency (per second) of spontaneous brief depolarization of the membrane at different temperatures

Temp. ...	30°C	35° C	38° C
No. 1	0·12	0·32	0·40
2	0·06	0·11	0·20
3	0·21	0·40	0·59
4	0·18	0·30	0·51
5	0·08	0·21	0·44
6*	0·25	0·44	0·61
7*	0·20	0·49	0·60
8*	0·28	0·41	0·59

* With stimulation at 0·1 c/s.

Since the frequency of the brief spontaneous depolarizations varied greatly between individual cells, the results are taken only from those experiments in which the micro-electrode was kept in the same cell during the change of temperature. At temperatures less than 25° C no spontaneous depolarization was observed, and at 30° C it appeared rarely. The investigation therefore covers the range between 30 and 38° C. Table 1 shows the effect of temperature on the frequency of spontaneous depolarizations under resting conditions, and during low-frequency hypogastric nerve stimulation (0·1/sec) for 60 sec. The mean Q_{10} was $3{\cdot}8 \pm 0{\cdot}61$.

Lowering the temperature from 35 to 30° C reduced also the amplitude of the spontaneous depolarizations. Below 30° C the amplitude was usually not more than 5 mV. An example is shown in Fig. 1 at 36° C (*d*) and 30° C (*e*). The rate of rise and fall of the depolarizations was reduced. However, the duration did not exceed twice the normal duration.

Junction potentials

Figure 2 shows the junction potentials recorded from the same cell stimulated repetitively (0·5/sec) through the hypogastric nerve, at two different temperatures (36 and 20° C). The latency increased in this cell from 24 msec at 36° C to 36 msec at 20° C, the stimulating electrodes on the nerve fibres being 10 mm from the impaled cell. The rising phase was prolonged from 25 msec at 36° C to 85 msec at 20° C. The maximum rate of depolarization at 36° C was 0·32 V/sec for the first and 0·8 V/sec for

the fifth junction potential produced by stimulation at 0·5 sec. At 20° C the rate of depolarization was only 0·12 V/sec for the first and 0·15 V/sec for the fifth junction potential. Facilitation can be expressed as the ratio (fifth junction potential):(first junction potential). It was 1·9 at 36° C, but only 1·1 at 20° C.

Figure 3 shows the effect of temperatures (20–38° C) on the amplitude and 'half duration' (from $\frac{1}{2}$ maximum to peak) of the rising and falling phase of the junction potential produced by hypogastric nerve (*A*) and field stimuli (*B*) at 10 V and 0·1 msec pulse duration. The stimulating

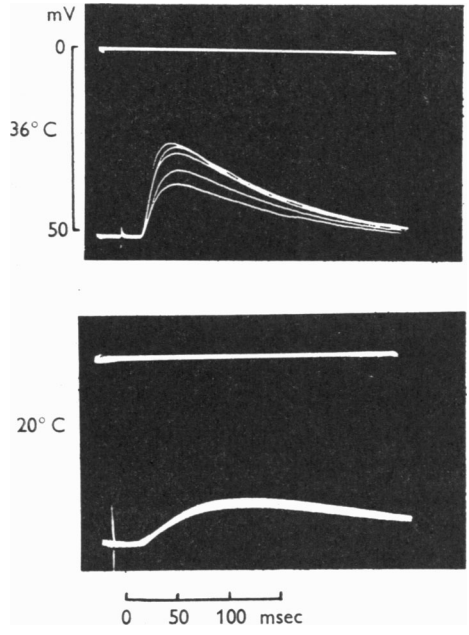

Fig. 2. Junction potentials recorded in response to repetitive hypogastric nerve stimulation (0·5 c/s, 10 V, 0·01 msec) at 36 and 20° C. Each record shows superimposed five junction potentials.

electrodes were placed on the hypogastric nerve fibres at 20 mm, and on the muscle fibre for field stimulation at 1–2 mm, from the impaled cell. The effects of temperature were nearly the same with both types of stimulation. A minor difference was in the lowest temperature at which junction potentials could be elicited by the two forms of stimulation. At 21–20° C hypogastric nerve stimulation with 0·01 msec pulses failed to elicit junction potentials, while field stimulation was still effective and only failed at 18° C. The Q_{10} (20–32° C) of the amplitude, 'half duration' of the rising and falling phase and the maximum rates of rise and fall of the junction potentials produced by hypogastric nerve stimulation were

calculated as 2·2, 0·39, 0·63, 5·7 and 3·5, respectively, and the Q_{10} for junction potentials produced by field stimulation were calculated as 2·2, 0·34, 0·62, 6·4 and 3·9. The Q_{10} values of the latencies to evoke the junction potentials by hypogastric nerve and field stimulations were calculated as 0·50 and 0·55, respectively.

Figure 4 shows the effect of temperature on the facilitation of junction potentials produced by low-frequency field stimulation (0·1 to 1 c/s, 10 V, and 0·05 msec pulse duration). The maximum amplitude, after repetitive

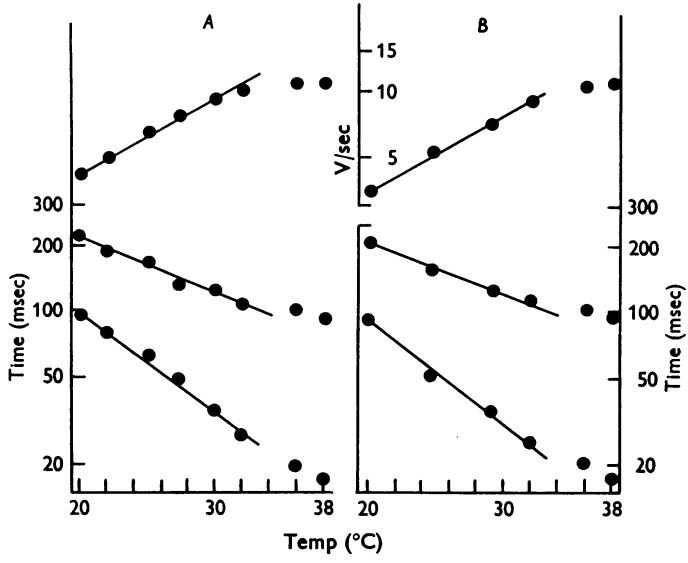

Fig. 3. Effect of temperature on amplitude (upper curve) and durations of the falling (middle curve) and rising phase (lower curve) of the junction potentials, recorded in response to hypogastric nerve stimulation (10 V, 0·05 msec) *A*, and field stimulation of the muscle *B*. Semi-log. scales.

stimulation at five different rates and four different temperatures is shown in Fig. 4*a*.

At 36° C stimulation at 0·1 c/s does not usually increase the amplitude of junction potentials, but facilitation occurs at frequencies from 0·25 c/s upwards. Figure 4*a* shows that lowering of temperature to 20° C reduces the size of the junction potential and prevents facilitation even at the higher frequencies of stimulation.

The amplitude and the rate of rise of junction potentials produced in the same cell by the first to the tenth stimulus at four different temperatures is shown in (*b*) and (*c*) (1 c/s, 10 V, and 0·05 msec duration). The rise times remained the same during repetitive stimulations, so that the changes in maximum rate of rise are proportional to the changes in

amplitude of the junction potentials at the same temperature (Fig. 4*b*, *c*). The Q_{10} of the amplitude and rates of rise and fall of the tenth junction potential, at 1 c/s, was higher (2·7, 7·9 and 4·6, respectively) than the Q_{10} of the first (2·2, 6·4 and 3·9, respectively).

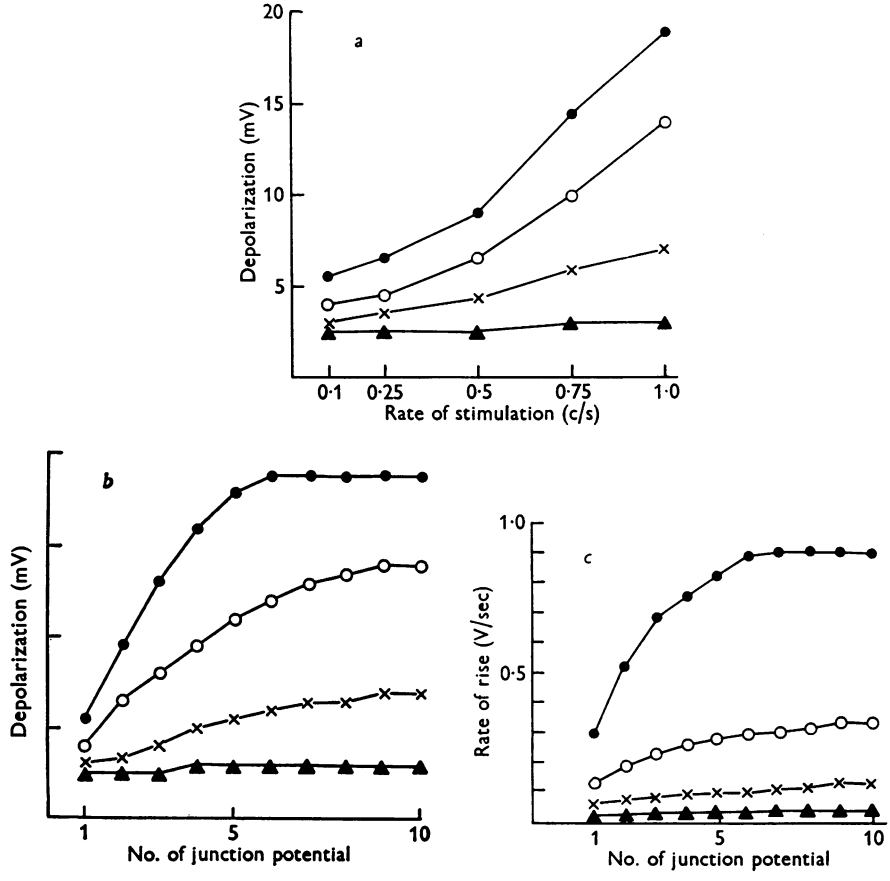

Fig. 4. The effect of temperature on the facilitation of the junction potentials evoked by stimulation (10 V, 0·05 msec). Temperatures as indicated (36 ●, 30 ○, 25 ×, and 20° C ▲). *a* shows the maximum depolarization of the membrane after facilitation was complete. Abscissa, rate of stimulation (0·1–1·0 c/s); ordinate, maximum depolarization. *b* abscissa, number of junction potential; ordinate, depolarization (mV); *c* as *b*, ordinate, rate of rise (V/sec). For details see text.

Effect of temperature on the smooth muscle membrane

The effects of temperature on the membrane potential and the action potential are illustrated in Fig. 5. Action potentials were elicited by single field stimuli to the muscle. At 36° C a stimulus of 1 msec triggered the spike, but when the temperature was lowered to 20° C the threshold for

spike generation increased and the pulse duration was increased to 5 msec. The mean membrane potential measured in 5 different preparations was highest (62·5 mV ± 0·81 S.E.) at 32° C, 48 mV ± 0·73 at 20° C, and 58 mV ± 0·59 at 36° C. Between 22 and 32° C the mean Q_{10} for the membrane potential was 1·3. The mean spike amplitude was 72 mV ± 0·75 at 36° C and fell to 42 mV ± 0·93 at 20° C, but in the range from 38 to 32° C the 'overshoot' potential was scarcely affected. Between 26 and 36° C the

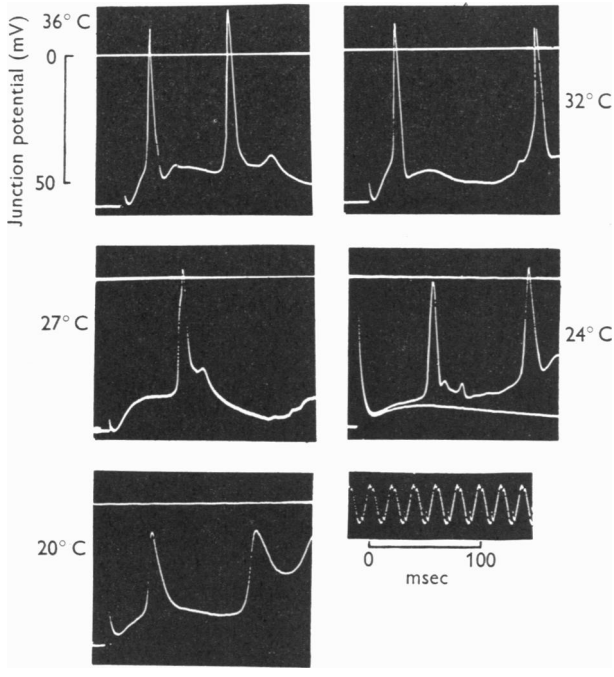

Fig. 5. The effect of temperature on the junction potential and spikes evoked by field stimulation (10 V; 1 sec, 36, 32 and 27° C, and 5 msec, 24 and 20° C). Continuous line shows zero potential. Note, at 24° C failure of first junction potential to trigger spike. The second spike produced by stimulation shows higher amplitude than the first spike; this may be due to the movement of the tissue.

mean Q_{10} for the spike amplitude was 1·5. Below 25° C the overshoot potential could not be observed. In contrast, the rising and the falling phases of the spike were progressively slowed as the temperature was lowered from 38 to 26° C. The 'half-duration' of the spike was prolonged from 5·8 msec at 36° C to 21 msec at 20° C. The maximum rates of rise and fall of the spike fell from 18 and 14 V/sec at 36° C, to 2·4 and 2·1 V/sec at 20° C. The main results of this investigation on each of the different stages of the process of transmission of excitation from the nerve to muscle cell are summarized in Table 2.

TABLE 2. Mean Q_{10} on the hypogastric-nerve–vas-deferens preparation

	Frequency	Amplitude	RP	RR	FP	RF
Brief spontaneous depolarization (30–38° C)	3·8	—	—	—	—	—
Junction potential (20–32° C)						
Single N*	—	2·2	0·39	5·7	0·63	3·5
Single F**	—	2·2	0·34	6·4	0·62	3·9
At max. facil. F**	—	2·7	—	7·9	—	4·6
Action potential (25–36° C)	—	1·5	0·53	2·8	0·52	2·9
Membrane potential (22–32° C)	—	1·3	—	—	—	—

* N indicates junction potential evoked by hypogastric nerve stimulation; ** F indicates junction potential evoked by field stimulation of the muscle. RP Rising phase at half amplitude; RR rate of rise; FP falling phase at half amplitude; RF rate of fall.

DISCUSSION

The mechanism of the miniature end-plate potential (m.e.p.p.) in skeletal muscle has been extensively studied (see review by Katz, 1962); it probably involves the spontaneous release of quantal units of acetylcholine from the nerve terminal without electrical changes in the nerves. The question remains whether the spontaneous brief depolarizations in the vas deferens are analogous to the m.e.p.p. The present results show that temperature changes have a similar effect on the frequency and duration of both the m.e.p.p. of some striated muscles and the spontaneous brief depolarizations. Thus, the Q_{10} value for the frequency of the spontaneous brief depolarizations was 3·8, a value within the range (3–5) reported for the m.e.p.p.'s of frog skeletal muscle (Fatt & Katz, 1952; Takeuchi, 1958) and the rat diaphragm (Liley, 1956). The one exception is the muscle of the guinea-pig diaphragm, where the Q_{10} for the m.e.p.p. was reported to be 2·1 (Brooks, 1956).

The total duration of the spontaneous brief depolarizations is much longer than that of the m.e.p.p. perhaps because the time taken to inactivate noradrenaline might be much longer than that taken to inactivate acetylcholine (Brown & Gillespie, 1957). However, the effect of temperature on the duration of the spontaneous brief depolarizations in the vas deferens is similar to that observed for the m.e.p.p. of skeletal muscle (Boyd & Martin, 1956; Li, 1958). Lowering the temperature prolongs both the m.e.p.p. and the spontaneous brief depolarization.

Despite these similarities, the origin of the spontaneous brief depolarization remains uncertain. Noradrenaline may be released from a number of different nerve terminals ending on the same cell or from numerous points along the fine terminal nerve filaments running within the muscle, or the autonomic ground plexus. Electronmicrographs show in many places a close apposition of the nerve and muscle cell membrane without

interposition of Schwann cells (Hillarp, 1959; Burnstock & Holman, 1961; Richardson, 1962). The present results again confirmed earlier observations (Burnstock & Holman, 1962*a*, *b*) that the brief depolarizations vary greatly in amplitude. Multiple innervation could give rise to a variety of different amplitudes solely because of the different distances between the nerve terminals and the micro-electrode. We have no means of determining this distance. Further, we cannot exclude the possibility that the large brief depolarization may result from electrotonic spread of a spike from more distant structures (e.g. Fig. 1*c*).

The effect of temperature on the amplitude and the rising phase of the junction potential in the vas deferens is similar to that on the end-plate potential (e.p.p.) in curarized skeletal muscle (Eccles, Katz & Kuffler, 1941; Fatt & Katz, 1952; Takeuchi, 1958). The slower rising and falling phases of the junction potential compared with the e.p.p. could be due to the different chemical transmitter and different processes for inactivation.

Repetitive stimulation of the nerve fibres enhanced the amplitude of the junction potentials, i.e. facilitation occurred. It is likely that this is due to an increased release of chemical transmitter with successive nerve impulses (Burnstock *et al.* 1964). This process may be suppressed by cooling.

SUMMARY

1. The effects of temperature on excitation in the hypogastric-nerve–vas-deferens preparation of the guinea-pig were observed with the micro-electrode technique. Temperatures of the bathing solution were varied from 20 to 40° C.

2. The mean frequency of the spontaneous brief depolarizations of the muscle membrane was 0·31/sec at 36° C and the Q_{10} was 3·8 between 30 and 40° C. The amplitude was lowered and the duration of the rising and falling phase was prolonged by cooling.

3. The effects of temperature on the junction potentials produced by hypogastric nerve stimulation and by field stimulation of the muscle were identical.

(*a*) When the temperature was lowered to 20° C, no facilitation was produced by repeated stimulation up to 1 sec.

(*b*) The amplitude was reduced and the duration of the rising and falling phase of the junction potential was prolonged by cooling.

4. The Q_{10} of the amplitude and the rates of rise and fall of the junction potentials at the point of maximum facilitation were 2·7, 7·9 and 4·6, respectively. These Q_{10} values were higher than for a single junction potential (2·2, 6·4 and 3·9, respectively).

5. The membrane potentials and the spike amplitude were reduced by lowering the temperature below 32° C; the overshoot potential could not be observed below 25° C. The Q_{10} of the membrane potential and the spike amplitude were 1·3 and 1·5, respectively. The rates of rise and fall of the spike fell from 36 to 20° C, the Q_{10} being 2·6 and 2·7, respectively.

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