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TEMPERATURE AND INHIBITORY JUNCTIONAL TRANSMISSION IN GUINEA-PIG ILEUM

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The effects of temperature on the inhibitory junctional potential (i.j.p.) and the electrotonic potential, recorded in the circular smooth muscle of the guinea-pig ileum, were studied with intracellular microelectrodes. The amplitude and time course of the i.j.p. were both dependent on the ambient temperature, the i.j.p. becoming smaller and more prolonged as the temperature was lowered. In contrast, the electrotonic potential was not much affected by temperature. The atropine-resistant 'late' depolarization was also dependent on temperature. These results are consistent with the mechanism of release, of the nonadrenergic noncholinergic inhibitory transmitter, being sensitive to temperature and with the inhibitory conductance change, underlying the i.j.p., being long compared to the membrane time constant of the circular smooth muscle.

Introduction The summation of subthreshold excitatory and inhibitory postsynaptic potentials forms the fundamental process by which a single neurone can integrate a number of converging inputs. The initiation of an action potential in the postsynaptic cell is determined by the nature and frequency of the subthreshold potentials. At the neuromuscular junction the endplate potential is associated with a brief conductance change of a few milliseconds, the time course and spread of the endplate potential along the muscle fibre being determined by the passive properties of the muscle fibre (Fatt & Katz, 1951). On the other hand junctional potentials, excitatory and inhibitory, recorded in smooth muscle fibres have generally been associated with a conductance change that lasts throughout the recorded potential transient (300 to 500 ms) (Bennett, 1972). Recently, however, long-lasting excitatory junctional potentials have been recorded in several smooth muscles which result from conductance changes shorter than their membrane time constants (<250 ms) (Bywater & Taylor, 1978; Hirst & Neild, 1978; Supernant, 1978). In the present experiments the effects of temperature on the electrotonic potential and the inhibitory junctional potential (i.j.p.), recorded in the circular muscle of the guinea-pig small intestine, are consistent with the inhibitory conductance change being long compared to the membrane time constant of the circular smooth muscle. The duration of the inhibitory conductance change could not be determined, being confounded by the presence of a 'late', atropine-resistant depolarization (Bernath, Bywater, Holman, Supernant & Taylor, 1977; Lang, 1979).

Methods A thin strip (2 mm wide) of guinea-pig small intestine, cut in the circular direction, was mounted between two large extracellular polarizing electrodes; a portion of the strip protruded, through a small hole in one of the electrodes, into a recording chamber (Abe & Tomita, 1968; Holman, Taylor & Tomita, 1977). Short current pulses (0.2 to 0.5 ms), of strong intensities, were used to stimulate the intramural nerve fibres, while long pulses (1 to 2 s), of weak intensities, were used to polarize the smooth muscle fibres. Intracellular recordings were made from the circular smooth muscle with glass micropipettes filled with 2 M KCl and placed 1 mm from the nearest stimulating electrode. Physiological saline flowed continuously through the stimulating and recording chambers, atropine sulphate (0.12 μg/ml) was present in all experiments. The temperatures used were 25, 30 and 35°C; preparations were equilibrated for 30 min at a new temperature before recordings were made. Eight or sixteen responses to either long polarizing pulses or short transmural stimuli were averaged on a Biomac 1000 and then plotted on linear coordinates. Changes with temperature in the time to half-maximal amplitude of the electrotonic potential were assumed to reflect changes in the membrane time constant, the length constant being previously suggested to be insensitive to temperature (Bolton, 1974). The decay phase of the i.j.p. could not be described by a single exponential owing to the presence of the 'late' depolarization. Thus, changes in the time course of the i.j.p. with temperature were quantified by changes in the peak amplitude, latency (stimulus artifact to 10% peak amplitude), time to peak amplitude (10% to 90% peak amplitude) and half-amplitude duration.

Results Lowering the temperature from 35° to 25°C significantly decreased the amplitude of the i.j.p. ($P < 0.01$, Student's t test) (see Table 1) without any significant change in the resting membrane potential (Lang, 1979). Assuming a linear relationship

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between i.j.p. amplitude and temperature, a Q_{10} of 1.7 was obtained. The time course of the i.j.p. was also slower at 25°C. The latency, time to peak amplitude and half-amplitude duration of the i.j.p. all significantly increased when the temperature was lowered ($P < 0.01$), having Q_{10}s of 2.7, 2.1 and 1.9 respectively (see Table 1). It should be noted that the 'late' depolarization was also dependent on temperature, decreasing in amplitude with cooling and sometimes disappearing at 25°C. This 'late' depolarization is not 'myogenic rebound' as it is still present when the i.j.p. is blocked with apamine (Vladimiora & Shuba, 1978) or when the membrane potential of the smooth muscle fibres is displaced close to the 'reversal potential' of the i.j.p. (Bernath *et al.*, 1977). On the other hand, the time to half maximal amplitude of the electrotonic potential did not significantly change with cooling, being 48 ± 8 (7) ms at 35°C and 69 ± 6 (6) ms at 25°C ($P > 0.05$).

Discussion The decrease in amplitude and increase in latency of the i.j.p. with cooling are consistent with a mechanism of release of inhibitory transmitter which is sensitive to temperature, the release of transmitter being reduced and prolonged when the temperature is lowered (Katz & Miledi, 1965). If the activation of the terminals of the inhibitory nerves and the release and diffusion of inhibitory transmitter to the postjunctional membrane were similar to those at adrenergic smooth muscle junctions (<20 ms; Bennett, 1972), a large portion of the latency before the i.j.p. must be associated with the postjunctional reactions which give rise to the inhibitory conductance change. The large Q_{10}s of the latency and time to peak amplitude of the i.j.p. are consistent with a chemical reaction being the rate-limiting step in the activation of the inhibitory conductance change. Much lower Q_{10}s would be expected if the diffusion of the inhibitory transmitter to the postjunctional receptors was the rate-limiting step in the activation of the inhibitory conductance change.

If the inhibitory conductance change was short, compared to the membrane time constant, the decay of the i.j.p. would be 'passive', being dependent on the membrane time constant and showing a temperature-dependence similar to that of the electrotonic potential. As the temperature-dependence of the half-amplitude duration of the i.j.p. was greater than the electrotonic potential, it can be suggested that the time course of the i.j.p. was not determined by the passive properties of the circular smooth muscle. The simplest explanation of the effects of temperature on the half-amplitude duration seems to be that the inhibitory conductance change is long compared to the membrane time constant, i.e. that there is an 'active' decay of the i.j.p. Such a high temperature-dependence also suggests that the rate-limiting step in the deactivation of the inhibitory conductance change is a chemical reaction and not simply diffusion of the inhibitory transmitter from the postjunctional receptor region. Alternatively, the high Q_{10} of the half-amplitude duration could merely reflect the temperature dependence of the 'late' depolarization, the half-amplitude duration being reduced as the contribution of the 'late' depolarization to the potential transient is increased with temperature. However, as the decay of the i.j.p. could not be described by a single exponential at 25°C, when the 'late' depolarization has disappeared, it seems likely that the duration of the conductance change, underlying the nonadrenergic, noncholinergic i.j.p., is long compared to the membrane time constant. Thus, while excitatory junctional potentials may result from 'short' conductance changes of the postjunctional membrane, the advantages of 'long-lasting' inhibitory conductance changes, keeping the smooth muscle fibres from their thresholds, can still be envisaged.

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Table 1 Effect of temperature on the time course of the inhibitory junctional potential

Temperature (°C)	*Amplitude* (mV)	*Latency* (ms)	*Time to peak amplitude* (ms)	*Half-amplitude duration* (ms)
25	8.1 ± 0.8* (13)	213 ± 11 (16)	187 ± 11 (16)	667 ± 34 (16)
30	11.7 ± 1.8 (3)	112 ± 9 (3)	122 ± 10 (4)	429 ± 12 (4)
35	13.4 ± 1.2 (16)	80 ± 4 (13)	90 ± 6 (13)	337 ± 6 (13)

* Mean ± one standard error of the mean. Number of observations are given in parentheses.

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