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THE EFFECTS OF CALCIUM AND MAGNESIUM IONS, TEMPERATURE AND REPETITIVE STIMULATION ON INHIBITORY JUNCTIONAL TRANSMISSION IN SMOOTH MUSCLE OF GUINEA-PIG SMALL INTESTINE

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1 The effects of calcium and magnesium ions and temperature on the peak amplitude of the nonadrenergic, noncholinergic inhibitory junction potential, evoked by a single stimulus, or paired transmural stimuli, were examined in the circular muscle of guinea-pig small intestine.

2 The peak amplitude of the inhibitory junction potential (i.j.p.) could be decreased by lowering the external concentration of calcium or by raising the external magnesium concentration (at 25°C).

3 At 25°C, the second of a pair of i.j.ps was larger than the first at short intervals (<0.2 s), but smaller at larger intervals (0.2 to 20 s). Enhancement of the second (test) response decayed exponentially with a time constant of 87 ms. Depression of the test i.j.p. was maximal at 0.4 s and then recovered exponentially with a time constant of 11 s.

4 In low calcium or high magnesium solution, depression of the test i.j.p. decreased without any change in the rate of recovery from depression.

5 Despite the slow rate of recovery from depression after a single conditioning response, transmitter output could be maintained during low-frequency repetitive stimulation.

6 The peak amplitude of the i.j.p. increased as the temperature was raised to 35°C (Q_{10} = 1.5).

7 In contrast to the neuromuscular junction, the depression of the second of a pair of i.j.ps decreased as the temperature was raised. At 35°C the test i.j.p. was larger than the conditioning i.j.p. for most stimulus intervals (1 to 20 s).

8 The results suggest that the rate of replenishment of the store of inhibitory transmitter is sensitive to both temperature and repetitive stimulation.

Introduction

Inhibitory junction potentials (i.j.ps) can be recorded in the circular muscle of the guinea-pig small intestine in response to transmural stimulation in the presence of atropine, or to distension of an orally-situated segment of small intestine (Hirst & McKirdy, 1974). These potentials are blocked by tetrodotoxin, but are not affected by cholinergic or adrenergic antagonists such as atropine or guanethidine (Bennett, 1972) or by extrinsic denervation (Furness, 1969a). I.j.ps have been shown to be due to an increased membrane conductance for potassium ions (Bennett, Burnstock & Holman, 1963; Tomita, 1972). The i.j.p. was originally reported to be insensitive to the external concentration of calcium and magnesium (Hidaka & Kuriyama, 1969). However, Holman & Weinrich (1975) showed that the peak amplitude of the i.j.p. increased as the external concentration of calcium was raised or as the magnesium concentration was lowered. The amplitude and time course of the summed hyperpolarization recorded with the sucrose gap, in response to repetitive field stimulation (30 Hz), have been reported to increase with temperature (Jager & Den Hertog, 1974). In the present experiments the effects of calcium/magnesium and temperature on the release of the noncholinergic, nonadrenergic inhibitory transmitter, evoked by single and paired transmural stimulating pulses, have been examined in more detail. The effects of low-frequency repetitive stimulation have also been examined.

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Methods

Guinea-pigs of either sex were stunned, bled and a segment of mid small intestine excised. A piece (1 to 2 cm long) of this was cut along its mesenteric border and pinned in an organ bath with the serosal surface uppermost. Physiological saline (see Table 1), gassed with 95% CO_2 and 5% O_2, flowed continuously through the organ bath (2 ml) at a rate of 2 ml/min. The saline was kept at 25°C unless otherwise stated. Atropine sulphate (0.06 to 0.12 μg/ml) was present in all experiments. Intramural nerves were stimulated by two fine platinum electrodes, one placed in the base of the organ bath, the other directly above the preparation. Both electrodes were insulated with Araldite leaving only their tips exposed. Supramaximal pulses of 0.2 ms duration were delivered to stimulate as many of the nerve axons as possible, using voltages which did not produce direct stimulation of the smooth muscle. Intracellular recordings were made from the circular muscle layer with glass microelectrodes filled with 2 to 3 M KCl, having resistances of 40 to 100 megohms and generally placed 3 to 5 mm from the stimulating electrodes.

The concentration of calcium and magnesium was varied between 0.25 and 25 mM and 1 and 25 mM respectively (see Table 1). Each preparation was exposed to a control solution before and after exposure to a test solution. All preparations were allowed to equilibrate for at least 15 to 30 min in the new solution before recordings were made. In some cases a serial change in the ionic composition was made to keep the time necessary to reach a new steady state to a minimum (e.g. 2.5, 1.25, 0.25, 2.5 mM Ca^{2+} or 1, 5, 15, 1 mM Mg^{2+}). The total concentration of divalent ions was usually not allowed to fall below 2 mM to avoid changes in excitability of the inhibitory nerves or the smooth muscle (Frankenhauser & Hodgkin, 1957). In other experiments the temperature was varied between 25 and 35°C (25, 27.5, 30 and 35°C) in normal solution. Again all preparations were exposed to the control temperature (25°C) before and after exposure to a different temperature. In all cases an experiment was discarded if the observations in the control solutions before and after the procedure were not comparable.

Assuming that the i.j.p. results from a long conductance increase (ΔG) for potassium ions (Tomita, 1972) and that the Nernst potential for potassium (E_K) was −90 mV (Casteels, 1969) a correction for non-linear summation was applied to all inhibitory responses such that

$$\text{i.j.p.}' = \text{i.j.p.}\left(1 - \frac{\text{i.j.p.}}{V_0}\right)^{-1}$$

where i.j.p.′ is the 'corrected' i.j.p. amplitude and $Vo = RMP - E_K$ (RMP = resting membrane potential) (Martin, 1955). If ΔG is assumed to reflect directly the release of inhibitory transmitter, then

$$\frac{\Delta G}{G} = \frac{\text{i.j.p.}'}{V_0},$$

where G is the resting membrane conductance. The peak amplitudes of the i.j.p., in the various calcium/magnesium solutions, were therefore corrected for nonlinear summation and averaged. At least eight (8 to 32) i.j.ps were included in each mean value. The mean amplitude of the 'corrected' i.j.p. was then expressed as a fraction of the mean 'corrected' i.j.p. amplitude recorded in normal solution and this fraction was termed the normalized i.j.p. amplitude. During pairs of transmural stimuli the 'corrected' ampli-

Table 1 Composition of physiological saline

	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	$H_2PO_4^-$ (mM)
Normal Ca^{2+}	146	5	2.5	2	134	25	1
1/8 Ca^{2+}	146	5	0.31	2	129.2	25	1
1/5 Ca^{2+}	146	5	0.5	2	130	25	1
1/2 Ca^{2+}	146	5	1.25	2	131.5	25	1
2 Ca^{2+}	146	5	5	2	139	25	1
4 Ca^{2+}	133.5	5	10	2	149	12.5*	1
10 Ca^{2+}	133.5	5	25	2	179	12.5*	1
Normal Mg^{2+}	146	5	2.5	1	132	25	1
2 Mg^{2+}	146	5	2.5	2	134	25	1
5 Mg^{2+}	146	5	2.5	5	140	25	1
10 Mg^{2+}	146	5	2.5	10	150	25	1
15 Mg^{2+}	146	5	2.5	15	160	25	1

* HCO_3^- was lowered in high calcium solutions to avoid the precipitation of $CaCO_3$.

tude of the second (test) i.j.p. was expressed as a fraction of the 'corrected' first (conditioning) i.j.p. and plotted against the conditioning-test stimulus interval. Thus at long intervals, when the i.j.ps did not summate, the effects of stimulus interval on the relative release of inhibitory transmitter after the test impulse can be expressed by

$$\frac{\Delta G_2}{\Delta G_1} = \frac{\text{i.j.p.}'_2}{\text{i.j.p.}'_1}$$

where ΔG_1, and ΔG_2 are the conductance changes associated with the first and second inhibitory responses respectively and i.j.p.$'_1$ and i.j.p.$'_2$ are the corrected amplitudes of these same responses. At shorter intervals, when the i.j.ps partially summate, the same equation was used but the amplitude of the second response was obtained by the method of Thies (1965). At intervals less than 0.5 s the twin responses summed into a smooth hyperpolarization (i.j.p.$_{1+2}$). Responses to single and twin responses were then averaged on a Biomac 1000. The averaged peak amplitudes were corrected for nonlinear summation and the relative amplitude of the test i.j.p. was obtained by subtraction. Thus

$$\frac{\Delta G_2}{\Delta G_1} = \frac{\text{i.j.p.}'_{1+2} - \text{i.j.p.}'_1}{\text{i.j.p.}'_1}.$$

Correction for nonlinear summation of responses smaller than 8 mV proved not to alter the test-conditioning ratio significantly.

Results

Single transmural stimulation

The small intestine preparation was usually spontaneously active at 37°C. Thus the temperature was lowered to 25°C and atropine sulphate was added to reduce the contractions of the smooth muscle which occurred spontaneously or as a result of transmural stimulation of excitatory cholinergic nerves. Preparations which remained spontaneously active under these conditions were discarded. Steady resting membrane potentials between −55 and −60 mV (e.g. −58.5 ± 0.73 (±s.e.), $n = 78$ cells from 11 preparations) were recorded, no spontaneous excitatory or inhibitory potentials were observed. Upon transmural stimulation i.j.ps, 5 to 15 mV in peak amplitude, were recorded with latencies of about 200 ms. It should be noted that following the i.j.p. a late atropine-resistant depolarization was sometimes seen (cf. Bernath, Bywater, Holman, Supernant & Taylor, 1977). Preparations in which this depolarization was small (< 5 mV), after a single stimulus, were generally used in the present experiments.

Effects of calcium and magnesium

In Figure 1 the effects of varying the external concentrations of calcium or magnesium on the peak amplitude of the i.j.p. can be seen. As the calcium concentration was raised from 0.31 to 25 mM (with magnesium concentration 2 mM) the peak amplitude of the i.j.p. was increased (Figure 1a). The data from seventeen experiments are shown in Figure 1b, in which the mean normalised i.j.p. amplitude has been plotted against the external concentration of calcium ($[Ca^{2+}]_0$). The i.j.ps were first corrected for nonlinear summation. The mean amplitude of the i.j.p. in normal (2.5 mM calcium, 2 mM magnesium) solution was 11.6 ± 1.6 mV. It can be seen that as the concentration of calcium was increased the normalized amplitude of the i.j.p. increased, reaching a plateau at a $[Ca^{2+}]_0$ of 10 mM. Conversely, as the external concentration of magnesium was increased (with calcium concentration 2.5 mM), the peak amplitude of the i.j.p. decreased until no membrane response could be elicited in 25 mM magnesium. These effects were readily reversible, the amplitude of the i.j.p. returned to its control level when the preparations were rewashed in normal (1 mM) magnesium solution (see Figure 1c). A plot of the normalized i.j.p. amplitude (after correlation for nonlinear summation) against the external concentration of magnesium ($[Mg^{2+}]_0$), over the range of 1 to 15 mM, revealed that the normalized i.j.p. amplitude decreased with magnesium. In these eight experiments the average amplitude of the i.j.p. in normal (1 mM Mg^{2+}, 2.5 mM Ca^{2+}) solution was 10.1 ± 1.7 mV.

During the alterations in the external concentration of magnesium there was no appreciable change in the resting membrane potential (< 5 mV). When the external concentration of calcium was lowered from 2.5 mM to 0.31 mM, three out of nine preparations showed a depolarization (5 to 10 mV) of the average resting membrane potential. When the concentration of calcium was raised above 2.5 mM the membrane potential hyperpolarized. In most cases this hyperpolarization was less than 10 mV. A plot of the average change in the membrane potential against the logarithm of the calcium concentration revealed an 8.3 mV change for a ten fold change in the external concentration of calcium. In making the corrections for nonlinear summation, changes in the resting membrane potential was taken into account only when potential shifts were larger than 5 mV. The late depolarization was also dependent on the external concentration of calcium and magnesium, decreasing in amplitude as the concentration of calcium was lowered or as the magnesium was raised.

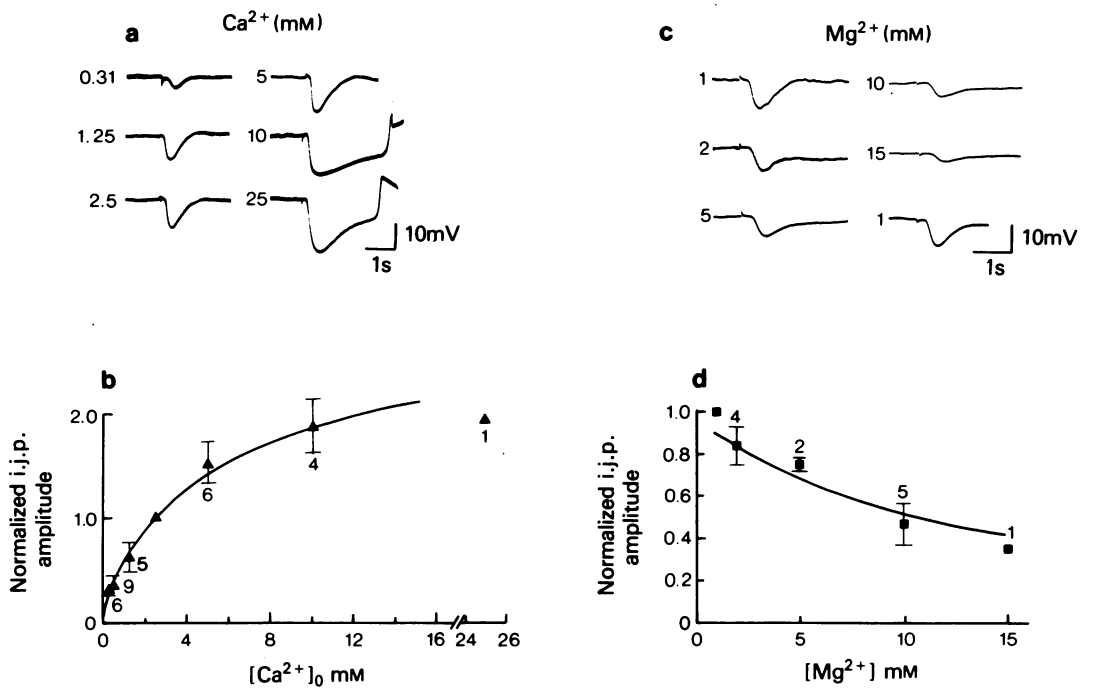

Figure 1 Effect of calcium and magnesium on the i.j.p. recorded with intracellular electrodes from the guinea-pig small intestine at 25°C. (a) Effect of varying the external calcium on the peak amplitude of the i.j.p. It can be seen that the amplitude of the i.j.p. increased as the external concentration of calcium was raised. Note also that the late depolarization increased in amplitude as the calcium was raised. Calibration bars apply to all traces. (b) Plot of normalized i.j.p. amplitude against the external concentration of calcium ($[Ca^{2+}]_0$) representing the data of 17 experiments. Each triangle represents the averaged normalized i.j.p. amplitude for each $[Ca^{2+}]_0$. The vertical bars represent s.e. mean, while the number of experiments at each $[Ca^{2+}]_0$ is shown below. The mean amplitude of the i.j.p. in 2.5 mM calcium solution was 11.6 ± 1.6 mV. (c) Effect of varying the external concentration of magnesium on the peak amplitude of the i.j.p. The peak amplitude of the i.j.p. decreased as the external concentration of magnesium was increased. Calibration bars apply to all traces. (d) Plot of normalized i.j.p. amplitude against the external concentration of magnesium ($[Mg^{2+}]_0$) representing the data of 10 experiments. Vertical bars represent s.e. mean at each $[Mg^{2+}]_0$, the number of experiments in each mean is shown above. The mean amplitude of i.j.p. in 1 mM magnesium solution was 10.1 ± 1.7 mV.

The effects of calcium and magnesium on the peak amplitude of the i.j.p. were consistent with a competitive action of these ions for a specific receptor site which initiates the release of inhibitory transmitter (cf. del Castillo & Katz, 1954). Application of the equations developed by Dodge & Rahamimoff (1967), for the co-operative action of calcium ions in triggering the release of transmitter, revealed that the experimental points could be described by the equation

$$\text{Normalized i.j.p.} = L\left(\frac{[Ca^{2+}]}{1 + [Ca^{2+}]/K_1 + [Mg^{2+}]/K_2}\right)^R.$$

L (0.8) is a constant incorporating the total number of 'transmitter-releasing' receptors in the inhibitory nerve terminals, K_1 (4.2 mM) and K_2 (4.6 mM) are the dissociation constants of the Ca-receptor and Mg-receptor complexes respectively and R (0.8) is the co-operative number (Dodge & Rahamimoff, 1967). This equation has been represented by the continuous lines in Figure 1.

Paired simulation

Figure 2a shows an example of the responses to paired transmural stimuli, at four large stimulus intervals, in normal solution (at 25°C). As the stimulus interval decreases from 16 to 1.5 s, the amplitude of the test i.j.p. became smaller relative to the conditioning i.j.p. When the relative amplitude of the test i.j.p. (corrected for nonlinear summation and expressed as

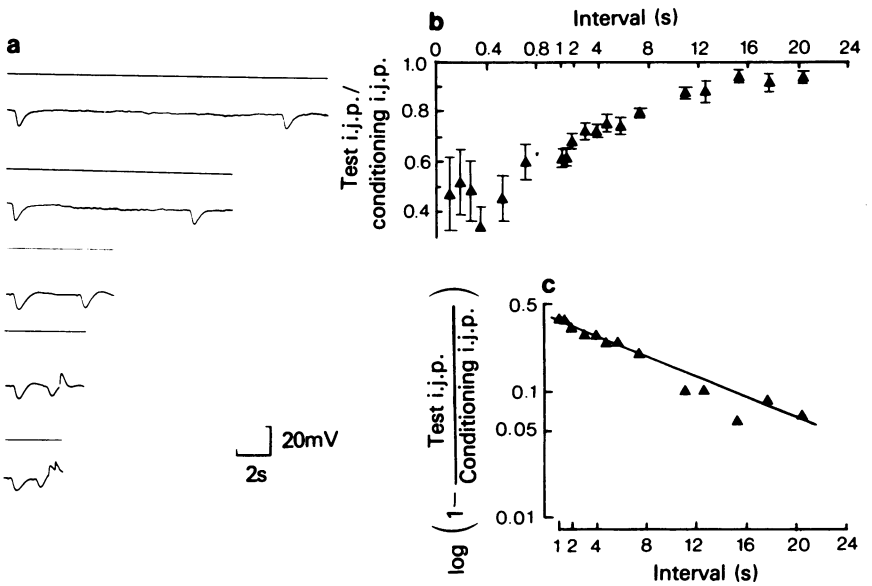

Figure 2. (a) Effect of stimulus interval on the amplitude of the second of a pair of i.j.ps. As the stimulus interval was decreased from 16 to 1.5 s the amplitude of the second (test) i.j.p. became smaller relative to the first (conditioning) i.j.p. Note that as the stimulus interval is reduced the amplitude of the second late depolarization is facilitated. At short intervals the late depolarization sometimes caused a contraction of the smooth muscle preparation, dislodging the recording electrode. Continuous straight line represents a zero transmembrane potential. Calibration bars apply to all traces. (b) Plot of the relative amplitude of the test i.j.p. (expressed as a fraction of the conditioning i.j.p.) against the conditioning-test interval representing the data from seventeen experiments. Each point is the mean of 8 to 25 observations. Vertical lines represent s.e. means. Note that the interval axis is expanded between 0 and 1 s for clarity. (c) Semilogarithmic plot of recovery from depression of the test i.j.p. (1 − test i.j.p./conditioning i.j.p.) against conditioning-test interval. The straight line was obtained by linear regression analysis. Thus the recovery from depression could be described by a single exponential with a time constant of 11 s.

a fraction of the conditioning i.j.p.) was plotted against the stimulus interval, maximal depression of the test i.j.p. could be seen at a stimulus interval of about 0.4 s (Figure 2b). Depression of the test i.j.p. recovers to within 0.95 of the conditioning i.j.p. at intervals greater than 20 s. Plotting the recovery phase of the depression of the test i.j.p. on semilogarithmic co-ordinates yielded a straight line with a time constant of 11 s (Figure 2c). Extrapolation of this line to zero time gave zero time depression (D_0) of 0.6. The time constant of recovery from depression and the D_0 are somewhat larger than those reported at skeletal neuromuscular junctions at similar temperatures (cf. Takeuchi, 1958; Betz, 1970; Christensen & Martin, 1970).

The effects of short-intervals on the second of paired responses were observed in three experiments. In Figure 3a the averaged response of eight i.j.ps is compared to the averaged response of eight pairs of i.j.ps (stimulus intervals ranging between 10 and 100 ms). A plot of the relative amplitude of the 'corrected' test i.j.p. against the conditioning-test interval on semilogarithmic co-ordinates revealed that the decline in the amplitude of the test i.j.p. could be described by a single exponential with a time constant of 87 ms (Figure 3b). Although the test i.j.p. in Figure 3b was still larger than the conditioning i.j.p. at 200 ms the test i.j.p. was usually depressed relative to the conditioning i.j.p. at intervals longer than 200 ms.

Effects of calcium and magnesium

When the amplitude of the i.j.p. was progressively decreased, by reducing the external concentration of calcium or by increasing the magnesium concentration, the amplitude of the second of a pair of i.j.ps became larger relative to the first i.j.p. In Figure 4 the relative amplitude of the test i.j.p. is plotted against the conditioning-test stimulus interval when in normal (2.5 mM Ca^{2+}, 1 mM Mg^{2+}; solid triangles) solution, in high magnesium (2.5 mM Ca^{2+}, 10 mM Mg^{2+}; open squares) solution and in low calcium (0.5 mM Ca^{2+};

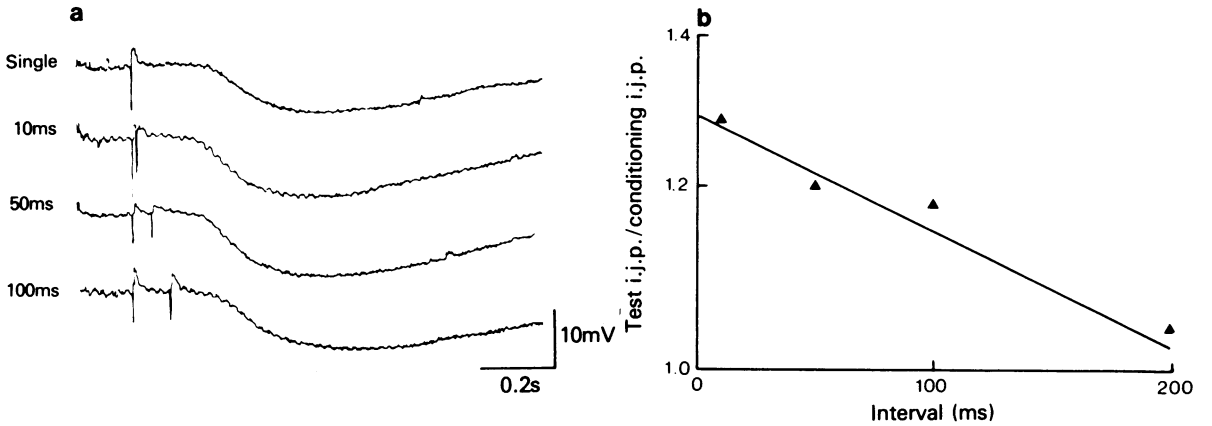

Figure 3 (a) Effect of short conditioning-test interval on the averaged amplitude of a pair of i.j.ps. The averaged response of 8 single i.j.ps is compared to the averaged response of 8 pairs of i.j.ps at stimulus intervals ranging between 10 and 100 ms. As the stimulus interval decreased the relative amplitude of the test i.j.p., obtained by subtracting the single response from the paired responses, became larger. (b) Semilogarithmic plot of the relative peak amplitude of the test i.j.p. (expressed as a fraction of the conditioning i.j.p.) against stimulus interval. Straight line fitted by linear regression analysis. The decline in the facilitated amplitude of the test i.j.p. can be described by a single exponential with a time constant of 87 ms.

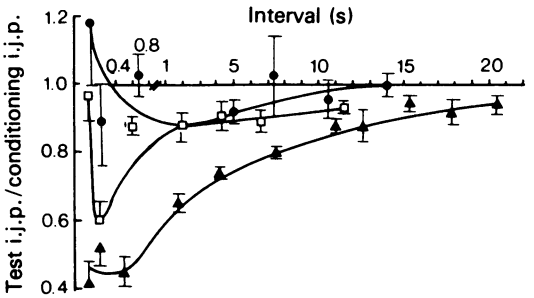

Figure 4 Comparison of the depression of the second of a pair of i.j.ps when in normal (2.5 mM Ca^{2+}, 1 mM Mg^{2+}; ▲) solution, high magnesium (2.5 mM Ca^{2+}, 10 mM Mg^{2+}; □) solution and low calcium (0.5 mM Ca^{2+}, 1 mM Mg^{2+}; ●) solution. The normal plot is the data of 17 experiments, the high magnesium plot is of 6 experiments and the low calcium plot is the data of 10 experiments. Each point is the mean of 8 to 25 experimental observations. The vertical lines represent s.e. mean. It can be seen that the depression of the test i.j.p. is decreased in high magnesium or low calcium solution for most stimulus intervals. In low calcium solution the amplitude of the test i.j.p. was larger than the first i.j.p. at short intervals. Note the expanded scale between 0 and 1 s. Curves drawn by eye.

1 mM Mg^{2+}; solid circles) solution. In 10 mM Mg^{2+} solutions the amplitude of the i.j.p. was about 50% of that in normal solution while in 0.5 mM Ca^{2+} the i.j.p. was about 30% of the control. It can be seen that, for comparable stimulus intervals, the depression of the test i.j.p. was progressively less in 10 mM Mg^{2+} and 0.5 mM Ca^{2+} solution than in normal solution. In 0.5 mM Ca^{2+} solution the test i.j.p. became larger than the conditioning i.j.p. at short intervals, although this was very variable. This facilitation however was short lived, lasting less than 400 ms. When plotted on semilogarithmic co-ordinates the time constant of recovery from depression was 10 s in normal and high magnesium solution but 7 s in low calcium solution. The decrease in the time constant in low calcium solution probably reflects the variability involved in comparing the relative amplitudes of small membrane responses during conditions of low transmitter release. At zero time, D_0 was 0.19 and 0.14, when in high magnesium and low calcium solution respectively.

Effects of temperature

The effects of temperature on the release of inhibitory transmitter after transmural stimulation were examined in eight experiments. As the temperature was

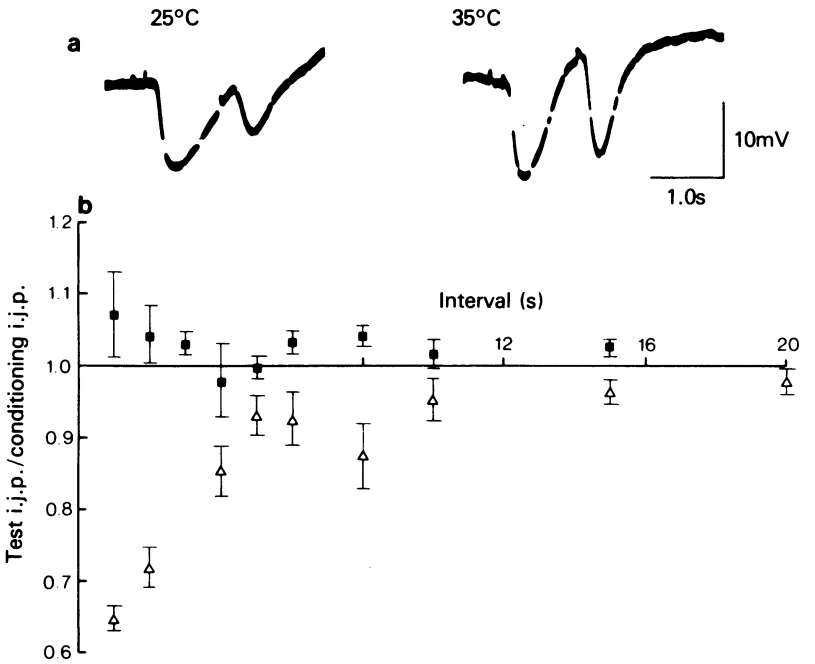

Figure 5 (a) Comparison of the amplitude of the second of a pair of i.j.ps (interval: 1 s) at 25° and 35°C. Note that as the temperature is increased the test i.j.p. becomes larger relative to the conditioning i.j.p. Calibration bars apply to both traces. (b) Plot of the relative amplitude of the test i.j.p. at 25° and 35°C against the conditioning-test interval. Each point is the mean of 5 to 16 experimental observations, one s.e. mean is represented by a vertical line. The depression of the test i.j.p. at 25°C (△) was similar to that previously described, while at 35°C (■) the amplitude of the test i.j.p. remains larger than the conditioning i.j.p. for most stimulus intervals. The conditioning and test i.j.ps were not corrected for non-linear summation.

progressively raised from 25°C to 35°C, the peak amplitude of the i.j.p. increased from 8.2 ± 1.2 mV (8 = no. of experiments) to 12.1 ± 1.3 (7). The mean amplitudes of at least eight (8 to 27) i.j.ps from each experiment were averaged. Assuming a linear relationship between temperature and the peak amplitude of the i.j.p., a Q_{10} of 1.5 was obtained by linear regression analysis. In this set of experiments the resting membrane potential was not significantly increased ($P > 0.05$) by the increase in temperature, being −54.8 ± 2.4 mV (11) at 25°C and −58.8 ± 2.3 mV (11) at 35°C.

In contrast to the observations at 25°C, the amplitude of the second of a pair of i.j.ps was equal to or larger than the first at 35°C at all intervals (Figure 5). The effects of temperature could be graded. In one experiment the depression of the test i.j.p. at 30°C was smaller than that observed at 25°C (for intervals 1 to 10 s). The rate of recovery from depression however increased with temperature, the time constant of recovery was 10.3 s at 25°C and 7.6 s at 30°C. At 35°C the test i.j.p. was again larger than the conditioning i.j.p. over the same interval range.

At short intervals, the depression of transmitter release after a conditioning impulse might be 'masked' at 35°C because the faster decay and the larger late depolarization, after the conditioning i.j.p. (see Figure 5a), would create an increase in the driving potential of the test i.j.p. When the changes in the driving potential between the inhibitory responses are accounted for, during the corrections of the conditioning and test responses for nonlinear summation, the relative amplitude of the i.j.p. still increased as the temperature was raised. In one experiment repetitive recordings of pairs of i.j.ps (interval: 1 s) were made at 25°, 30° and 34°C. The amplitudes of the responses were corrected for nonlinear summation and changes in the driving potential, the relative amplitude of the test i.j.p. was then calculated and averaged. This mean was compared to the relative amplitude of the test i.j.p. not corrected for nonlinear summation (Figure 6). It can be seen that these cor-

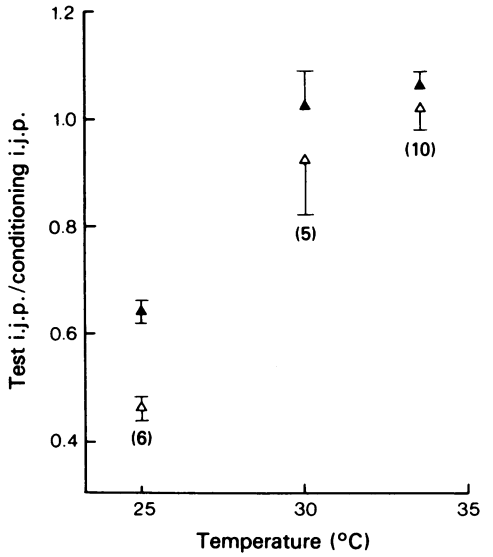

Figure 6 Plot of the mean amplitude of the test i.j.p. (expressed as a fraction of the conditioning i.j.p.) against temperature: (▲) observed relative amplitude of the test i.j.p.; (△) relative amplitude of the test i.j.p. after correction for nonlinear summation and changes in the driving potential between the conditioning and test responses (see text). The number of observation are shown in parentheses, vertical lines represent s.e. means. It should be noted that the relative amplitude of the test i.j.p. increased with temperature with or without correction for nonlinear summation.

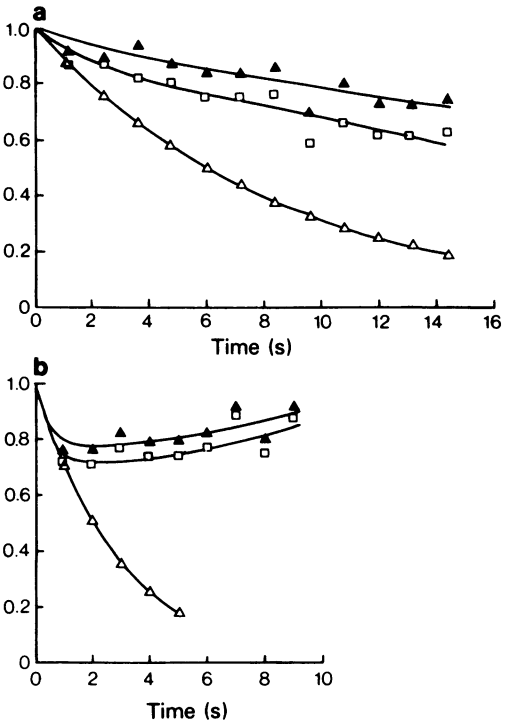

Figure 7 Two plots of the decline of the amplitude of the i.j.p. (expressed as a fraction of the first i.j.p.), during trains of stimuli at low frequencies (a: 1.2 Hz; b: 1 Hz), against time. The experimentally observed change in the amplitudes of successive i.j.ps is represented by (▲). Successive i.j.ps corrected for nonlinear summation and the concurrent 'slow' depolarization (see text) are represented by (□) while the decline in the successive i.j.p. amplitudes, as predicted by the simple depletion model (see text), is represented by (△). Lines drawn by eye.

rections tended to reduce the estimate of the relative amplitude of the test i.j.p., the reduction being larger at 25°C than at 34°C. However the relative amplitude of the test i.j.p. still increased as the temperature was raised. A similar procedure using twin responses at an interval of 2 s (i.e. no summation of responses) revealed a similar increase in the relative amplitude of the test i.j.p. Also, it should be noted that any conductance increase associated with the late depolarization, after the conditioning i.j.p., would tend to reduce the amplitude of the test i.j.p. Thus the amplitude of the test i.j.p. at short intervals would tend to underestimate the release of inhibitory transmitter.

Repetitive stimulation

The results of the paired-stimuli experiments (large D_0 and a slow rate of recovery from depression) at 25°C, suggested that the amplitude of the i.j.p. would quickly 'rundown' during repetitive stimulation, even at low frequency stimulation. Surprisingly this was not the case. During short trains of low-frequency (< 1.0 Hz) repetitive stimulation the amplitude of the i.j.p. (expressed as a fraction of the first i.j.p.), not corrected for nonlinear summation, did not continue to decrease, but usually reached a steady amplitude after about the third stimulus (Figure 7a, solid triangles). In some cases an apparent recovery from depression was seen, the amplitude of the successive i.j.ps returned to control levels during the train of stimulation (Figure 7b, solid triangles). During these trains of stimuli a slow depolarization (4 to 8 mV) of the membrane potential, between the inhibitory responses, was sometimes seen (cf. Bernarth *et al.*, 1977). Such a depolarization would create an increase in the driving potential of the successive inhibitory responses and therefore might be responsible for the apparent recovery. The change in the resting membrane potential can be accounted for when the ampli-

tudes of the successive i.j.ps are corrected for non-linear summation. Application of this correction to all of the i.j.ps in the two examples in Figure 7 has been represented by the open squares. It can be seen that, after the correction for nonlinear summation and the slow depolarization, the relative amplitudes of the successive i.j.ps were more depressed, but the recovery from depression still occurred during repetitive stimulation.

At the neuromuscular junction a 'simple' depletion model was suggested to predict the depression of transmitter release during a train of stimuli. Assuming that all stimuli released a constant fraction of transmitter from the store of available transmitter and that the replenishment of this store (mobilization) was slow and constant, the rate of decline of the amplitude of successive i.j.ps can be predicted from the depression of the second of a pair of i.j.ps at the same stimulus interval (Takeuchi, 1958) (Figure 7, open triangles). There is little agreement between the decline in the amplitude of the i.j.p. predicted by the simple depletion model and the change in the amplitude of the successive i.j.ps even after correction for nonlinear summation and the slow depolarization.

When the preparation was transmurally stimulated at high frequencies (10 Hz) the resultant hyperpolarization was not maintained. The hyperpolarization usually decayed during a train of stimuli, possibly due to the action of an atropine-resistant excitatory transmitter (Bernath *et al.*, 1977). Moreover after a period of repetitive stimulation a post-stimulus contraction was usually recorded (Bernath *et al.*, 1977). Thus it was usually impossible to maintain a successful microelectrode impalement during and after a train of repetitive stimulation. A study of the possible facilitatory processes, during and after high-frequency stimulation (cf. the neuromuscular junction), was therefore not possible.

Discussion

The effects of temperature and the divalent cations, calcium and magnesium, on the amplitude of the i.j.p., recorded in the guinea-pig small intestine, have been examined. The results with paired and repetitive stimulation were complicated by the presence of a late depolarization (Bernath *et al.*, 1977). At 25°C the late depolarization was not always present after a single stimulus, but could be elicited after repetitive stimulation. At 35°C the late depolarization was usually present. As the time course and magnitude of the conductance change underlying the late depolarization remains unknown, the effects of the late depolarization on the amplitude of successive i.j.ps cannot be determined. The effects of calcium and magnesium ions on the amplitude of the i.j.p. were basically similar to those reported by Holman & Weinrich (1975), except that the i.j.p. recorded in the present experiments was more sensitive to the changes in the external concentration of magnesium (see Figure 1d, cf. Figure 4 Holman & Weinrich, 1975). It is curious therefore that the i.j.p., recorded in the guinea-pig jejenum, has been reported to be insensitive to the external concentration of calcium and magnesium (Hidaka & Kuriyama, 1969). The results presented here are consistent with a competitive antagonism of these two cations for a receptor site which initiates the release of inhibitory transmitter (del Castillo & Katz, 1954; Dodge & Rahamimoff, 1967).

During pairs of transmural stimuli the amplitude of the test i.j.p. was facilitated at intervals less than 0.2 s and depressed at larger intervals (up to 20 s). The magnitude of the depression (D_0) and the rate of recovery from depression after a single conditioning stimulus were somewhat larger and slower than that reported at the neuromuscular junction at a similar temperature (25°C) (Takeuchi, 1958; Betz, 1970; Christensen & Martin, 1970). As the external concentration of calcium is reduced or as the magnesium concentration raised, the depression of the test i.j.p. is decreased without any change in the rate of recovery from depression. This is consistent with the models of depression postulated at the neuromuscular junction in which the depression of transmitter release, after a single conditioning stimulus, reflects a partial depletion of the 'readily-releasable' store of transmitter (Takeuchi, 1958) and the rate of recovery from depression reflects the rate of replenishment (mobilization of transmitter from other stores or synthesis of new transmitter) of transmitter (Elmqvist & Quastel, 1965).

Even though the paired-stimulus experiments, at 25°C, suggested that there is a slow recovery from depression, transmitter output can be maintained, sometimes recovering to prestimulation levels, during low-frequency repetitive stimulation (Figure 7 lower plot). The stimulus frequencies used (<1.2 Hz) were such that it was unlikely that there was any summation of the effects of facilitation. Additional facilitatory processes however have been shown to be present at the neuromuscular junction during short trains of low-frequency repetitive stimuli (Magleby & Zengel, 1976a, b). If the rate of recovery from depression remained slow any increase in the release of inhibitory transmitter, during repetitive stimulation, would be expected to deplete further the releasable store of transmitter. As the release of transmitter can be maintained, it seems likely that the rate of replenishment of the transmitter store is increased during repetitive stimulation. A similar recovery from depression of transmitter release, during repetitive stimulation, has been reported at a synapse in *Aplysia* where the maintenance of transmitter output has been

attributed to a transient increase in the release of transmitter, coupled with an increased rate of mobilization (Schlapfler, Woodson, Tremblay & Barondes, 1976).

At the inhibitory junction the amplitude of the i.j.p. and the relative amplitude of second of a pair of i.j.ps both increased as the temperature was raised. The electrotonic potential, elicited by large extracellular polarizing plates (Abe & Tomita, 1968) was not much affected (R.J. Lang, unpublished observations). This is in direct contrast to the skeletal neuromuscular junction where an increase in temperature generally increases the depression of the test response, owing to the enhanced release of transmitter during the conditioning impulse (Takeuchi, 1958), and decreases the effects of facilitation (Balnave & Gage, 1974). Thus the increase in the relative amplitude of the test i.j.p. with temperature can be best explained by a rate of replenishment which is highly sensitive to temperature. Such an enhanced rate of replenishment, coupled with an increased probability of transmitter release (as suggested by the increased amplitude of the i.j.p.), might account for the facilitated amplitude of the test i.j.p. at 35°C. The facilitation of the i.j.p. at 35°C has been previously reported in the guinea-pig colon during short trains of low-frequency repetitive stimulation (Furness, 1969b).

The effects of temperature and repetitive stimulation on the release of inhibitory transmitter are also consistent with the activation of a second, more rapid process of replenishment of the releasable store of transmitter (Kusano & Landau, 1975). If the rate of this 'fast' process was proportional to the rate of depletion of the releasable store, a 'feedback' mechanism can be envisaged to balance the release and replenishment of the releasable store. Presumably the fast recovery phase is not activated after a single stimulus at 25°C, the recovery from depression occurring with the slow time course observed. At 35°C the enhanced release of transmitter after a single stimulus may reduce the concentration of the transmitter in the releasable store to its 'threshold' concentration which triggers the rapid process of replenishment. Low-frequency repetitive stimulation (at 25°C) may also reduce the releasable store to its threshold level, triggering the rapid process of mobilization and giving rise to the recovery from depression during the train of impulses.

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