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SLOW EXCITATORY SYNAPTIC POTENTIALS RECORDED FROM NEURONES OF GUINEA-PIG SUBMUCOUS PLEXUS

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SUMMARY

1. Intracellular recordings made from neurones of guinea-pig submucous plexus revealed three types of synaptic input: cholinergic excitatory synaptic potentials (fast e.p.s.p.s) of 50–80 ms duration, inhibitory synaptic potentials (i.p.s.p.s) of 1 s duration, and non-cholinergic, non-adrenergic slow e.p.s.p.s which lasted for 15–20 s.

2. A single stimulus was sufficient to elicit the slow e.p.s.p. in all neurones in which this synaptic input was present.

3. Slow e.p.s.p.s were recorded in those neurones which also displayed i.p.s.p.s. Both the i.p.s.p. and the slow e.p.s.p. appeared in an all-or-none fashion and were not affected by alterations in the stimulus strength.

4. The inhibitory as well as the slow excitatory synaptic potentials reversed close to the K^+ equilibrium potential, indicating that the i.p.s.p. is due to an activation of K^+ conductance while the slow e.p.s.p. is due to its inactivation.

5. Evidence is presented which suggests the slow e.p.s.p. may be generated at a synapse located some distance from the soma, presumably at a dendritic location.

6. Only those cells which showed slow e.p.s.p.s responded to substance P with a depolarization which mimicked the slow e.p.s.p.

INTRODUCTION

The mammalian enteric nervous system which lies within the gut wall comprises two ganglionic plexuses, the myenteric plexus and the submucous plexus. Neurones located in these plexuses are responsible for co-ordinating gastrointestinal motility by integrating the information received by enteric afferent nerves and eventually exciting, or inhibiting, neurones which cause the appropriate excitation or inhibition of the muscularis externa (smooth muscle layers) (Bennett & Burnstock, 1968; Costa & Furness, 1976; Hirst, 1979; Wood, 1981). The neural activity which occurs in the enteric nervous system has been studied mainly in the myenteric plexus (e.g. Wood, 1970, 1981; North, 1973; Nishi & North, 1973; Hirst, Holman & Spence, 1974). In the myenteric plexus, cholinergic excitatory synaptic potentials (e.p.s.p.s) appear to be the main form of synaptic input, although slow depolarizations, which may be due to the release of substance P or 5-hydroxytryptamine or both are occasionally

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initiated by a single stimulus and, more often, during repetitive, high frequency stimulation (Wood & Mayer, 1979*a*, *b*; Johnson, Katayama & North, 1980; Johnson, Katayama, Morita & North, 1981).

Only a few studies on the synaptic activity of submucous plexus neurones have been reported (Hirst & McKirdy, 1975; Hirst & Silinsky, 1975; Edwards, Hirst & Silinsky, 1976; Neild, 1981). Hirst and these co-workers recorded two types of synaptic input onto neurones of the submucous plexus: cholinergic e.p.s.p.s and inhibitory synaptic potentials (i.p.s.p.s) which could be mimicked by the application of either noradrenaline or dopamine (Hirst & Silinsky, 1975). In the present experiments further electrophysiological characterization of neurones lying in the submucous plexus of the guinea-pig small intestine was carried out. In addition to cholinergic e.p.s.p.s and i.p.s.p.s, single transmural stimuli initiated non-cholinergic, non-adrenergic slow e.p.s.p.s. Two types of neurones which did not receive synaptic input have also been identified. This report describes some properties of the various synaptic potentials recorded from neurones in the submucous plexus. The following report deals with those cells in which no synaptic input could be demonstrated.

METHODS

Experiments were carried out on preparations of submucous plexus obtained from the small intestine of young guinea-pigs (200–300 g). The methods of dissection, recording and stimulation used were essentially those previously described by Hirst & McKirdy (1975). The mucosa was stripped from segments (8–12 mm long by 5–7 mm wide) of the small intestine, and the submucous plexus pulled away from the underlying circular smooth muscle. The plexus was pinned out, mucosal surface down, in an organ bath (vol. 0·3 ml) through which was passed continuously at 4 ml/min physiological saline of composition (mM): Na^+, 147; K^+, 5; Mg^{2+}, 2; Cl^-, 134; Ca^{2+}, 2·5; HCO_3^-, 25; $H_2PO_4^-$, 1; glucose, 8; gassed with 95% O_2/5% CO_2. Temperature was maintained at 37 °C. Only those preparations to which no visible circular smooth muscle strands adhered were used in these experiments. The organ bath was placed on the stage of an inverted microscope which allowed visualization of nodes of ganglion cells or outlines of individual neurones within a node using ×200 or ×400 magnification. Transmural stimulation was accomplished via a pair of fine platinum wires placed above and below the preparation. Intracellular recordings were made using 2 M-KCl fibre-filled micro-electrodes with resistances of 80–120 MΩ.

Impalement of a submucous plexus neurone usually resulted in an immediate voltage deflexion of −20 to −40 mV followed by an increase in membrane potential to approximately −60 to −68 mV over the next 10 s–5 min. Estimates of input resistance made at this time were in the range of 80–200 MΩ. However, over the next 5–10 min the membrane potential almost invariably decreased by about 2–7 mV and this was associated with a concomitant 5–40% decrease in the amplitude and time course of the electrotonic potential. Thereafter the electrical properties of the neurone often remained constant for recording periods up to 8 h. For these reasons the final criteria used for accepting a recording for purposes of further analysis were: (1) that a stable recording be maintained for > 45 min at a resting potential of $\geqslant -55$ mV, with no decrease in input resistance over that observed after the initial 5–15 min of recording, (2) that the final value for the input resistance be $\geqslant 80\%$ of the maximum initial value observed and (3) that the amplitude of action potentials evoked by passing depolarizing current pulses (20–150 ms duration, $1–5 \times 10^{-10}$ A) through the micro-electrode remain greater than 55–58 mV for the duration of the impalement.

If a change in permeability to a single ion (for example, K^+) is responsible for a change in membrane potential, providing that the membrane potential change is slow compared to the membrane time constant, then the relationship between the fractional conductance change and the amplitude of the response can be obtained from the equivalent circuit theory (e.g. Katz, 1966; Hubbard, Llinas & Quastel, 1969) to yield the following: $(R'/R) = -[\Delta v/(E_K - E_m)] + 1$; where Δv is the amplitude of the response, E_K and E_m are K^+ equilibrium potential and resting potential,

and R and R' are values of input resistance measured at rest and at Δv, respectively. Further assumptions, descriptions and full derivation of this relationship can be found in Ginsborg (1973, 1976) and Morita, North & Tokimasa (1981). Predicted values shown in Fig. 6 were obtained from this equation.

Solution changes were made by means of a hydraulic valve placed close to the inflow of the organ bath. When checked with a dye solution and photometric recorder, complete re-equilibration was found to occur within 20–40 s after switching solutions.

Intracellular recordings were stored on magnetic tape; except where noted all records of membrane potentials shown in the Figures were produced from computer files of the data after they had been sampled at a rate of 8000 points per sweep (1–60 sweeps). Records of slow e.p.s.p.s were initially sampled at 2–4 ms intervals to obtain the full time course of the response and then re-sampled in consecutive segments at 0·3 ms intervals. Records of substance P responses were initially sampled at 15 ms intervals and re-sampled at consecutive 0·1 ms intervals. Sampling rate for all other records was 0·3 ms.

The following drugs were used: tetrodotoxin (TTX; Sankyo), tubocurarine chloride (Calbiochem), hexamethonium chloride (Sigma), phentolamine mesylate (Regitin, Ciba), atropine sulphate (DBL), methysergide maleinate (Sandoz), noradrenaline (1-arterenol bitartrate, Sigma) and substance P (Sigma). Solutions of altered K^+ concentration were made by changing the NaCl concentration to maintain normal osmolarity. Altered Cl^- solution was made by substituting NaCl with Na isethionate.

Tests of significance were made using Student's t test. In all cases n refers to number of preparations. All means are expressed as mean $\pm$S.E. of the mean.

RESULTS

General observations

Intracellular recordings were obtained from a total of 235 ganglion cells in which the duration of the impalement (at least 15 min) was sufficient to allow an initial examination of both the electrical properties of the cell and its response to transmural stimulation. This report describes results obtained from 181 cells which showed various types of synaptic potentials in response to transmural stimulation; quantitative data were obtained from fifty of these cells during impalements maintained for 1–8 h (see Methods). No synaptic input or evidence of antidromic activation could be recorded in any neurone at distances greater than 4 mm from the stimulating electrode; therefore all data described here were obtained from ganglion cells within 3·2 mm from the stimulating electrodes.

Input resistances, determined from the slope of the current–voltage relation, and the time constant of the cell membrane, determined from the final exponential decay phase of the electrotonic potential recorded in response to small ($0{\cdot}5–1 \times 10^{-10}$ A) inward current pulses, were 98 ± 5 MΩ and $16 \pm 0{\cdot}9$ ms respectively ($n = 44$). No obvious differences in the passive membrane properties were noted in neurones which received different types of synaptic input. Current–voltage relationships were found to be linear for membrane hyperpolarizations to 15–35 mV and for membrane depolarizations to 5–10 mV ($n = 13$). A decrease in membrane resistance was observed in all cells when the membrane was hyperpolarized by more than 35–40 mV from resting potential and in some cells at membrane potentials depolarized by more than 10 mV from resting potential.

Single transmural stimuli (duration 0·05–0·2 ms) initiated three distinct types of post-synaptic potentials: fast, nicotinic excitatory synaptic potentials (fast e.p.s.p.s; Fig. 1 *A*, upper trace); inhibitory synaptic potentials (i.p.s.p.s; Fig. 1 *A*, middle trace);

and slow excitatory synaptic potentials (slow e.p.s.p.s) whose minimum total duration was 12–15 s (Fig. 1*A*, lower trace). The slow e.p.s.p. was considered to be due to the release of a transmitter substance because it was blocked by the application of TTX (Fig. 1*B*) and was depressed in a dose-dependent manner by lowering the external Ca^{2+} and/or increasing external Mg^{2+} concentration ($n = 22$).

Fast e.p.s.p.s were initiated in all cells displaying a synaptic input (181 cells); in ninety-two cells this was the only type of synaptic input that could be demonstrated by transmural stimulation. In addition to fast e.p.s.p.s, inhibitory synaptic potentials were recorded from eighty cells; in sixty-four of these cells slow e.p.s.p.s were also initiated. Slow e.p.s.p.s were recorded in a further five cells in which no i.p.s.p. was recorded in response to nerve stimulation.

TABLE 1. Effects of noradrenaline (10^{-5} M) on membrane potential recorded from submucous plexus neurones

		Post-synaptic effect of noradrenaline		
Response	Number of cells	Cells showing no effect	Cells showing hyperpolarization	Amount of hyperpolarization
Fast e.p.s.p. only	18	15	3	24 ± 3 mV
Fast e.p.s.p. + i.p.s.p.	2	0	2	23 mV; 19 mV
Fast e.p.s.p. + i.p.s.p. + slow e.p.s.p.	23	0	23	22·1 ± 0·9 mV
Fast e.p.s.p. + slow e.p.s.p.	5	0	5	20 ± 2 mV

Since the method of transmural stimulation used in these experiments would not be expected to excite all of the presynaptic axons to any one neurone, the absence of a specific synaptic potential could not be used as evidence of the anatomical absence of a synapse. Hirst & Silinsky (1975) found that i.p.s.p.s could only be recorded from submucosal neurones which were hyperpolarized by the ionophoretic application of noradrenaline. Therefore, in this study the change in membrane potential in response to noradrenaline was also used in an attempt to distinguish further between neurones that possessed inhibitory input and those that did not.

A total of forty-eight cells were examined in this way (Table 1). In all of the cells noradrenaline resulted in a dose-dependent decrease in the amplitude of the fast e.p.s.p., probably due to a presynaptic inhibition of transmitter release via α_2-receptor activation (A. Surprenant, unpublished observations). Noradrenaline also caused a dose-dependent hyperpolarization of all cells displaying i.p.s.p.s; the peak membrane hyperpolarization in response to 10^{-5} M-noradrenaline was 22·1 ± 0·9 mV (twenty-five cells, $n = 18$). Similar applications of noradrenaline, in concentrations up to 10^{-4} M, produced no change in membrane potential in fifteen of eighteen cells which showed only fast e.p.s.p.s. The membrane potentials of the other three cells were hyperpolarized by noradrenaline, with the peak hyperpolarization in response to 10^{-5} M-noradrenaline being 24 ± 3 mV. The failure to record an i.p.s.p. in these cells probably reflects the placement of the stimulating electrode relative to the inhibitory axon. Noradrenaline (10^{-5} M) also hyperpolarized the membrane by 20 ± 2 mV of all five neurones in which a slow e.p.s.p. but no i.p.s.p. was evoked by transmural stimulation. In view of these results it is suggested that slow excitatory synaptic input (as well as inhibitory synaptic input) occurs only in those neurones which also hyperpolarize in response to noradrenaline.

In the remaining four cells a single stimulus initiated the fast e.p.s.p. followed by a further e.p.s.p. of 3–8 mV amplitude whose total duration was some 100–450 ms. The latency of this e.p.s.p. was approximately 70–100 ms after the stimulus and it usually arose during the final decay of the fast e.p.s.p. These e.p.s.p.s would appear to be similar to those also briefly noted by Hirst & McKirdy (1975). Atropine (10^{-7} M), but not hexamethonium (2×10^{-4} M) or curare (2×10^{-4} g/ml), completely abolished this response within 1 min of application (three of three cells examined). The four cells in which these muscarinic e.p.s.p.s were recorded did not show i.p.s.p.s or slow e.p.s.p.s. The scarcity of such muscarinic e.p.s.p.s observed in the present study precluded further investigation.

Some properties of the synaptic potentials recorded from submucous plexus neurones

Fast e.p.s.p.s. The occurrence of multiple nicotinic synaptic inputs to neurones of the submucous plexus has been described by Hirst and co-workers (Hirst & McKirdy, 1975; Hirst & Silinsky, 1975). In the present study fast e.p.s.p.s were examined in some detail in eighteen cells in which no other synaptic input was initiated by transmural stimulation.

In many of these cells a just-threshold stimulus intensity evoked a fast e.p.s.p. which was suprathreshold for initiation of an action potential. A single stimulus evoked an initial e.p.s.p. or action potential followed by a series of further fast e.p.s.p.s over the next 40–100 ms. When an impalement was made in a neurone which lay within one to three nodes from the stimulating electrode, action potentials initiated by antidromic activation of nerve fibres were often recorded (see Hirst & McKirdy, 1975). In ten of the eighteen cells examined a single stimulus evoked the initial series of fast e.p.s.p.s and a late fast e.p.s.p. which occurred 1·7–2·2 s after the stimulus. This late fast e.p.s.p. followed each stimulus at a constant latency in any one cell. Other late e.p.s.p.s of variable latencies between 0·3 and 1·0 s were observed in many cells but this '2 s latency', stimulus-locked, late fast e.p.s.p. emerged as the only consistent pattern in this series of experiments. Spontaneous fast e.p.s.p.s occurred at variable but often high (0·1–10/s) frequencies in the majority of cells which received synaptic input (Surprenant, 1984). Hexamethonium (2×10^{-4} M) and curare (2×10^{-4} g/ml) rapidly and reversibly abolished all fast e.p.s.p.s.

I.p.s.p.s. Many of the findings reported by Hirst & McKirdy (1975) and Hirst & Silinsky (1975) concerning the i.p.s.p.s recorded from submucous plexus neurones were confirmed in the present study. Transmural stimulation evoked an i.p.s.p. which followed the fast e.p.s.p. in approximately half of all cells possessing a synaptic input. In these experiments the stimulus intensity which was just threshold for initiation of an i.p.s.p. was always higher than that required for initiation of the fast e.p.s.p. In the majority of cells examined (total of thirty-one cells from twenty-eight preparations) increasing the stimulus intensity did not change the amplitude of the i.p.s.p. (within ± 2 mV). However, a definite 'step' increase in the i.p.s.p. amplitude was observed in two cells when the stimulus intensity was progressively increased; in both cells two steps were observed. These results support the view of Hirst & McKirdy (1975) that submucous plexus neurones which receive inhibitory synapses probably receive only one (or, at most, two) inhibitory inputs.

A single stimulus evoked an i.p.s.p. of 4–18 mV in amplitude whose time-to-peak (10–90 %) was 80–230 ms, and whose half-width was 450–700 ms (nineteen cells, fourteen preparations) (Fig. 1*A*, middle trace). The i.p.s.p.s recorded in the present study were faster than those reported in the studies of Hirst and co-workers (Hirst

& McKirdy, 1975; Hirst & Silinsky, 1975); this may be due to the higher temperature used in the present experiments.

Phentolamine (10^{-8}–5×10^{-6} M) reversibly depressed the amplitude of the i.p.s.p. in a dose-dependent fashion (fifty-two cells, thirty-two preparations). The i.p.s.p. evoked by a single stimulus was completely blocked by 5×10^{-7} to 5×10^{-6} M-phentolamine. Phentolamine, up to 3×10^{-5} M, did not alter the time course or amplitude of the directly evoked action potentials or the membrane properties of the cells even when present in the bathing fluid for up to 7 h. This drug was therefore used in many of these experiments to inhibit the i.p.s.p. in order to examine the characteristics of the slow e.p.s.p.

Again, late fast e.p.s.p.s, notably the '2 s latency' late fast e.p.s.p. was observed in several neurones which possessed inhibitory, as well as slow excitatory, synaptic potentials.

During prolonged impalements of cells showing inhibitory input, spontaneous i.p.s.p.s began to occur after approximately 3–5 h, their frequency being 1–3/min. Spontaneous i.p.s.p.s had time courses and amplitudes identical to the i.p.s.p. evoked in the same cell by a single stimulus and were associated with an equivalent conductance change. Spontaneous i.p.s.p.s were reversibly abolished by TTX (10^{-7} g/ml) or 10^{-6} M-phentolamine ($n = 5$). No apparent deterioration in the membrane properties or alterations in evoked synaptic potentials were noted at these times. However, as spontaneous i.p.s.p.s have not been recorded in any cell during the initial 1–3 h of an impalement it is felt that the spontaneous i.p.s.p.s recorded in the present study most likely reflect some subtle deterioration of the preparation.

Slow e.p.s.p.s

Responses to single stimuli. In a large proportion (81 %) of neurones which received an inhibitory synaptic input, a single transmural stimulus also elicited a very long-lasting (10–20 s) membrane depolarization, the slow e.p.s.p. (Figs. 1–4). A single stimulus was sufficient to initiate a slow e.p.s.p. in all cells possessing this type of synaptic input (sixty-nine cells, fifty-two preparations). In most cells the stimulus intensity required to initiate the slow e.p.s.p. was similar to that required for initiation of the i.p.s.p. However, in a minority of cells the slow e.p.s.p. was evoked at an appreciably lower stimulus intensity than that required for the i.p.s.p. For example, the neurone from which the record of Fig. 1*A* (lower trace) was obtained showed rheobase values for the fast e.p.s.p., the i.p.s.p. and the slow e.p.s.p. of 4·2, 7 and 8 V respectively, whereas the records of Fig. 6*C* were obtained in a neurone whose corresponding rheobase values were 3, 15 and 6 V respectively. Like the i.p.s.p., the amplitude of the slow e.p.s.p. did not change (within 1·5 mV) with increases in the stimulus intensity ($n = 7$). It was also noted that the slow e.p.s.p., but not the fast e.p.s.p., failed in an all-or-nothing manner when TTX was added to the bathing fluid ($n = 3$) (Fig. 1*B*).

Little variation in the time course of slow e.p.s.p.s recorded from different preparations in response to single stimuli was observed. The averaged slow e.p.s.p., recorded in response to twenty stimuli delivered once a minute, had an amplitude of $6{\cdot}7 \pm 0{\cdot}4$ mV, a time-to-peak amplitude (measured from stimulus artifact) of $2{\cdot}7 \pm 0{\cdot}1$ s and a half-duration of $8{\cdot}4 \pm 0{\cdot}4$ s ($n = 19$). The amplitude of the slow e.p.s.p. occasionally exceeded threshold for action potential initiation; repetitive spike discharge then ensued. Since the spike activity obviously prevented measurements

of the underlying slow e.p.s.p., neurones displaying slow e.p.s.p.s and low thresholds (less than 10–12 mV depolarization from resting potential) were not examined further in this study.

As already described, single stimuli often evoked stimulus-locked late fast e.p.s.p.s. When these occurred on the rising phase of the slow e.p.s.p. they often initiated action potentials. Therefore, low doses of curare (10^{-6} g/ml) were sometimes used to block the late fast e.p.s.p. during studies on the time course of the slow e.p.s.p.

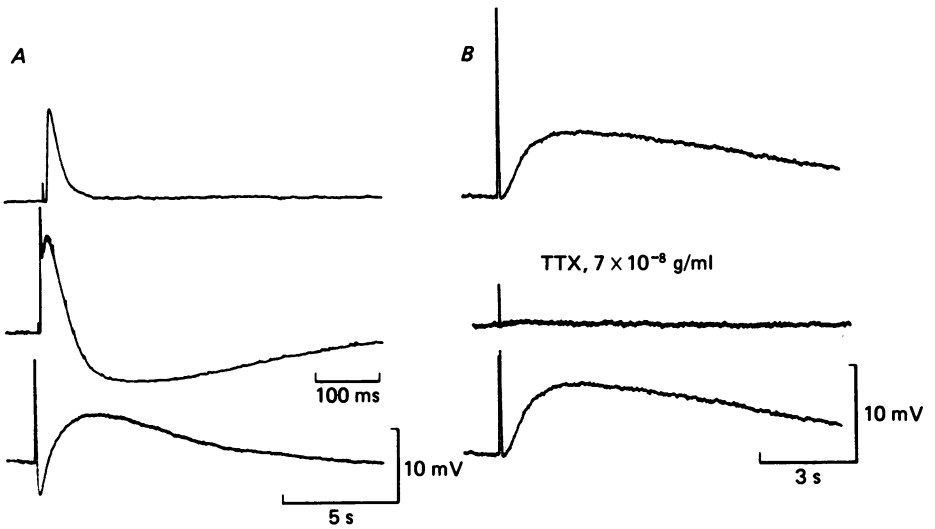

Fig. 1. Three types of synaptic potentials recorded from guinea-pig submucous plexus neurones. *A*, fast e.p.s.p. (upper trace), fast e.p.s.p. + i.p.s.p. (middle trace), fast e.p.s.p. + i.p.s.p. + slow e.p.s.p. (lower trace); note slower time base in lower trace. Each trace is the average of ten responses to single stimuli delivered once a minute. Records obtained from three different neurones. Single stimuli often initiated multiple fast e.p.s.p.s, some of which were suprathreshold for action potential initiation (middle trace); total spike amplitude not shown in this trace. *B*, intracellular records obtained from submucous plexus neurone before, during and after TTX perfusion. The slow e.p.s.p. failed in an all-or-none manner in the presence of TTX. Full amplitude of fast e.p.s.p. is not shown in the bottom trace.

Responses to repetitive stimuli. In those cells in which a slow e.p.s.p. but no i.p.s.p. could be demonstrated, repetitive stimulation at frequencies between 5–20 Hz (for 0·1–2 s) increased the amplitude of the slow e.p.s.p., with the maximum amplitude occurring after the 2nd–8th stimulus. An example is shown in Fig. 2 where slow e.p.s.p.s can be seen to reach approximately maximum amplitude by the 2nd–3rd pulse at a frequency of 20 Hz (Fig. 2*A* and *B*). Similar findings were obtained in all neurones demonstrating a slow e.p.s.p. when the i.p.s.p. was suppressed by the addition of phentolamine (Fig. 2*B*). When the duration of stimulation was less than 500–800 ms (at frequencies of 5–20 Hz), no significant difference between the amplitude of the slow e.p.s.p. recorded in normal solution and in phentolamine was observed ($n = 7$). Throughout the course of the present experiments the duration of stimulation was less than 800 ms for all frequencies (5–20 Hz) applied.

Ionic requirements for the slow e.p.s.p.

Reversal potential. The polarity of the slow e.p.s.p., as well as the i.p.s.p., could be reversed by passing hyperpolarizing current through the micro-electrode. As shown in Fig. 3*A*, the amplitude of both the i.p.s.p. and the slow e.p.s.p. evoked by single stimuli were decreased when the membrane was hyperpolarized and were inverted at membrane potentials more negative than −85 to −95 mV. A linear relationship between the amplitude of the synaptic potentials and the membrane potential at which they were evoked was observed (Fig. 3*B*). In nine of ten neurones examined

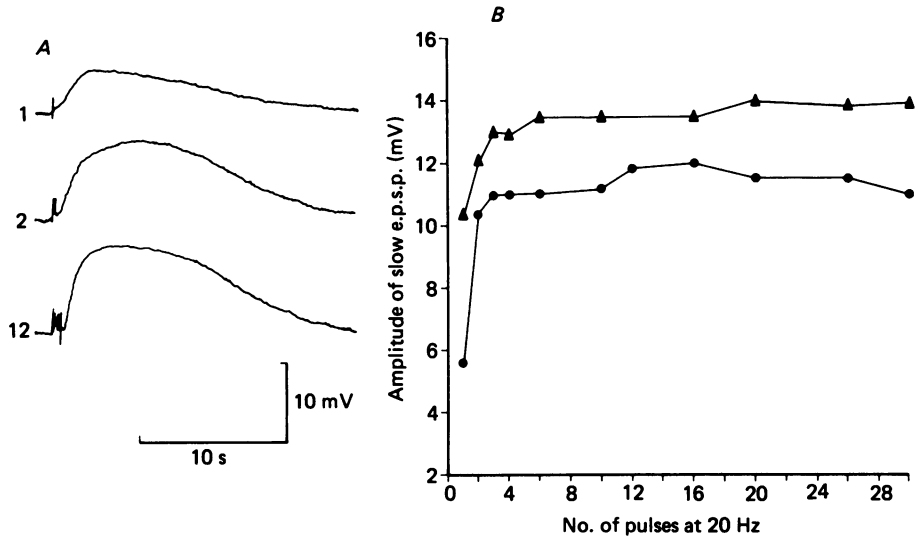

Fig. 2. Effect of repetitive stimulation on amplitude of slow e.p.s.p. *A*, slow e.p.s.p. recorded in response to 1, 2 and 12 stimuli at a frequency of 20 Hz. Each trace is the average of five responses, all stimuli delivered at 1 min intervals. Curare (2×10^{-4} g/ml) was present to abolish fast e.p.s.p.s. No i.p.s.p. was recorded in this neurone. *B*, two examples of the relation between amplitude of slow e.p.s.p. and repetitive stimulation. ●, data obtained from neurone shown in *A*; ▲, data from a neurone in which the i.p.s.p. had been blocked by phentolamine. The maximum amplitude of the slow e.p.s.p. was reached by the 2nd–8th pulse (*A* and *B*).

in this way the reversal potential for the i.p.s.p., determined from plots of amplitude as a function of membrane potential, was found to be about 4–8 mV less negative than the reversal potential for the slow e.p.s.p. recorded in the same cell (Fig. 3*B*). In some of these experiments reversal of the i.p.s.p. was clearly evident at a membrane potential where the slow e.p.s.p. remained a depolarizing response (e.g. Fig. 6*C*, inset). It was also noted that the i.p.s.p. reversal potential fell within 1–2 mV of the membrane potential at which the undershoot of the directly evoked action potential was seen to disappear ($n = 3$). There was no difference in the value of the reversal potential of the slow e.p.s.p. determined in the same cell before and after the fast e.p.s.p. and the i.p.s.p. were completely abolished by high concentrations of curare and phentolamine ($n = 3$).

Effects of changing external K^+ and Cl^- concentrations. When external K^+ was elevated beyond 15–20 mM all synaptic potentials were usually abolished, probably due primarily to depolarization of the nerve terminals. The effects of altered K^+ concentration on the slow e.p.s.p. and the i.p.s.p. were investigated in the following manner. Membrane potential was altered by passing constant current through the micro-electrode. The response evoked by single transmural stimuli delivered once a minute was recorded at seven to ten different membrane potential levels in normal (5 mM) K^+ and 5–15 min after switching to a new external K^+ concentration; five

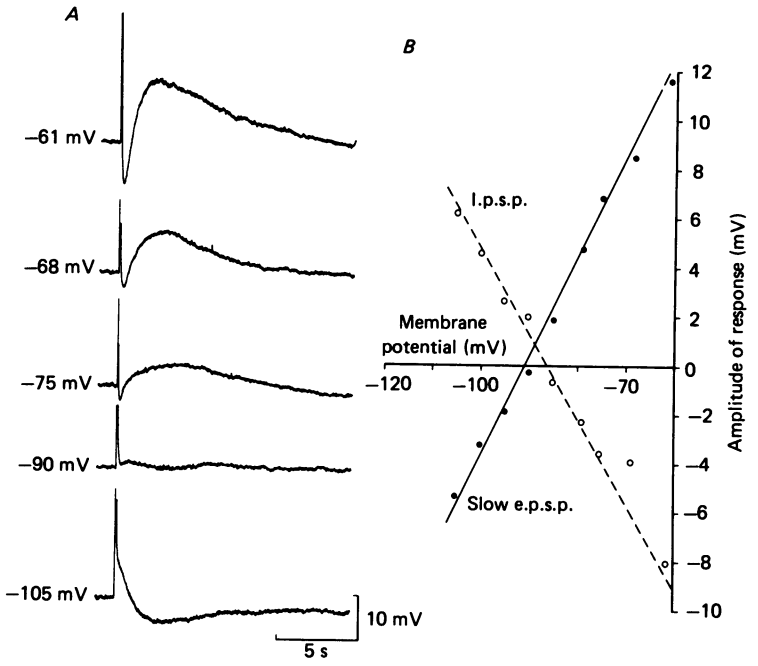

Fig. 3. Effect of hyperpolarizing the membrane by passing inward current through the micro-electrode on the amplitude and polarity of the i.p.s.p. and slow e.p.s.p. *A*, records obtained at various levels of membrane hyperpolarizations from resting potential (uppermost trace). Both i.p.s.p. and slow e.p.s.p. were decreased and then inverted by membrane hyperpolarization. The fast e.p.s.p.s increased in amplitude over these values and have been truncated for clarity. *B*, amplitude of i.p.s.p. (○) and slow e.p.s.p. (●) recorded in *A* plotted against membrane potential at which they were evoked. Note apparent reversal potential for slow e.p.s.p. is 5 mV more negative than i.p.s.p. reversal potential.

different K^+ concentrations, between 0·5 and 10 mM, were tested on each cell. Between changes in the K^+ concentration, normal solution was present for 10–15 min during which time reversal potentials were again determined. Results were discarded if the reversal potential in normal solution, for either synaptic potential, changed by ± 4 mV during the course of the experiment. This procedure was carried out before and after the fast e.p.s.p. and the i.p.s.p. had been inhibited by 75–100% (by curare and phentolamine) and no difference in the slow e.p.s.p. amplitudes were observed. Records obtained during one of these experiments are shown in Fig. 4*A*, and the slow

e.p.s.p. amplitude as a function of membrane potential obtained from this experiment for three K^+ concentrations is shown in Fig. 4*B*. At any membrane potential low external K^+ increased and elevated external K^+ decreased, the amplitude of the slow e.p.s.p. ($n = 6$). A linear relationship between the reversal potential of the slow e.p.s.p.

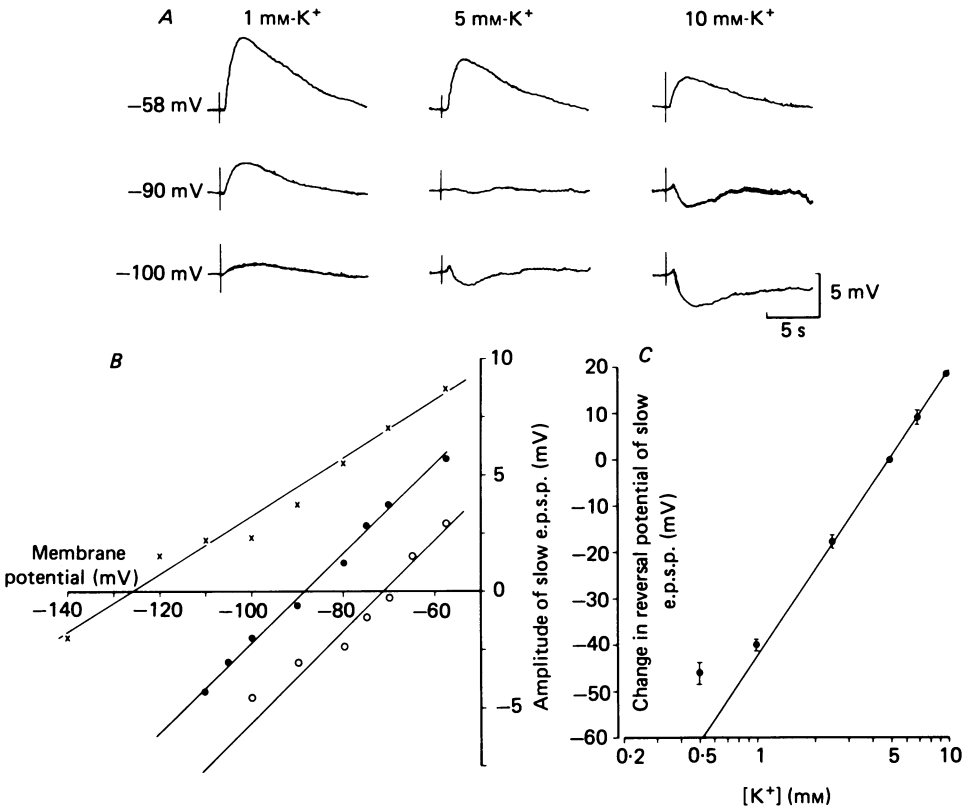

Fig. 4. Effect of altering external K^+ concentration on the amplitude (*A* and *B*) and reversal potential (*C*) of the slow e.p.s.p. *A*, recordings obtained from one neurone in low (1 mM), normal (5 mM) and elevated (10 mM) K^+ concentration. Single stimuli were delivered once a minute and slow e.p.s.p.s recorded at seven to ten different levels of membrane potential at each K^+ concentration. Records obtained at three membrane potential levels are shown in *A*. Curare (2×10^{-4} g/ml) and phentolamine (10^{-5} M) present to abolish fast e.p.s.p. and i.p.s.p. *B*, data from experiment in which responses shown in *A* were recorded are plotted for 1 mM-K^+ (×), 5 mM-K^+ (●) and 10 mM-K^+ (○). Low $[K^+]$ increased and high $[K^+]$ decreased slow e.p.s.p. amplitude and changed the reversal potential. The resting potential recorded during this experiment did not change (±3 mV) for external $[K^+]$ between 1 and 10 mM. *C*, relation between change in reversal potential of slow e.p.s.p. and logarithm of external $[K^+]$ for all experiments ($n = 6$). Straight line calculated from the Nernst equation: $E_K = 61 \log [K^+]_o/[K^+]_i$, where $[K^+]_i = 155$ mM. Deviation from the predicted relation is apparent at $[K^+]_o$ less than 1 mM.

and the logarithm of the external K^+ concentration was obtained in all six experiments for external K^+ of 1–10 mM (Fig. 4*C*). For these K^+ concentrations the observed values were in good agreement with those predicted by the Nernst equation when the internal K^+ was assumed to be 155 mM.

An obvious deviation from the predicted Nernst relation was apparent at external K^+ concentrations less than 1·0 mM (Fig. 4*C*). The most likely cause of this deviation is experimental error since it was usually not possible to pass sufficient current through the micro-electrode to actually reverse the synaptic potential at concentrations less than 1 mM; therefore the value of the reversal potential was determined by extrapolation. Alternatively, it may be that alterations in internal K^+ concentration occurred at the very low external K^+ levels.

No change in the resting membrane potential or the reversal potential for the slow e.p.s.p. was noted when the external Cl^- concentration was reduced to 1/2 or 1/10 normal ($n = 4$).

Identical results were obtained for the changes in the i.p.s.p.s recorded during the same experiments; i.e. the amplitude of the i.p.s.p. was a function of the external K^+ but not Cl^- concentration and the relation between the i.p.s.p. reversal potential and external K^+ concentration could be predicted by the Nernst equation for external K^+ of 1–10 mM ($n = 6$).

These experiments indicate that the conductance changes which occur during the i.p.s.p. and the slow e.p.s.p. (see below) probably involve a permeability change to only one ion: K^+.

Conductance changes associated with slow e.p.s.p.s and i.p.s.p.s

The amplitude and time course of electrotonic potentials evoked by passing brief (60–150 ms) hyperpolarizing current pulses through the recording electrode always decreased during the i.p.s.p. and usually increased during the slow e.p.s.p. (Fig. 5). These conductance changes were independent of membrane potential; that is, when the membrane potential was held at levels sufficiently negative so that less than ± 2 mV change in potential followed a single stimulus, the resulting changes in the electrotonic potentials were similar to those which occurred when the i.p.s.p. and slow e.p.s.p. were initiated from the resting potential ($n = 4$). These results indicate the observed conductance changes were probably generated by the action of the transmitters and were not due simply to polarization of the membrane.

When i.p.s.p.s of similar amplitude were compared, the relative decrease in input resistance (as a proportion of resting value) appeared to be roughly the same for all cells. In contrast, for slow e.p.s.p.s of similar amplitude the relative increase in input resistance showed considerable variation from cell to cell. On occasions no measurable change in input resistance was observed at the peak of the slow e.p.s.p. when the amplitude was less than 6–8 mV. No decrease in the input resistance was ever observed during the slow e.p.s.p. For these reasons, peak conductance changes associated with the i.p.s.p. and the slow e.p.s.p. were compared with those predicted as described in the Methods, assuming the conductance changes were due solely to turning on (during the i.p.s.p.) and turning off (during the slow e.p.s.p.) G_K.

Measurements of input resistances were obtained only from those cells in which the current–voltage relation was linear for membrane depolarizations up to 12–16 mV from resting potential and for membrane hyperpolarizations up to 20–25 mV from resting potential. Electrotonic potentials were recorded before and during transmural stimulation (1–10 pulses at 20 Hz) which evoked i.p.s.p.s and slow e.p.s.p.s of increasing amplitude; ten to twenty responses were averaged at each stimulus

duration before and after the i.p.s.p. had been depressed or abolished by the addition of phentolamine (Fig. 5). Reversal potentials for the i.p.s.p. and the slow e.p.s.p. were determined for each cell studied and these values were used to predict the relation between amplitude and resistance ratios.

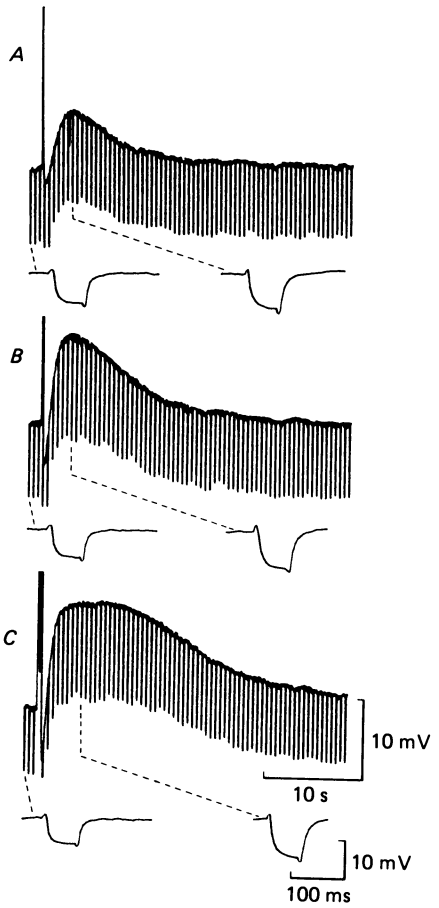

Fig. 5. Conductance decrease associated with the slow e.p.s.p. Electrotonic potentials evoked by passing hyperpolarizing current pulses (10^{-10} A) through the micro-electrode at a frequency of 2 Hz were recorded before and during slow e.p.s.p.s evoked at 1 min intervals by 1 (*A*), 2 (*B*) or 8 (*C*) nerve stimuli (at 20 Hz). Each trace is the average of ten responses. Electrotonic potentials recorded before and at the peak of each response (as indicated by dashed lines) are shown on faster sweep speed under each response. Although not shown on fast speed, it can be seen that the amplitude of the electrotonic potential is decreased during the i.p.s.p., indicating a conductance increase during this synaptic potential.

Results obtained in three experiments are shown in Fig. 6*A–C* and from all experiments in Fig. 6*D* ($n = 8$). In only one cell did the observed and predicted values for both the i.p.s.p. and the slow e.p.s.p. coincide (Fig. 6*A*). It can also be seen in Fig. 6*A* that the reversal potentials for the i.p.s.p. and the slow e.p.s.p. were identical

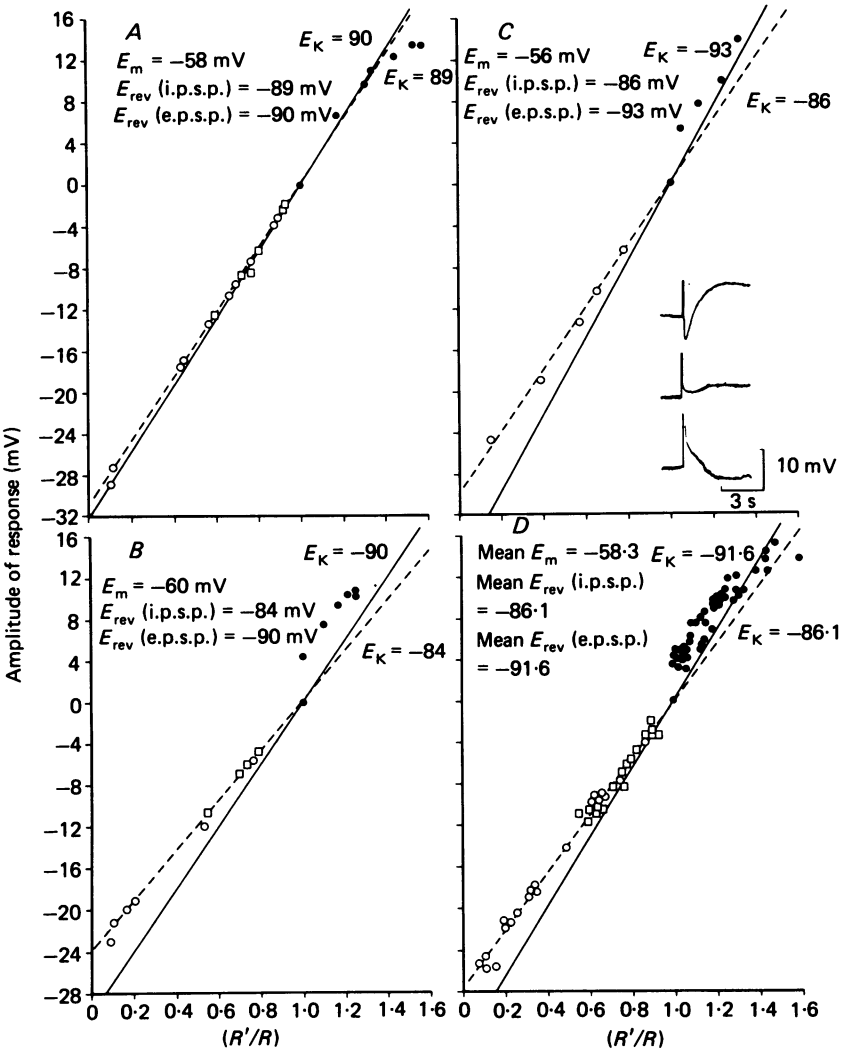

Fig. 6. Relation between amplitude of i.p.s.p. (○, □) and slow e.p.s.p. (●) and proportional conductance change (as R'/R, where R and R' are input resistance before and at peak of response). Results from three individual experiments are shown in *A–C*, and from all experiments in *D*. In each graph the continuous line represents the relation predicted if E_K = reversal potential for slow e.p.s.p.; dashed line calculated assuming E_K = i.p.s.p. reversal potential. Lines calculated from equation given in Methods. In all experiments there was no significant difference between the observed and predicted values for the i.p.s.p., assuming E_K = i.p.s.p. reversal potential (*A–D*). ○, values obtained in normal solution; □, values obtained when i.p.s.p. was depressed but not abolished by low doses of phentolamine. The values observed for the slow e.p.s.p. were significantly different ($P < 0{\cdot}001$) from either of the predicted relations in nine of ten cells examined (*B–D*). In only one case did observed and predicted values for the slow e.p.s.p. coincide (*A*); note i.p.s.p. and slow e.p.s.p. reversal potentials were indentical in this cell. Inset in *C* shows records obtained during this experiment at three levels of membrane potential (−61, −91 and −106 mV, top to bottom). In the middle record it is clear that the i.p.s.p. but not the slow e.p.s.p. has reversed. All data obtained from cells whose current–voltage relation remained linear over the range of membrane potentials examined.

in this cell. For all other cases the conductance changes recorded during the i.p.s.p. were not significantly different from those predicted from the i.p.s.p. reversal potential (Fig. 6*D*). On the other hand, the observed conductance changes associated with the slow e.p.s.p. deviated significantly from the predicted relationship (Fig. 6*B–D*).

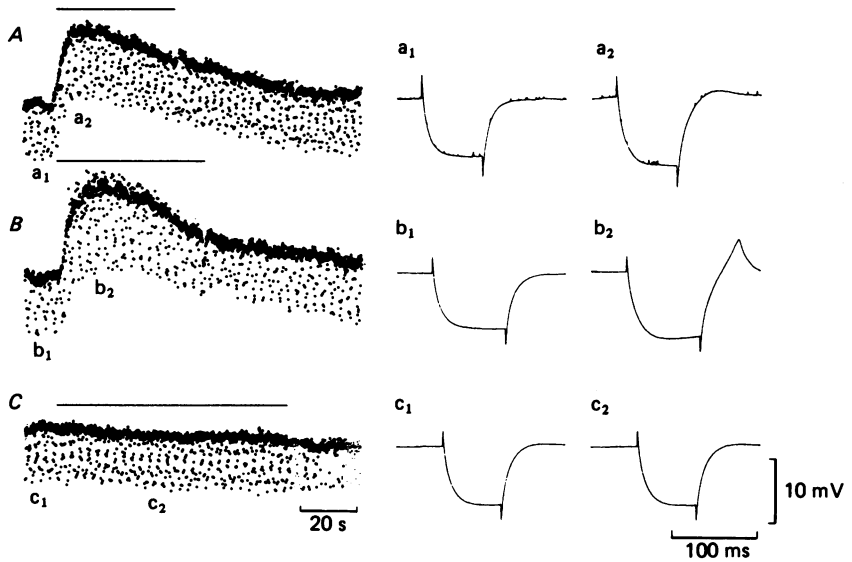

Fig. 7. Substance P depolarized neurones which received a slow excitatory synaptic input (*A* and *B*) but had no effect on neurones lacking slow excitatory input (*C*). *A* and *B*, perfusion with 10^{-8} M-substance P (*A*) and 10^{-7} M-substance P (*B*) caused a depolarization of the membrane of this neurone in which single stimuli evoked both fast and slow e.p.s.p.s as well as i.p.s.p.s. *C*, perfusion with 5×10^{-7} M-substance P had no effect on this neurone in which only fast e.p.s.p.s were evoked. Duration of perfusion is indicated by lines above each trace. Recordings were obtained from neurones which appeared to be adjacent in the same node. Electrotonic potentials evoked at a frequency of 3 Hz were recorded throughout these experiments. They are distorted in *A–C* because of the slow sampling rate (see Methods) but are shown in a_1–c_2 after the records were re-sampled (where indicated) at a fast rate. Average of thirty successive electrotonic potentials are shown before (a_1, b_1, c_1) and during (a_2, b_2, c_2) substance P perfusion. The action potential, which was initiated on cessation of some of the pulses is attenuated in b_2 due to the averaging procedure.

Some pharmacological properties of the slow e.p.s.p.

The amplitude and time course of the slow e.p.s.p. was unaffected by prolonged (5–7 h) perfusion with curare (10^{-6} to 5×10^{-4} g/ml) ($n > 28$), atropine (10^{-7} to 10^{-5} g/ml) ($n = 8$) or phentolamine (to 5×10^{-5} M) ($n > 30$). In seven of eight cells examined, hexamethonium (2×10^{-4} M) completely blocked the fast e.p.s.p. but had no effect on the slow e.p.s.p. when present for up to 3 h. However, on one occasion this concentration of hexamethonium reversibly and repeatedly (in three trials) depressed the slow e.p.s.p. by 80%, the fast e.p.s.p. by 100% and the i.p.s.p. by 25–60%. This synaptic depression was not accompanied by any apparent changes in the membrane properties of the cell.

When the preparation was perfused with substance P (10^{-9} to 5×10^{-7} M) for periods of 0·5–3·0 min a dose-dependent depolarization of the membrane was observed in all neurones exhibiting slow e.p.s.p.s (twelve cells, $n = 5$) (Fig. 7*A* and *B*). After the initial depolarization, the membrane began to repolarize in the continued presence of substance P; i.e. desensitization to substance P was apparent (Fig. 7*A* and *B*). During perfusion with substance P, single transmural stimuli,

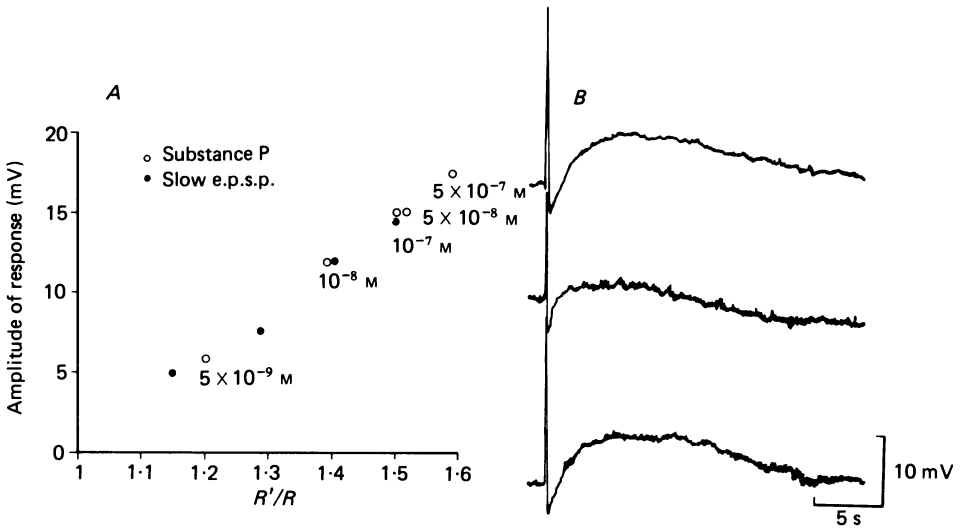

Fig. 8. *A*, amplitude of the slow e.p.s.p. (●) and substance P depolarization (○) plotted against the proportional conductance change (as R'/R). Data obtained from experiment in which records of Fig. 7*A* and *B* are shown; slow e.p.s.p.s were evoked in the same neurone. Amplitude of slow e.p.s.p. was varied by increasing the number of stimuli delivered (at 20 Hz) from one to eight. Slow e.p.s.p.s were recorded in normal solution. It can be seen that similar changes in conductance were observed. *B*, effects of substance P on amplitude of synaptic potentials. Resting potential in normal solution was −59 mV. Synaptic potentials recorded before (upper), 3 min in 5×10^{-7} M-substance P containing solution (middle), at which time the membrane had repolarized to −54 mV, and 10 min after wash-out (lower). In this experiment substance P decreased the amplitudes of the fast and slow e.p.s.p.s by 31 % and 69 % respectively, and increased the i.p.s.p. by 9 % over control amplitudes. Recordings obtained from a different neurone than that shown in *A*.

applied after the membrane potential had returned to within 3–6 mV of the resting potential, initiated fast and slow e.p.s.p.s whose amplitudes were decreased compared with control. Inhibitory synaptic potentials recorded at the same time showed a slight increase in amplitude (Fig. 8*B*). At the highest concentration of substance P examined (5×10^{-7} M) the amplitudes of the fast e.p.s.p. and slow e.p.s.p. were *reduced* by $39 \pm 3{\cdot}5$ % and $77 \pm 2{\cdot}4$ %, respectively, over control, while the i.p.s.p. was *increased* by $8{\cdot}4 \pm 2{\cdot}4$ % (five cells, $n = 4$). It seems likely that some of the depression of the fast e.p.s.p., as well as the increase in the i.p.s.p., may have been due to the depolarized (3–6 mV) membrane potential. All effects were reversible within 6 min of wash-out of substance P, by which time the membrane had returned to within 1–2 mV of its original potential (Fig. 8*B*).

The conductance changes recorded from one neurone during evoked slow e.p.s.p.s and substance P depolarizations are shown in Fig. 8*A*. It can be seen that the conductance changes associated with the substance P depolarization and with the slow e.p.s.p.s were identical for responses of equal amplitude. Similar results were obtained in two other experiments.

Substance P (5×10^{-7} M) was completely without effect on neurones in which no inhibitory or slow excitatory synaptic input could be demonstrated by nerve stimulation (eight cells, three preparations) (Fig. 7*C*). These cells were also tested for post-synaptic noradrenaline sensitivity and none were hyperpolarized by this drug. Higher concentrations of substance P were not examined.

These results suggest that receptors which are activated by substance P are present only on the post-synaptic membrane of those neurones which possess a slow excitatory synaptic input.

DISCUSSION

At least three types of synaptic input are commonly activated by a single stimulus applied to nerve fibres of the submucous plexus. In about half of the neurones studied cholinergic fast e.p.s.p.s of 50–80 ms duration were followed by an i.p.s.p. of about 1 s duration, followed, in turn, by a non-cholinergic, non-adrenergic slow e.p.s.p. which listed for 15–20 s. Both the i.p.s.p. and the slow e.p.s.p. appeared in an all-or-none manner and were not altered by further increases in the stimulus intensity. These results suggest that submucous plexus neurones receive multiple cholinergic excitatory input but only a single or very limited number of inhibitory and slow excitatory axons. However, no attempt was made to stimulate all of the nerves which enter a single node and, in these experiments, only one or two interganglionic fibre tracts (from a total of four to seven per node) would have been activated during transmural stimulation.

The majority of submucous neurones which showed slow e.p.s.p.s also showed i.p.s.p.s. Moreover, all neurones which displayed i.p.s.p.s or slow e.p.s.p.s (or both) were hyperpolarized by noradrenaline. The possibility thus arises that the inhibitory transmitter substance and the slow excitatory transmitter are present in and released from the same nerve fibre. Two observations argue against such an arrangement: (1) five cells which were hyperpolarized by noradrenaline failed to display an i.p.s.p. during transmural stimulation which did evoke a slow e.p.s.p.; (2) in some neurones the stimulus intensity required to initiate a slow e.p.s.p. was considerably lower than that required to initiate an i.p.s.p. It is therefore tentatively suggested that transmitters which give rise to the i.p.s.p. and the slow e.p.s.p. are found in separate nerves synapsing onto a common neurone.

Both the i.p.s.p. and the slow e.p.s.p. were depressed and eventually reversed when the membrane was hyperpolarized; these synaptic potentials were associated with decreases and increases, respectively, in input resistance, and the reversal potentials for both the i.p.s.p. and the slow e.p.s.p. were related to the external K^+ concentration in a manner that could be predicted by the Nernst equation. These results suggest that the i.p.s.p. is due to an increased G_K and the slow e.p.s.p. to a decreased G_K. If the i.p.s.p. and the slow e.p.s.p. are, indeed, due solely to respective activation and

inactivation of G_K, their reversal potentials should be identical to each other and equal to the K^+ equilibrium potential. However, in the majority of experiments the slow e.p.s.p. reversal potential was about 4–8 mV more negative than the i.p.s.p. reversal potential or that of the undershoot of the directly evoked action potential, which is known to be due to activation of G_K (Hodgkin & Huxley, 1952; Dodge & Frankenhauser, 1960). Moreover, the conductance decrease associated with the slow e.p.s.p. was 10–35 % less than predicted from the equivalent circuit theory (Ginsborg, 1973, 1976; Morita *et al.* 1981), while predicted and observed conductance increases associated with the i.p.s.p. were identical. The simplest explanation for these findings is that the inhibitory synapse is located on the soma whereas the synapse or synapses giving rise to the slow e.p.s.p. is usually located some distance from the soma, presumably on the dendrites. A shift in the apparent reversal potential to levels more negative than the true equilibrium potential and a 'shunting' of the dendritic conductance change are qualitatively consistent with dendritic synaptic locations (Rall, 1967; Smith, Wuerker & Frank, 1967; Jack, Noble & Tsien, 1975). Computational experiments, making use of a compartmental model (Rall, 1964, 1967; Jack & Redman, 1971), showed that the observed shift in the slow e.p.s.p. reversal potential is also quantitatively compatible with the observed discrepancy in the conductance ratios (J. B. Chapman & A. Surprenant, unpublished observations).

Substance P produced a membrane depolarization associated with a conductance decrease in only those submucous plexus neurones which displayed slow e.p.s.p.s. Neurones in which no slow e.p.s.p. or i.p.s.p. could be elicited by nerve stimulation and which additionally were not hyperpolarized by noradrenaline were found to be completely insensitive to substance P. Although these results yield little information regarding the identity of the transmitter responsible for the slow e.p.s.p., they do provide strong evidence that substance P receptors are specifically limited to neurones which possess slow excitatory synaptic input. Moreover, the specificity of the substance P response observed in the present study and the findings that substance-P-containing nerve terminals, whose cell bodies are located in the myenteric plexus, are present in the guinea-pig submucosal plexus do make substance P an obvious candidate for further investigation (Furness & Costa, 1980; Schultzberg, Hokfelt, Nilsson, Terenius, Rehfeld, Brown, Elde, Goldstein & Said, 1980; Jessen, Polak, VanNoorden, Bloom & Burnstock, 1980).

Slow excitatory potentials recorded from neurones of the guinea-pig myenteric plexus have been proposed to be due to the release of 5-hydroxytryptamine (5-HT) (Wood & Mayer, 1979*a*, *b*) or substance P (Katayama & North, 1978; Katayama, North & Williams, 1979; Morita, North & Katayama, 1980), although neither agonist has been shown to be specific for cells which display the slow e.p.s.p. (Johnson *et al.* 1981). Hirst & Silinsky (1975) and Neild (1981) have shown that 5-HT iontophoresis onto submucous plexus neurones caused a short duration (35–150 ms) membrane depolarization in all cells and an increase in amplitude of the 5-HT potential when the membrane was hyperpolarized; also, the 5-HT potential was blocked by curare. These results make 5-HT an unlikely candidate for the role of the neurotransmitter causing the slow e.p.s.p. in the submucous plexus.

This study has shown that in some submucous plexus neurones only fast e.p.s.p.s. are recorded while in other cells an inhibitory synaptic potential follows the initial

fast e.p.s.p. and precedes the slow e.p.s.p. Interactions clearly occur among all three synaptic inputs: it has been suggested that the inhibitory transmitter may reduce the release of acetylcholine from nicotinic synapses (Edwards *et al.* 1976). Preliminary studies have indicated that the action of the inhibitory transmitter is also capable of overcoming the depolarization of the slow e.p.s.p. (A. Surprenant, personal observations). Furthermore, late fast e.p.s.p.s often reach threshold for action potential initiation when they occur during the depolarization of the slow e.p.s.p. Further electrophysiological investigations of submucous plexus neurones should provide a better understanding of the functional implications of such synaptic interactions.

It has been pointed out that a single stimulus was sufficient to evoke a slow e.p.s.p. in all submucous plexus neurones which received this type of synaptic input. Thus, it would seem reasonable to expect the slow e.p.s.p. of the submucous plexus to play a significant role in the co-ordination of gastrointestinal motility.

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REFERENCES

BENNETT, M. R. & BURNSTOCK, G. (1968). Electrophysiology of the innervation of intestinal smooth muscle. In *Handbook of Physiology*, section 6, *Alimentary Canal*, ed. CODE, C. F., pp. 1709–1732. Washington D.C.: Am. Physiol. Soc.

DODGE, F. A. & FRANKENHAUSER, B. (1960). Membrane currents in isolated frog nerve fibre under voltage clamp conditions. *J. Physiol.* **143**, 76–90.

COSTA, M. & FURNESS, J. B. (1976). The peristaltic reflex: an analysis of the nerve pathways and their pharmacology. *Naunyn-Schmiedebergs Arch. Pharmacol.* **294**, 47–60.

EDWARDS, F. R., HIRST, G. D. S. & SILINSKY, E. M. (1976). Interaction between inhibitory and excitatory synaptic potentials at a peripheral neurone. *J. Physiol.* **259**, 647–663.

FURNESS, J. B. & COSTA, M. (1980). Types of nerves in the enteric nervous system. *Neuroscience* **5**, 1–20.

GINSBORG, B. L. (1973). Electrical changes in the membrane in junctional transmission. *Biochim. biophys. Acta* **300**, 289–317.

GINSBORG, B. L. (1976). Physiology of the autonomic nervous system. In *Frog Neurobiology*, ed. LLINAS, R. & PRECHT, W., pp. 151–168. Berlin: Springer-Verlag.

HIRST, G. D. S. (1979). Mechanisms of peristalsis. *Br. med. Bull.* **35**, 263–268.

HIRST, G. D. S., HOLMAN, M. E. & SPENCE, I. (1974). Two types of neurones in the myenteric plexus of duodenum in the guinea-pig. *J. Physiol.* **236**, 303–326.

HIRST, G. D. S. & MCKIRDY, H. C. (1975). Synaptic potentials recorded from neurones of the submucous plexus of guinea-pig small intestine. *J. Physiol.* **249**, 369–385.

HIRST, G. D. S. & SILINSKY, E. M. (1975). Some effects of 5-hydroxytryptamine, dopamine and noradrenaline on neurones in the submucous plexus of guinea pig small intestine. *J. Physiol.* **251**, 817–832.

HODGKIN, A. L. & HUXLEY, A. F. (1952). Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol.* **116**, 449–472.

HUBBARD, J. I., LLINAS, R. & QUASTEL, D. M. J. (1969). *Electrophysiological Analysis of Synaptic Transmission*. London: Arnold.

JACK, J. J. B., NOBLE, D. & TSIEN, R. W. (1975). *Electric Current Flow in Excitable Cells*. Oxford: Oxford University Press.

JACK, J. J. B. & REDMAN, S. J. (1971). An electrical description of the motoneurone, and its application to the analysis of synaptic potentials. *J. Physiol.* **215**, 321–352.

JESSEN, K. R., POLAK, J. M., VANNOORDEN, S., BLOOM, S. R. & BURNSTOCK, G. (1980). Peptide-contaning neurones connect the two ganglionated plexuses of the enteric nervous system. *Nature, Lond.* **283**, 391–393.

JOHNSON, S. M., KATAYAMA, Y. & NORTH, R. A. (1980). Slow synaptic potentials in neurones of the myenteric plexus. *J. Physiol.* **301**, 505–576.

JOHNSON, S. M., KATAYAMA, Y., MORITA, K. & NORTH, R. A. (1981). Mediators of slow synaptic potentials in the myenteric plexus of the guinea-pig ileum. *J. Physiol.* **320**, 175–186.

KATAYAMA, Y. & NORTH, R. A. (1978). Does substance P mediate slow synaptic excitation within the myenteric plexus? *Nature, Lond.* **274**, 387–388.

KATAYAMA, Y., NORTH, R. A. & WILLIAMS, J. T. (1979). The action of substance P on neurones of the myenteric plexus of the guinea pig ileum. *Proc. R. Soc.* B **206**, 191–208.

KATZ, B. (1966). *Nerve, Muscle and Synapse*. New York: McGraw Hill.

MORITA, K., NORTH, R. A. & KATAYAMA, Y. (1980). Evidence that substance P is a transmitter in the myenteric plexus. *Nature, Lond.* **287**, 151–152.

MORITA, K., NORTH, R. A. & TOKIMASA, T. (1981). The calcium-activated potassium conductance in guinea pig myenteric neurones. *J. Physiol.* **329**, 341–354.

NEILD, T. O. (1981). The action of 5-hydroxytryptamine and possible 5-hydroxytryptamine antagonists on neurones of the guinea pig submucous plexus. *Gen. Pharmacol.* **12**, 281–284.

NISHI, S. & NORTH, R. A. (1973). Intracellular recording from the myenteric plexus of the guinea pig ileum. *J. Physiol.* **321**, 471–491.

NORTH, R. A. (1973). The calcium dependent slow after-hyperpolarization in myenteric plexus neurons with tetrodotoxin-resistant action potential. *Br. J. Pharmac.* **49**, 709–711.

RALL, W. (1964). Theoretical significance of dendritic trees for neuronal input–output relations. In *Neural Theory and Modelling*, ed. REISS, R. F., pp. 73–97. Stanford University Press.

RALL, W. (1967). Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. *J. Neurophysiol.* **30**, 1138–1168.

SCHULTZBERG, M., HOKFELT, T., NILSSON, G., TERENIUS, L., REHFELD, J. F., BROWN, M., ELDE, R., GOLDSTEIN, M. & SAID, S. (1980). Distribution of peptide and catecholamine-containing neurons in the gastrointestinal tract of rat and guinea-pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine-β-hydroxylase. *Neuroscience* **5**, 689–744.

SMITH, T. G., WUERKER, R. B. & FRANK, K. (1967). Membrane impedance changes during synaptic transmission in cat spinal motoneurons. *J. Neurophysiol.* **30**, 1072–1096.

SURPRENANT, A. (1984). Two types of neurones lacking synaptic input in the submucous plexus of guinea-pig small intestine. *J. Physiol.* **351**, 363–378.

WOOD, J. D. (1970). Electrical activity from single neurons in Auerbach's plexus. *Am. J. Physiol.* **219**, 159–169.

WOOD, J. D. (1981). Physiology of the enteric nervous system. In *Physiology of the Gastrointestinal Tract*, ed. JOHNSON, L. R., pp. 1–37. New York: Raven Press.

WOOD, J. D. & MAYER, C. J. (1979*a*). Intracellular study of tonic-type enteric neurons in guinea-pig small intestine. *J. Neurophysiol.* **42**, 569–581.

WOOD, J. D. & MAYER, C. J. (1979*b*). Serotonergic activation of tonic type enteric neurons in guinea-pig small intestine. *J. Neurophysiol.* **42**, 582–593.