J. Physiol. (1982), **325**, *pp.* 145–159
With 10 *text-figures*
Printed in Great Britain

NON-CHOLINERGIC EXCITATORY TRANSMISSION IN INFERIOR MESENTERIC GANGLIA OF THE GUINEA-PIG: POSSIBLE MEDIATION BY SUBSTANCE P

BY N. J. DUN AND Z. G. JIANG*

From the Department of Pharmacology, Loyola University, Stritch School of Medicine, 2160 South First Avenue, Maywood, IL 60153, U.S.A.

(*Received 13 August 1981*)

SUMMARY

1. Repetitive stimulation of guinea-pig hypogastric nerves elicited, in addition to the fast cholinergic excitatory potential, a slow depolarization lasting for seconds to minutes in neurones of the isolated inferior mesenteric ganglion.

2. The slow depolarization which could be elicited at a frequency as low as 1–2 Hz for several seconds was not blocked by cholinergic antagonists, but was eliminated in a low Ca^{2+} solution; it was termed henceforth the non-cholinergic excitatory potential.

3. When the membrane potential was manually clamped, the non-cholinergic potential was associated with three types of membrane resistance change: an increase, a delayed increase and a biphasic change consisting of an initial decrease followed by an increase.

4. In the majority of neurones, conditioning hyperpolarization augmented the non-cholinergic depolarization; in a few neurones, moderate hyperpolarization depressed the latter, whereas stronger hyperpolarization unmasked a low depolarization.

5. The non-cholinergic response was markedly attenuated in the presence of exogenously applied substance P; it was partially suppressed by luteinizing hormone-releasing hormone.

6. Non-cholinergic depolarization could be elicited in the same neurone by stimulation of all four nerve trunks associated with the ganglion.

7. It is suggested that substance P, a peptide, may be the transmitter responsible for the generation of the non-cholinergic potential and that it may be released from collateral endings of primary sensory neurones, thus providing a functional connexion between sensory and autonomic neurones.

INTRODUCTION

The importance of prevertebral ganglia in the modulation of gastrointestinal motility has been emphasized (Szurszewski & Weems, 1976; Szurszewski, 1981). The neurones in these ganglia receive synaptic inputs arising from the central nervous

* Visiting Scholar from the First Medical College of Shanghai, the People's Republic of China.

system as well as from the peripheral viscera, suggesting that they may be involved in reflex modulation of gastrointestinal activity (Crowcroft & Szurszewski, 1971; Crowcroft, Holman & Szurszewski, 1971; Szurszewsi, 1981). The pathways and the means by which the peripheral inputs are transmitted to the prevertebral ganglion cells have not been entirely clarified.

Crowcroft & Szurszewski (1971) suggested that these pathways include a reflex constituted by axons emanating from cholinergic neurones situated in the wall of the gastrointestinal tract and their synapses with neurones of the inferior mesenteric ganglia. The observation that repetitive stimulation of hypogastric nerves elicited in the cells of the inferior mesenteric ganglia a long-lasting membrane depolarization raises the possibility that this response may also participate in the local reflex-control of gastrointestinal activity. As this depolarizing response was not sensitive to cholinergic antagonists, but abolished in a low Ca^{2+} solution (Neild, 1978), it was referred to as the non-cholinergic excitatory potential (Dun & Karczmar, 1979). In the present study, the synaptic pathways, electrophysiological properties and the transmitter possibly involved in the generation of this non-cholinergic potential have been further characterized.

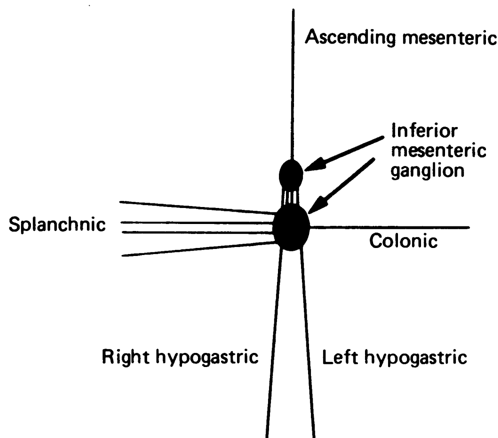

Fig. 1. Schematic diagram of a guinea-pig inferior mesenteric ganglion and associated nerves. The inferior mesenteric ganglion consists of two discrete ganglia interconnected by strands of nerve fibres. The left and right hypogastric, ascending mesenteric and colonic nerves usually consist of a single nerve trunk, whereas the splanchnic nerves are composed of seven to ten separate nerve trunks.

METHODS

Adult male albino guinea-pigs (250–300 g) were stunned and killed by a sharp blow on the head. The inferior mesenteric ganglia, their left and right hypogastric nerves, and, in several experiments, the major branches of three other nerves, i.e. splanchnic (consisting of seven to ten separate nerve trunks), ascending mesenteric and colonic (Fig. 1; see also Crowcroft & Szurszewski, 1971), were rapidly excised and transferred to the recording chamber. The ganglia were superfused with a Krebs solution of the following composition (mM): NaCl, 117; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 25; NaH_2PO_4, 1·2 and glucose, 11·5. The solution was equilibrated with 95% O_2 and 5% CO_2, and pre-warmed to 35–36 °C.

Intracellular recordings were obtained from neurones of isolated ganglia by means of fibre-containing glass micro-electrodes filled with 3 M-KCl, having a tip resistance of 30–60 MΩ (Dun

& Nishi, 1974; Dun & Minota, 1981). Transmembrane current was passed through the recording electrode utilizing a bridge circuit of the preamplifier (WPI-701). The nerve trunks were carefully dissected free of connective tissues and drawn into separate suction electrodes for electrical stimulation. The potential changes were recorded on a Tektronix oscilloscope and on a Gould Brush pen recorder (model 2200). The results were expressed as mean ± S.D. The Figures were reproduced from the tracings of the pen recorder. The following compounds were used: atropine sulphate, hexamethonium bromide, D-tubocurarine chloride (Sigma Co.), substance P and luteinizing hormone-releasing hormone (Peninsula Laboratories).

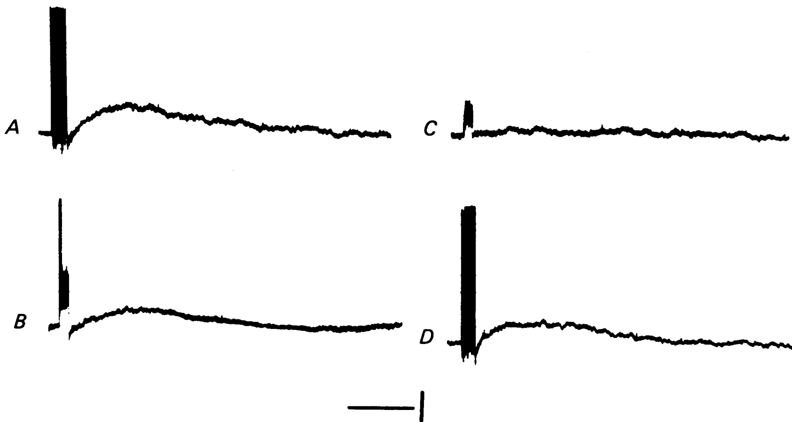

Fig. 2. Effects of low Ca^{2+} (0·25 mM)/high Mg^{2+} (12 mM) solution on the fast excitatory post-synaptic potentials (initial vertical tracings), the after-hyperpolarizations, and non-cholinergic excitatory potentials elicited by repetitive stimulation of hypogastric nerves (30 Hz, 5 sec). *A*: control responses; *B* and *C*: 5 and 10 min after superfusion the ganglion with low Ca^{2+} solution, respectively. Note that the fast excitatory potentials, the after-hyperpolarizations, and non-cholinergic potentials were markedly diminished in *B*, and abolished in *C*. *D*: 5 min after returning to Krebs solution. Both the fast excitatory and non-cholinergic potentials as well as the after-hyperpolarization have recovered to near control levels. The spike potentials were attenuated because of the limitation of the frequency response of the pen recorder. Calibration: 10 mV and 20 sec.

RESULTS

The non-cholinergic excitatory potential

Repetitive stimulation of hypogastric nerves (20–30 Hz, 1–5 sec) elicited in neurones of the inferior mesenteric ganglia a burst of action potentials which was followed in the majority of neurones by a hyperpolarization (Figs. 2, 3 and 6*A*). The hyperpolarizing response probably represented an after-hyperpolarization of the neurone following spike discharges, as it disappeared when the discharges were eliminated in a low Ca^{2+} solution (Fig. 2) or by D-tubocurarine (Fig. 3*C*; see also below). The initial hyperpolarization was followed in 169 of the 198 neurones examined by a slow depolarization. The slow depolarization was termed the non-cholinergic excitatory potential as it was not blocked by cholinergic antagonists (see below).

The synaptic delay of the slow non-cholinergic potential showed considerable variation from cell to cell; it ranged between 0·5 and 5 sec (see also Neild, 1978). The amplitude of the slow depolarization also varied considerably among individual neurones; it ranged from 2 to 16 mV, with a mean of 4·1 ± 0·3 mV (n = 169) when

measured at resting membrane potentials between −50 and −60 mV. The time course of the non-cholinergic depolarization was long-lasting, ranging from 20 sec to 4 min; the average was 54±26 sec. In a number of neurones, the slow depolarization was succeeded by a second hyperpolarization of long duration (Figs. 3–5 and 6*A*). The mean amplitude and duration of the latter were 2·4±1·3 mV and 76±34·6 sec, respectively ($n = 50$).

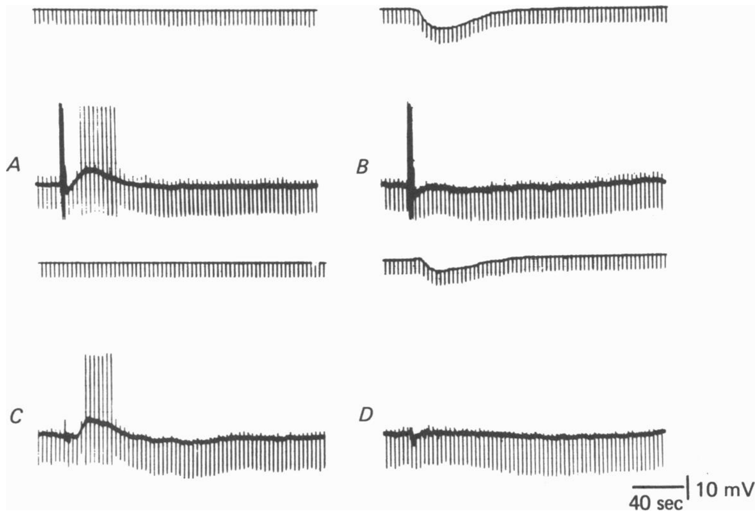

Fig. 3. Lack of effects of cholinergic antagonists on the slow non-cholinergic depolarization. *A*: control response elicited by repetitive (30 Hz, 4 sec) stimulation of hypogastric nerves. Note that the burst of discharges was followed by a hyperpolarization which was in turn succeeded by a slow membrane depolarization; the latter was followed by a second long-lasting hyperpolarization. Hyperpolarizing current pulses of 200 msec duration (upper tracing) were used to induce hyperpolarizing electrotonic potentials (lower tracing). Note that at the peak of the slow depolarization, several off spikes (also in *C*) could be seen following the applied hyperpolarization. *B*: the non-cholinergic potential was annulled by passage of hyperpolarizing current through the recording electrode. Note that membrane resistance during the initial phase of depolarization showed no detectable change, but increased thereafter. *C*: repetitive stimulation elicited the slow depolarization 20 min after superfusing the ganglion with D-tubocurarine (10 μM) and atropine (1 μM). Note that the second hyperpolarization was also not affected by cholinergic antagonists. *D*: membrane potential was manually clamped in the presence of cholinergic antagonists. The membrane resistance change in this case showed a similar pattern of change as in *B*. The recordings were taken from the same ganglion cell. Calibration: 10 mV, 0·5 nA and 40 sec.

The slow non-cholinergic depolarization as well as the spike discharges were reversibly abolished in a low Ca^{2+} (0·25 mM)/high Mg^{2+} (12 mM) solution (Fig. 2) in all of the ten cells tested. Superfusion of the ganglion cells with D-tubocurarine (10 μM) or hexamethonium (50 μM) blocked the spike discharges, indicating that the transmitter mediating these responses may be acetylcholine (ACh; see also Crowcroft & Szurszewski, 1971). On the other hand, D-tubocurarine and/or atropine (1 μM) did not affect the amplitude and time course of the non-cholinergic depolarization (Fig. 3). Moreover, membrane resistance changes associated with the non-cholinergic depolarization were not affected by the presence of cholinergic antagonists. For

example, in a manually clamped ganglion cell the membrane resistance increased, following a delay in the course of the slow depolarization (Fig. 3*B*); a similar pattern of membrane resistance change, i.e. a delayed increase after a period of no detectable change (Fig. 3*D*), was observed in the presence of cholinergic antagonists, which eliminated the spike discharges as well as the after-hyperpolarization (Fig. 3*C* and *D*).

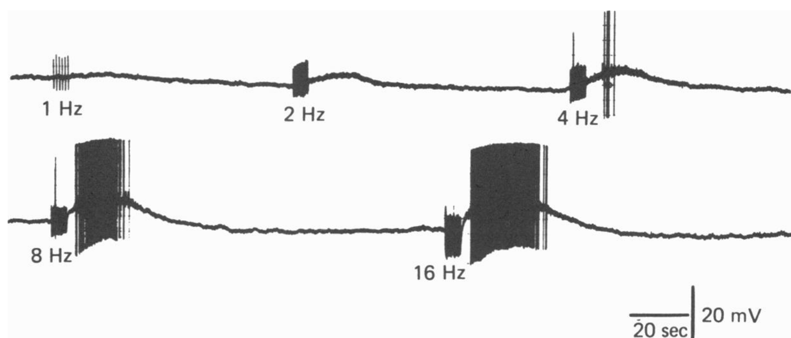

Fig. 4. The relationship between the non-cholinergic depolarization and stimulus frequency. The tracing constitutes a continuous recording from a single neurone. The peaks of spike discharges were cut off. Note that a small non-cholinergic depolarization could be induced at a frequency of 1 Hz for 5 sec. Spontaneous discharges occurred at the peak of non-cholinergic depolarization evoked at a frequency of 4 Hz, and became intensified at higher frequencies.

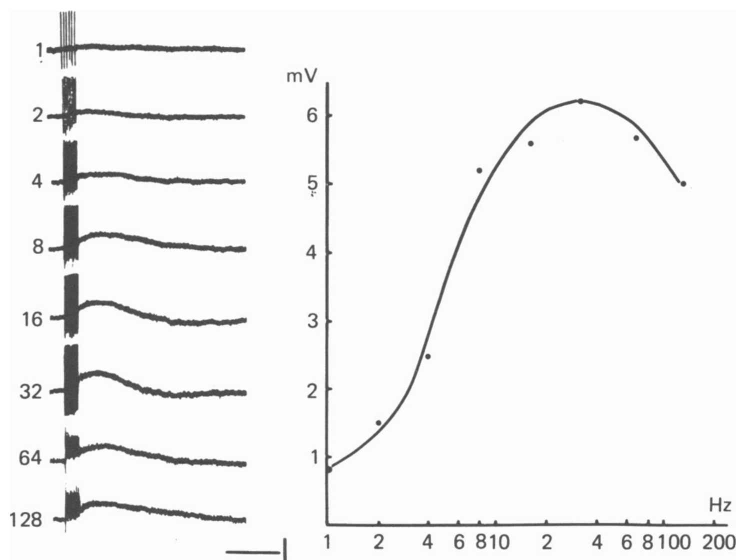

Fig. 5. The frequency–response relationship of non-cholinergic excitatory potential of a ganglion cell. The frequency (Hz) used to elicit the slow depolarization is shown on the left of each tracing. The graph on the right was plotted from the recording shown on the left. Note that the non-cholinergic depolarization was followed by a hyperpolarization when stimulated at the frequencies of 16 and 32 Hz. Ordinates: peak amplitude of non-cholinergic depolarization in mV. Abscissa: log frequency of stimulation. Calibration: 10 mV and 40 sec.

In subsequent experiments, cholinergic antagonists were not routinely added to the perfusing Krebs solution. Attempts were not made in the present study to correlate the size of fast e.p.s.p.s and the amplitude of the non-cholinergic potentials, as the strength of electrical stimulation employed was always supramaximal and invariabily caused spike potentials. It was noticed however that in a number of cells repetitive stimulation evoked only fast e.p.s.p.s, with no evidence of non-cholinergic transmission; conversely, the non-cholinergic potential in all cells tested was preceded by nicotinic e.p.s.p.s.

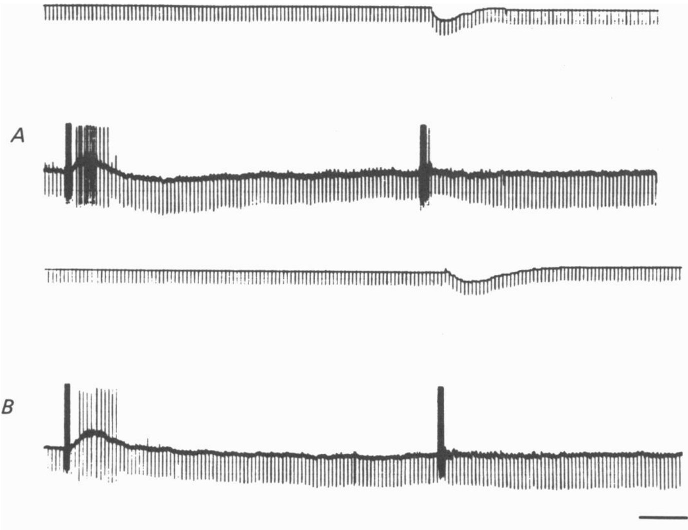

Fig. 6. Two types of membrane resistance change during the non-cholinergic depolarizations observed in two different ganglion cells *A* and *B*. The beginning of each lower tracing depicts the non-cholinergic potential elicited by repetitive stimulation (30 Hz, 4 sec). Hyperpolarizing electrotonic potentials (lower tracing) were induced by hyperpolarizing current pulses of 250 msec duration (upper tracing). The membrane resistance change during the slow depolarization was obscured by spontaneous discharges in cell *A* and off spikes in cell *B*. When the membrane depolarization was nullified by the application of hyperpolarizing current, the membrane resistance showed an initial decrease followed by an increase in cell *A*, whereas cell *B* exhibited a monophasic increase. Note that the non-cholinergic depolarization was followed by a hyperpolarization in cell *A*. Calibration: 10 mV, 0·5 ηA and 40 sec.

Another feature that was frequently encountered during the generation of non-cholinergic depolarization was the occurrence of spontaneous spike discharges and subthreshold oscillations (Figs. 4 and 8). The frequency and intensity of the discharges appeared to be related to the amplitude of the non-cholinergic depolarization (Fig. 4). These discharges were not affected by cholinergic nicotinic and muscarinic antagonists, but readily obliterated by returning the membrane potential to the resting level; thus, these discharges probably represented neuronal action potentials generated by membrane depolarization.

Frequency response

The minimal and optimal frequency of stimulation of the hypogastric nerves which induced non-cholinergic depolarization were determined in this series of experiments. In responsive neurones, a few seconds of stimulation at a frequency as low as 1 Hz effectively evoked a small but detectable depolarization. In the neurone shown in Fig. 4, the membrane depolarization induced by stimulation at 4 Hz for 5 sec was sufficiently large to exceed the threshold and generate spontaneous firing. In fourteen neurones examined, a maximal non-cholinergic depolarization was elicited by a 20–30 Hz stimulus applied for 2–5 sec. A typical experiment is shown in Fig. 5; in this particular cell, a measurable response was induced at a frequency of 2 Hz, and the maximal response was attained at about 30 Hz; the depolarization became progressively smaller at higher frequencies. Accordingly, the stimulus frequency in most of the experiments described in this study was set at 30 Hz.

Membrane resistance change

The neuronal input resistance change was estimated from the current–voltage relationship. Because the time course of the non-cholinergic depolarization was slow, input resistance change could be estimated under a manual voltage-clamp procedure, i.e. the depolarization was nullified by passage of hyperpolarizing current through the recording electrode. The membrane resistance change associated with non-cholinergic potential was complex and variable; three types of resistance change were observed in the forty-seven neurones tested. In the first case the membrane resistance showed little or no measurable change during the initial phase of the depolarization, but was increased after some 20–30 sec (Fig. 3*B* and *D*). The increase was generally long-lasting and persisted a few minutes after the membrane potential had returned to the resting level. This type of input resistance change was noticed in twenty-one neurones, and the mean increase was 21 %. A response of the second type was seen in another fifteen neurones; in this case, the membrane resistance showed a biphasic change consisting of a small but definite decrease followed by a sustained increase (Fig. 6*A*). The average decreases and increases were 16 % and 24 %, respectively. Finally, in the case of the remaining eleven neurones the input resistance showed a monophasic increase (Fig. 6*B*), which amounted on average to 22 %.

It is of interest to note that the second hyperpolarization that followed the non-cholinergic depolarization was frequently accompanied by an increase of membrane resistance; the mean increase was about 25 % ($n = 23$).

Relationship between membrane potential and non-cholinergic depolarization

The relationship between the amplitude of non-cholinergic depolarization and the membrane potential was investigated in forty-five neurones. In the large majority of neurones ($n = 32$), hyperpolarization increased the amplitude of the non-cholinergic depolarization; a typical experiment is shown in Fig. 7*A*. Single neuronal action potentials were induced by direct intracellular stimulation just prior to repetitive stimulation of hypogastric nerves, and the amplitude of the spike after-hyperpolarization was used as an indicator of the membrane potential level. Progressively increasing the membrane potential from -50 to -70 mV reduced the

amplitude of the after-hyperpolarizations (arrows), whereas the non-cholinergic response was markedly enhanced. Interestingly, the hyperpolarization that followed the non-cholinergic depolarization was also augmented (Fig. 7*A*). The mean equilibrium potential of the non-cholinergic response, as extrapolated from data obtained from fourteen cells, amounted to -39 ± 7 mV.

On the other hand, in thirteen other neurones moderate hyperpolarization caused either a depression ($n = 9$) or no detectable change ($n = 4$) of the amplitude of the

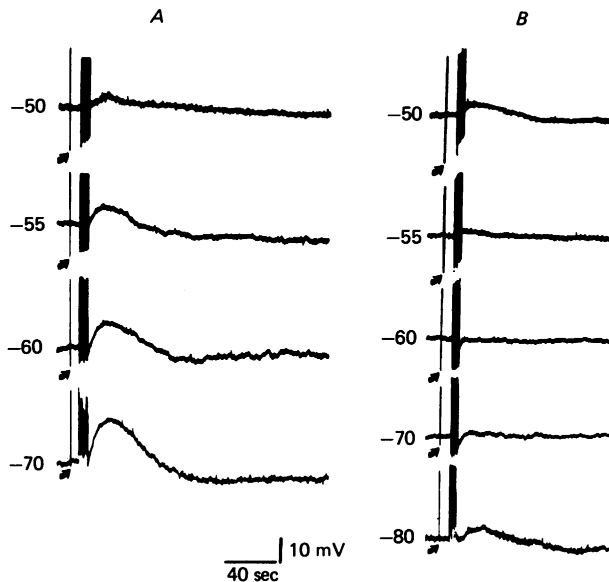

Fig. 7. Effects of conditioning hyperpolarization on non-cholinergic depolarization in two different ganglion cells, *A* and *B*. Single neuronal action potentials were elicited by direct intracellular stimulation just prior to repetitive stimulation of hypogastric nerves, and the amplitude of the after-hyperpolarization (arrow) was used as an indicator of the membrane potential level which is shown at the left of each tracing. Cell *A*: membrane hyperpolarization from -50 to -70 mV increased the non-cholinergic depolarization. Note also that the amplitude and time course of the hyperpolarization which followed the non-cholinergic depolarization were increased by conditioning hyperpolarization. Cell *B*: conditioning hyperpolarization from -50 to -60 mV reduced the non-cholinergic response, whereas further hyperpolarization unmasked the non-cholinergic response.

non-cholinergic depolarization; when stronger conditioning hyperpolarization (> -60 mV) was applied to these cells, it unmasked a slow depolarization that appeared to have been depressed by the preceding moderate hyperpolarization. A representative experiment is illustrated in Fig. 7*B*. In this particular neurone, moderate hyperpolarization of 10 mV nearly abolished the non-cholinergic response; however, further hyperpolarization unmasked rather than reversed the response.

Stimulation of other nerve trunks

The results described thus far were obtained from inferior mesenteric ganglion cells upon stimulation of the left and right hypogastric nerves. In a previous study, it was reported that cholinergic excitatory potentials could be evoked in neurones of the

inferior mesenteric ganglia by stimulation of the splanchnic, colonic and ascending mesenteric nerves, as well as the hypogastric (Crowcroft & Szurszewski, 1971). It appeared to be of importance to establish whether or not non-cholinergic depolarization could be similarly induced by stimulation of nerve trunks other than the hypogastric.

In the majority of neurones (seventeen out of twenty-two) stimulation of the major branches of any one of the four nerve trunks in question evoked a non-cholinergic potential in the same neurone; the potentials differed as to their amplitude and time

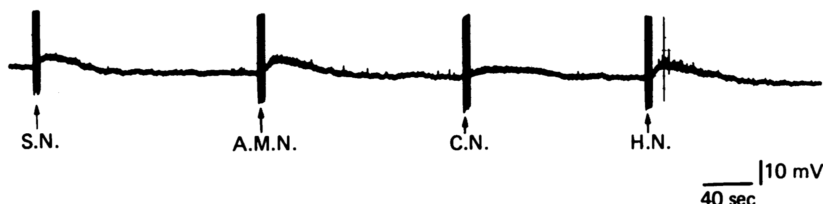

Fig. 8. Synaptic responses of a single ganglion cell to stimulation of four nerve trunks: splanchnic (S.N.), ascending mesenteric (A.M.N.), colonic (C.N.) and hypogastric (H.N.) nerves. Note that stimulation of these nerves (30 Hz, 4 sec) all elicited in the same neurone fast excitatory potentials, causing neurone spikes followed by a slow non-cholinergic depolarization.

course. A representative experiment is shown in Fig. 8. In this particular cell, the non-cholinergic depolarization evoked by stimulation of the colonic nerve was the smallest, and that elicited by the hypogastric nerves was the largest. It should be emphasized that this rank order was not applicable to other neurones; in fact, in some neurones the non-cholinergic depolarization elicited by stimulation of the colonic nerve was substantially greater than that evoked by hypogastric nerves. In the remaining five neurones, non-cholinergic potentials could be induced by stimulation of two or three nerve trunks.

It should be pointed out that the parameters of the slow depolarization elicited by the stimulation of the splanchnic, colonic and ascending mesenteric nerves, i.e. their amplitude, time course and membrane resistance changes, were similar to the parameters characterizing the slow non-cholinergic depolarization evoked by the stimulation of the hypogastric nerves.

Substance P and the non-cholinergic potential

Substance P was found to depolarize the neurones of the inferior mesenteric ganglia; the characteristics of this response were similar to those of the non-cholinergic potential (for detailed information see Dun & Minota, 1981; Minota, Dun & Karczmar, 1981). The effect of exogenously applied substance P on synaptically induced non-cholinergic depolarization was investigated in the present study.

The non-cholinergic potential in eight neurones studied was found to be markedly depressed or completely abolished in the presence of substance P; the results of a typical experiment are shown in Fig. 9. First, the non-cholinergic depolarization was elicited by stimulation of the hypogastric nerves; the membrane resistance showed an increase following the peak of non-cholinergic depolarization. Substance P (1 μM) was applied next to the ganglion, causing a large depolarization and intense neuronal

discharges; substance P depolarization was also accompanied by an increase of membrane resistance. The membrane potential gradually subsided in the continuous presence of substance P; stimulation of the hypogastric nerves at this time failed to evoke a detectable non-cholinergic potential. The non-cholinergic potential could be evoked again a few minutes after returning the ganglion to normal Krebs solution.

It should be stressed that substance P did not necessarily induce a similar membrane resistance change in all the eight neurones in question; in fact, the changes were similar to those observed in the course of the synaptically induced non-cholinergic response described above. In any given neurone the membrane resistance change associated with the substance P depolarization was similar to that associated with the non-cholinergic potential obtained in this particular cell.

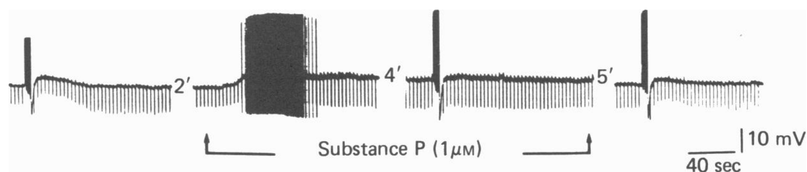

Fig. 9. Effects of exogenously applied substance P on the non-cholinergic depolarization. The tracing represents a continuous recording interrupted by intervals marked by numerals in minutes. The non-cholinergic depolarization was induced by repetitive stimulation of hypogastric nerves. Hyperpolarizing electrotonic potentials were induced by injection of hyperpolarizing current pulses of 200 msec duration. Note that there is an increase of membrane resistance following non-cholinergic depolarization. Application of substance P (1 μM) caused a marked depolarization and intense neuronal discharges. Also note that membrane resistance increased following substance P depolarization. In the continuous presence of substance P, membrane depolarization gradually subsided, and stimulation of hypogastric nerves elicited no detectable non-cholinergic depolarization. The latter recovered to nearly control level a few minutes after returning the preparation to Krebs solution.

Another peptide, luteinizing hormone-releasing hormone (LHRH) was also found to depolarize the neurones of the inferior mesenteric ganglia. The concentrations of LHRH (10–50 μM) effective in eliciting a membrane depolarization were approximately one hundred times higher than the effective concentrations of substance P (see Dun & Minota, 1981; Minota *et al.* 1981); furthermore, the resulting depolarizations were generally slow in onset and small in amplitude. The effects of exogenously applied LHRH on the synaptically induced non-cholinergic response were tested in four cells; the non-cholinergic responses were partially depressed after superfusing the ganglion cells for 10 min with LHRH (10–50 μM).

DISCUSSION

While the general features, i.e. time course, amplitude and pharmacological properties, of the non-cholinergic depolarization observed in the present study were similar to those described by Neild (1978), our results differ from those of the early report in two important aspects. First, contrary to the early report that 10 Hz was the lowest frequency necessary to evoke a non-cholinergic potential, a distinctive depolarization could be induced in responsive neurones at a frequency as low as 1–2 Hz.

Secondly, the membrane resistance change associated with the non-cholinergic depolarization was found to be more variable than that reported in the previous study, in which the slow depolarizations in the majority of neurones were accompanied by decreases in membrane resistance. Furthermore, the present study shows that the non-cholinergic potential can be elicited by stimulation of all four nerve trunks associated with the inferior mesenteric ganglion.

Three types of membrane resistance change were associated with the non-cholinergic depolarization in the manually clamped neurones. In about 50% of the neurones tested, the membrane resistance showed no appreciable change during the initial phase of the response, while it increased thereafter, this increase persisting for several minutes. In the case of the neurones of the second type, the membrane resistance showed a monophasic increase. Lastly, a biphasic change was noted in some neurones in which an initial transient decrease was followed by a long-lasting increase of resistance. These findings suggest that changes in conductance of several ions may underlie the non-cholinergic depolarization. These changes may include inactivation of potassium conductance (G_K) as suggested by Konishi, Tsunoo & Otsuka (1979). However, the observation that in a large number of neurones membrane hyperpolarization increases the non-cholinergic response is not what could be expected from simple G_K inactivation. It is true that in some neurones the non-cholinergic depolarization was depressed upon moderate hyperpolarization as expected if the depolarization was due to G_K inactivation; however, the finding that further hyperpolarization caused in some instances a reappearance of the non-cholinergic depolarization rather than causing a reversal indicates that even in these neurones the conductance change may involve multiple mechanisms rather than a single one. Moreover, in some neurones a decrease of membrane resistance was clearly evident. It is particularly noteworthy that multiple conductance changes may underlie the slow excitatory post-synaptic potential which is mediated by a muscarinic action of ACh, as well as the late slow excitatory post-synaptic potential which may be generated by the peptide LHRH of bullfrog sympathetic neurones; the changes in question may involve G_{Na}, G_K and G_{Ca} (Kuba & Koketsu, 1974, 1976; Jan, Jan, & Kuffler, 1980; Katayama, Inokuchi & Nishi, 1981; Nishi & Katayama, 1981). Hence, there is a close similarity between the muscarinic action on sympathetic neurones and that of several peptides. Indeed, it was shown recently by means of the voltage-clamp method that one common cellular mode of action of muscarine and the peptides LHRH and angiotensin II on sympathetic ganglion cells is the inactivation of a voltage-sensitive potassium current (Adams & Brown, 1980; Brown & Adams, 1980; Brown, Constanti & Marsh, 1980).

A few comments are pertinent with respect to the transmitter mediating the non-cholinergic depolarization. We showed previously that substance P, an undecapeptide, when applied to neurones of the inferior mesenteric ganglia caused a membrane depolarization the characteristics of which were similar to those of the non-cholinergic depolarization evoked by nerve stimulation (Dun & Karczmar, 1979; Dun & Minota, 1981; Minota *et al.* 1981). Depolarization induced by substance P was accompanied by either an increase or biphasic membrane resistance change, which may result from an increase of G_{Na} and a decrease of G_K (Dun & Minota, 1981; Minota *et al.* 1981); these mechanisms appear to be analogous to those involved

in synaptically induced non-cholinergic depolarization. More importantly, it was found in the present study that in any given neurone parallel changes of membrane resistance occur during the depolarization induced by substance P and by nerve stimulation. Moreover, the finding that the synaptically induced non-cholinergic potential was markedly diminished in the presence of exogenously applied substance P suggests that the latter and the transmitter mediating the non-cholinergic response may be acting on the same receptor.

On the other hand, LHRH, which is proposed to be the transmitter generating the late slow e.p.s.p. in bullfrog sympathetic neurones (Jan, Jan & Kuffler, 1979, 1980), appeared to be much less effective in depolarizing the inferior mesenteric ganglion cells than substance P. Furthermore, the presence of LHRH or a closely related peptide in mammalian sympathetic ganglia has not been reported, whereas dense networks of nerve fibres exhibiting substance P immunoreactivity have been shown in inferior mesenteric ganglia by immunohistofluorescent methods (Hökfelt, Elfvin, Schultzberg, Goldstein & Nilsson, 1977; Baker, Cuello & Matthews, 1980); also, the presence of high concentrations of substance P or a closely related peptide in these ganglia was confirmed by means of radioimmunoassay methods (Konishi *et al.* 1979; Gamse, Wax, Zigmond & Leeman, 1981). Furthermore, placing the ganglia in a high K^+ solution caused a release of substance P immunoreactivity in a Ca^{2+}-dependent manner (Konishi *et al.* 1979). The above findings, together with our electrophysiological data, appear to support the notion that substance P is a likely candidate in mediating the non-cholinergic excitatory potential in the inferior mesenteric ganglia of the guinea-pig. In this context, substance P was also suggested to be the mediator of the slow depolarization in myenteric neurones of the guinea-pig ileum (Katayama & North, 1978; Morita, North & Katayama, 1980). However, there is some evidence suggesting that serotonin, rather than a peptide, may be the transmitter in this preparation (Grafe, Mayer & Wood, 1979).

The present study led also to the novel finding of a long-lasting hyperpolarization that followed the non-cholinergic depolarization. The nature and origin of this response is at present not known. Whether or not it is a transmitter-mediated event or an after-potential remains to be investigated. It is interesting in this regard to note that a hyperpolarization was also observed in some neurones following depolarization induced by substance P (Dun & Minota, 1981).

The origin of the substance P-positive fibres giving rise to the non-cholinergic transmission in inferior mesenteric ganglia has not been firmly established. The results from several studies suggest that substance P-containing fibres in these ganglia may arise from sensory neurones of the spinal ganglia (Elfvin & Dalsgaard, 1977; Konishi *et al.* 1979; Baker *et al.* 1980). Indeed, substance P immunoreactivity has been localized in some small primary sensory neurones of the dorsal root ganglia (Hökfelt, Kellerth, Nilsson & Pernow, 1975). Furthermore, the observation that injection of capsaicin, a compound which depletes substance P from primary sensory neurones (Jessell, Iversen & Cuello, 1978), caused a marked reduction of immunoreactive substance P in prevertebral ganglia is consistent with the hypothesis that the peptide-containing fibres in the inferior mesenteric ganglion may originate from primary sensory neurones (Gamse *et al.* 1981). Our finding that stimulation of any of the four nerve inputs to the inferior mesenteric ganglion induced a non-cholinergic transmission in the same neurone agrees with the interpretation that the fibres may

be collaterals of sensory projections passing through the ganglion from the gastrointestinal tract to the dorsal root ganglion (see Fig. 10 for a hypothetical presentation of this concept). As substance P immunoreactivity was observed in neurones of the gastrointestinal tract (Nilsson, Larsson, Hakanson, Brodin, Pernow & Sundler, 1975), the possibility that some of the peptide-containing fibres in the inferior mesenteric ganglion may arise from these neurones cannot be excluded (Fig. 10; see also Baker *et al.* 1980).

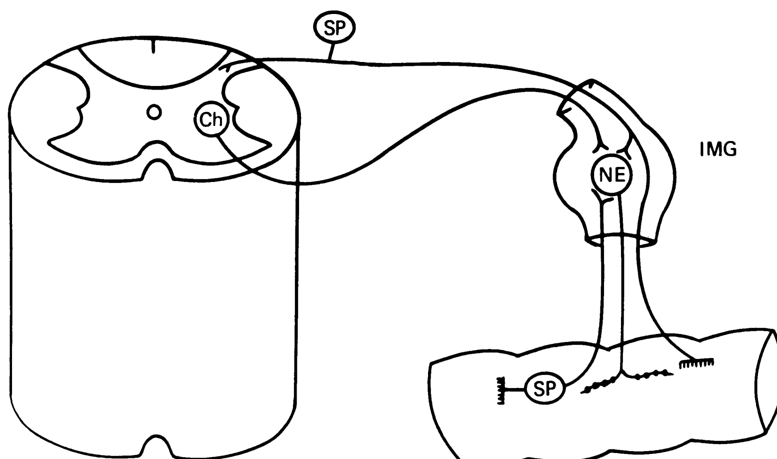

Fig. 10. Schematic diagram of two possible pathways of substance P-containing fibres in the inferior mesenteric ganglion (IMG) of the guinea-pig. Sensory fibres in the gastrointestinal tract may send collateral endings to neurones in the ganglion. Alternatively, some of the peptide-containing fibres may originate from neurones in the gastrointestinal tract. The symbols Ch, NE and SP denote cholinergic preganglionic neurones, noradrenergic sympathetic neurones and substance P-containing neurones, respectively.

The question whether or not substance P is released conjointly with ACh is interesting and remains to be investigated. The finding that the non-cholinergic potential was preceded by nicotinic transmission may be indicative of such a co-release phenomenon; however, it should be pointed out that in some instances repetitive stimulation evoked only fast e.p.s.p.s with no detectable non-cholinergic potential. Furthermore, immunohistochemical evidence for the co-existence of these two substances in the same neurone has not yet been obtained.

The non-cholinergic excitatory transmission in the inferior mesenteric ganglion may represent a physiologically significant event in view of the finding that it can be effectively induced at a frequency as low as 1–2 Hz. If indeed the substance P-containing fibres are sensory collaterals, excitation of sensory endings in the gastrointestinal tract may activate relatively readily the neurones of the inferior mesenteric ganglion, in addition to those in the dorsal horn of the spinal cord, via the release of substance P. As a corollary, the non-cholinergic transmission may function as a local reflex whereby sensory information is transmitted from the gastrointestinal tract to sympathetic neurones of the inferior mesenteric ganglion where, after appropriate processing inhibitory signals in the form of noradrenaline release are relayed back to the gut (Fig. 10).

We thank Dr A. G. Karczmar for valuable comments in preparing the manuscript. This study was supported in part by PHS Grant NS15848.

REFERENCES

ADAMS, P. R. & BROWN, D. A. (1980). Luteinizing hormone-releasing factor and muscarinic agonists act on the same voltage-sensitive K^+-current in bullfrog sympathetic neurones. *Br. J. Pharmac. Chemother.* **68**, 353–355.

BAKER, S. C., CUELLO, A. C. & MATTHEWS, M. R. (1980). Substance P-containing synapses in a sympathetic ganglion, and their possible origin as collaterals from sensory nerve fibres. *J. Physiol.* **308**, 76*P*.

BROWN, D. A. & ADAMS, P. R. (1980). Muscarinic suppression of a novel voltage-sensitive K^+-current in a vertebrate neurone. *Nature, Lond.* **283**, 673–676.

BROWN, D. A., CONSTANTI, A. & MARSH, S. (1980). Angiotensin mimics the action of muscarinic agonists on rat sympathetic neurones. *Brain Res.* **193**, 614–619.

CROWCROFT, P. J., HOLMAN, M. E. & SZURSZEWSKI, J. H. (1971). Excitatory input from the distal colon to the inferior mesenteric ganglion in the guinea-pig. *J. Physiol.* **219**, 443–461.

CROWCROFT, P. J. & SZURSZEWSKI, J. H. (1971). A study of the inferior mesenteric and pelvic ganglia of guinea-pigs with intracellular electrodes. *J. Physiol.* **219**, 421–441.

DUN, N. J. & KARCZMAR, A. G. (1979). Actions of substance P on sympathetic neurons. *Neuropharmacology* **18**, 215–218.

DUN, N. J. & MINOTA, S. (1981). Effects of substance P on neurones of the inferior mesenteric ganglia of the guinea-pig. *J. Physiol.* **321**, 259–271.

DUN, N. & NISHI, S. (1974). Effects of dopamine on the superior cervical ganglion of the rabbit. *J. Physiol.* **239**, 155–164.

ELFVIN, L.-G. & DALSGAARD, C. J. (1977). Retrograde axonal transport of horseradish peroxidase in afferent fibers of the inferior mesenteric ganglion of the guinea pig. Identification of the cells of origin in dorsal root ganglia. *Brain Res.* **126**, 149–153.

GAMSE, R., WAX, A., ZIGMOND, R. E. & LEEMAN, S. E. (1981). Immunoreactive substance P in sympathetic ganglia: distribution and sensitivity towards capsaicin. *Neurosci.* **6**, 437–441.

GRAFE, P., MAYER, C. J. & WOOD, J. D. (1979). Evidence that substance P does not mediate slow synaptic excitation within the myenteric plexus. *Nature, Lond.* **279**, 720–721.

HÖKFELT, T., ELFVIN, L.-G., SCHULTZBERG, M., GOLDSTEIN, M. & NILSSON, G. (1977). On the occurrence of substance P containing fibers in sympathetic ganglia: immunohistochemical evidence. *Brain Res.* **132**, 29–41.

HÖKFELT, T., KELLERTH, J. O., NILSSON, G. & PERNOW, B. (1975). Substance P: localization in the central nervous system and in some primary sensory neurons. *Science, N.Y.* **190**, 889–890.

JAN, Y. N., JAN, L. Y. & KUFFLER, S. W. (1979). A peptide as a possible tranmitter in sympathetic ganglia of the frog. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1501–1505.

JAN, Y. N., JAN, L. Y. & KUFFLER, S. W. (1980). Further evidence for peptidergic transmission in sympathetic ganglia. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5008–5012.

JESSELL, T. M., IVERSEN, L. L. & CUELLO, A. C. (1978). Capsaicin-induced depletion of substance P from primary neurons. *Brain Res.* **152**, 183–188.

KATAYAMA, Y., INOKUCHI, H. & NISHI, S. (1981). Voltage clamp study of the late slow excitatory postsynaptic current in bullfrog sympathetic ganglion cells. *Neurosci. Lett.* suppl. **6**, S63.

KATAYAMA, Y. & NORTH, R. A. (1978). Does substance P mediate slow synaptic excitation within the myenteric plexus? *Nature, Lond.* **274**, 378–388.

KONISHI, S., TSUNOO, A. & OTSUKA, M. (1979). Substance P and non-cholinergic excitatory synaptic transmission in guinea pig sympathetic ganglia. *Proc. Jap. Acad.* **55**, 525–530.

KUBA, K. & KOKETSU, K. (1974). Ionic mechanism of the slow excitatory postsynaptic potential in bullfrog sympathetic ganglion cells. *Brain Res.* **81**, 338–342.

KUBA, K. & KOKETSU, K. (1976). Analysis of the slow excitatory postsynaptic potential in bullfrog sympathetic ganglion cells. *Jap. J. Physiol.* **26**, 651–669.

MINOTA, S., DUN, N. J. & KARCZMAR, A. G. (1981). Substance P-induced depolarization in sympathetic neurons: not simple K-inactivation. *Brain Res.* **216**, 224–228.

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

NEILD, T. O. (1978). Slowly-developing depolarization of neurones in the guinea-pig inferior mesenteric ganglion following repetitive stimulation of the preganglionic nerves. *Brain Res.* **140**, 231–239.

NILSSON, G., LARSSON, L T., HAKANSON, R., BRODIN, E., PERNOW, B. & SUNDLER, F. (1975). Localization of substance P-like immunoreactivity in the mouse gut. *Histochemistry* **43**, 97–99.

NISHI, S. & KATAYAMA, Y. (1981). The non-cholinergic excitatory transmission in sympathetic ganglia. In *Adv. physiol. Sci.* **4**, Physiology of Excitable Membranes, ed. SALANSKI, J. pp. 323–327. Oxford: Pergamon Press.

SZURSZEWSKI, J. H. (1981). Physiology of mammalian prevertebral ganglia. *A. Rev. Physiol.* **43**, 53–68.

SZURSZEWSKI, J. H. & WEEMS, W. A. (1976). Control of gastrointestinal motility by prevertebral ganglia. In *Physiology of Smooth Muscle*, ed. BULBRING, E. & SHUBA, M. F. pp. 313–319. New York: Raven Press.