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INHIBITORY SYNAPTIC POTENTIALS RESULTING FROM α_2-ADRENOCEPTOR ACTIVATION IN GUINEA-PIG SUBMUCOUS PLEXUS NEURONES

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SUMMARY

1. Intracellular recordings were obtained from neurones of the guinea-pig submucous plexus. Inhibitory synaptic potentials (i.p.s.p.s) were compared with hyperpolarizations evoked by brief, local applications of noradrenaline and by superfusion with adrenoceptor agonists.

2. Hyperpolarizing potentials elicited by brief applications of noradrenaline were similar to the i.p.s.p. in latency of onset, amplitude, time course, conductance increase, reversal potential and ionic dependence. Both responses were blocked by low concentrations of Ba^{2+} and quinine.

3. 6-hydroxydopamine selectively and irreversibly abolished the i.p.s.p. and resulted in a complete loss of catecholamine fluorescent nerve fibres in the submucous plexus.

4. The α_2-adrenoceptor antagonists, phentolamine, yohimbine and RX781094, reversibly blocked the i.p.s.p. and the noradrenaline hyperpolarization. Prazosin, propranolol, atropine and naloxone had no effect on these responses.

5. Superfusion with noradrenaline and clonidine produced dose-dependent membrane hyperpolarizations. Noradrenaline and clonidine dose–hyperpolarization curves were shifted to the right in a parallel fashion by α_2-adrenoceptor antagonists.

6. Determination of the dissociation equilibrium constants for phentolamine, yohimbine and RX781094 showed that the hyperpolarization produced by noradrenaline perfusion is due to α_2-adrenoceptor activation.

7. It is concluded that the release of noradrenaline from sympathetic nerves activates post-synaptic α_2-adrenoceptors, resulting in the K^+ conductance increase which underlies the i.p.s.p. in submucous plexus neurones.

INTRODUCTION

Stimulation of the nerve supply to many lower vertebrate and mammalian peripheral ganglia can elicit three distinct types of post-synaptic potentials: a nicotinic excitatory synaptic potential (fast e.p.s.p.) of some 20–60 ms duration, followed by a 1–10 s long inhibitory synaptic potential (i.p.s.p.) which is often followed by a slow excitatory synaptic potential (slow e.p.s.p. or late slow e.p.s.p.)

lasting for 10 s to several minutes (e.g. bull-frog sympathetic ganglia: Koketsu & Nishi, 1967; Libet, Chichibu & Tosaka, 1968; Koketsu, 1969; Jan, Jan & Kuffler, 1979; Dodd & Horn, 1983; mammalian sympathetic ganglia: Eccles & Libet, 1961; Volle, 1966; Weight & Padgen, 1973; Cole & Shinnick-Gallagher, 1981; mudpuppy and cat parasympathetic ganglia: Hartzell, Kuffler, Stickgold & Yoshikami, 1977; Gallagher, Griffith & Shinnick-Gallagher, 1982; guinea-pig myenteric ganglia: Wood & Mayer, 1979; Johnson, Katayama & North, 1980). The i.p.s.p.s evoked in sympathetic and parasympathetic ganglia have been shown to be due to activation of post-synaptic muscarinic receptors which most often results in an increased K^+ conductance (Hartzell *et al.* 1977; Gallagher *et al.* 1982; Dodd & Horn, 1983; but see Nishi, 1974; Kuba & Koketsu, 1978).

Neurones of the guinea-pig submucous plexus also display this triad of synaptic potentials upon stimulation of their nerve supply (Mihara, Katayama & Nishi, 1983; Surprenant, 1984). Here too, the fast e.p.s.p. is nicotinic (Hirst & McKirdy, 1975; Neild, 1981). However, the i.p.s.p. recorded from the submucous plexus does not result from activation of muscarinic receptors, in that atropine does not inhibit, nor does acetylcholine ionophoresis mimic, the i.p.s.p. (Hirst & McKirdy, 1975; Neild, 1981; Surprenant, 1984). Rather, the transmitter responsible for the i.p.s.p. in submucous plexus neurones may be an amine which activates α-adrenoreceptors on the cell body since noradrenaline and dopamine ionophoresis mimic, and phentolamine inhibits, the i.p.s.p. (Hirst & Silinsky, 1975; Mihara *et al.* 1983; Surprenant, 1984).

In the present study i.p.s.p.s recorded from guinea-pig submucous plexus neurones were compared with membrane potential changes evoked by brief, local application of noradrenaline as well as by steady superfusion with other adrenoceptor agonists. In addition, the participation of α_2-adrenoreceptors in each of these membrane potential changes was investigated using appropriate α_2-antagonists.

METHODS

Preparations of submucous plexus were obtained from the small intestine of young guinea-pigs (200–300 g). Methods of dissection, intracellular recording and transmural stimulation have been described in detail previously (Hirst & McKirdy, 1975; Surprenant, 1984). The plexus was superfused with physiological saline of composition (mM): Na^+, 147; K^+, 5; Mg^{2+}, 2; Ca^{2+}, 2·5; Cl^-, 134; HCO_3^-, 25; $H_2PO_4^-$, 1; glucose 8; gassed with 95% O_2/5% CO_2; temperature was maintained at 37 °C.

Noradrenaline (0·1–10 mM), acetylcholine (10 mM) and KCl (500 mM), dissolved in the physiological saline solution, were applied by pressure ejection from micropipettes whose tip diameters were 4–10 μm. Duration and frequency of pressure application was varied; pressure was constant at 68 kPa (6·8 kPa = 1 lb./sq. in.). If any change in membrane potential was observed upon advancing the pressure pipette from outside the bathing fluid to within 10 μm of the impaled neurone and vice versa, this was taken as evidence of leakage; such pressure pipettes were discarded. In some experiments noradrenaline or acetylcholine was applied by ionophoresis. Ionophoretic pipettes were filled with 0·5 M-noradrenaline or 1 M-acetylcholine chloride and had tip resistances of 150–300 MΩ. Ejection current pulses of 5–50 nA for 1–50 ms were used. A backing current of 5 nA was applied when acetylcholine was in the pipette; none was necessary with noradrenaline ionophoresis.

The preparation was continuously superfused at 3 ml/min (bath volume 0·5 ml) from tap-selectable reservoirs of normal and drug-containing solutions. Approximately 30–40 s elapsed from turning the tap to entry of the changed solution into the bath; complete re-equilibration occurred within 2–3 min. Antagonists were present in the bathing solution for at least 7 min prior to and then throughout the period of superfusion with an agonist. Between drug applications normal solution was present for at least 15 min or until the membrane potential returned to control levels.

In vitro sympathectomy was carried out by the 6-hydroxydopamine (6-OHDA) method described by Aprigliano & Hermsmeyer (1976) and Cheung (1982). The formaldehyde–glutaraldehyde fluorescence histochemistry method of Furness, Costa & Wilson (1977) was used to demonstrate catecholamine fluorescence in the submucous plexus.

The following drugs were used: noradrenaline (1-arterenol bitartrate, Sigma); clonidine hydrochloride (Boehringer Inglelheim); adrenaline bitartrate (Sigma), isoprenaline bitartrate (Sigma); phenylephrine hydrochloride (Sigma); desmethylimipramine hydrochloride (DMI, Ciba-Geigy); phentolamine hydrochloride (Ciba-Geigy); RX781094 (idazoxan, Reckitt & Colman); yohimbine hydrochloride (Sigma); prazosin hydrochloride (Pfizer); propranolol hydrochloride (ICI); acetylcholine chloride (ACh, Sigma); 6-hydroxydopamine (6-OHDA, Sigma); and naloxone hydrochloride (Endo).

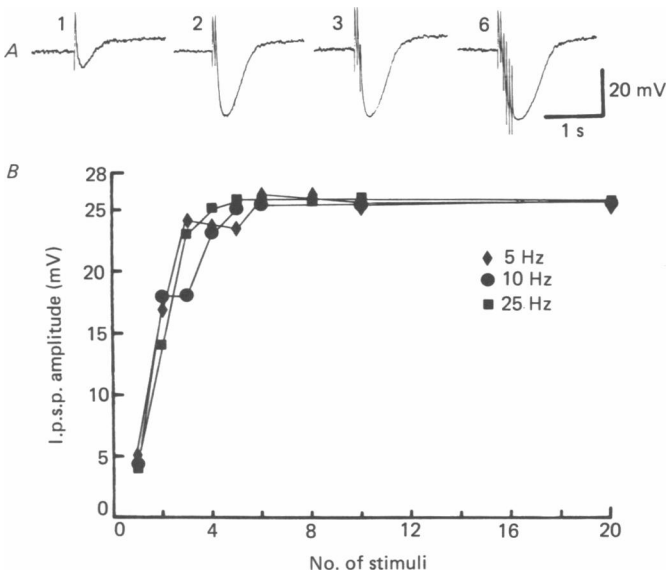

Fig. 1. Effect of repetitive stimulation on amplitude of i.p.s.p. *A*, i.p.s.p. recorded in response to 1, 2, 3 and 6 stimuli delivered at a frequency of 20 Hz. *B*, relation between amplitude of i.p.s.p. and number of stimuli at 5 Hz (▲), 10 Hz (●) and 25 Hz (■) for all neurones examined ($n = 11$). Maximum amplitudes were 26–32 mV in these cells.

RESULTS

Single and repetitive nerve stimulation evoked one to three distinct synaptic potentials (fast e.p.s.p.s, i.p.s.p.s, slow e.p.s.p.s) in over 90% (eighty-two of a total of ninety) of the submucous plexus neurones examined in this study; i.p.s.p.s were recorded from over 80% of the cells. All neurones which displayed i.p.s.p.s in response to transmural stimulation were also hyperpolarized by the application of noradrenaline (see Surprenant, 1984). Quantitative data described in this report were obtained from forty-seven neurones; the duration of these impalements was 2–12 h. Resting membrane potentials ranged from −54 mV to −63 mV, input resistances were 100–200 MΩ and membrane time constants were 9–30 ms.

I.p.s.p.s. As described previously, the i.p.s.p. evoked by a single stimulus appeared in an all-or-nothing manner and was not altered by increasing the stimulus intensity (Hirst & McKirdy, 1975; Surprenant, 1984). However, the amplitude of the i.p.s.p. was increased sharply by repetitive stimulation at frequencies of 5–25 Hz, with the

maximum amplitude being reached by the second to fifth stimulus (Fig. 1). While the amplitude of the i.p.s.p. evoked by a single stimulus showed considerable variation among cells (3–15 mV, $n = 23$ cells), the maximum amplitudes recorded in these experiments were quite similar, ranging from 26 to 32 mV. The maximum amplitude attained in response to repetitive stimulation was unrelated to the amplitude of the single-pulse i.p.s.p., but appeared to be solely a function of the resting membrane potential (see below). In addition, the same maximum amplitude was elicited by all frequencies examined (5–25 Hz) and was reached after approximately the same number of pulses (Fig. 1).

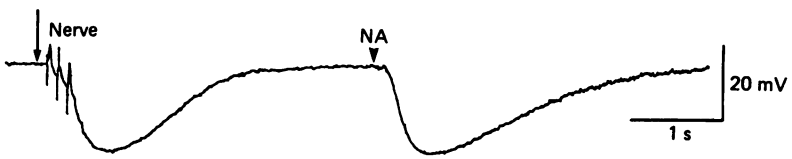

Fig. 2. I.p.s.p. evoked by 3 stimuli at 20 Hz (arrow) and hyperpolarizing potential evoked by a single 3 ms pressure ejection pulse of noradrenaline (NA; ▼). Concentration of noradrenaline in the pressure pipette was 1 mM.

Comparison of the i.p.s.p. and response to brief, local application of noradrenaline

Time course and ionic mechanism. When noradrenaline was applied by pressure ejection from a micropipette placed close (see below) to the impaled neurone, a hyperpolarization which closely resembled the i.p.s.p. was observed. An example is shown in Fig. 2 where it can be seen that the i.p.s.p. evoked by three stimuli (at 20 Hz) and the hyperpolarization evoked by a single 3 ms pressure pulse of noradrenaline were virtually identical. Similar results were obtained with ionophoresis of noradrenaline; however, as the noradrenaline potentials evoked by either method were similar, pressure ejection rather than ionophoresis was preferred in these studies because pressure ejection of noradrenaline produced more reliable and consistent effects.

The effects of altering the concentration of noradrenaline in the pipette, the position of the pipette and the frequency and duration of the pressure pulse were examined in a number of experiments. When the noradrenaline concentration in the pipette was less than 100 μM no response (± 3 mV) to pressure pulses (3–150 ms duration, 1–50 pulses at 6–20 Hz) was observed ($n = 4$). With concentrations of 100–250 μM-noradrenaline in the pipette, noradrenaline potentials evoked by single pressure pulses (3–10 ms duration), whose time courses and amplitudes were similar to the i.p.s.p., could be obtained provided the pipette tip was within 5–10 μm of the impaled neurone. Unfortunately this proximity also provoked an initial (depolarizing) pressure artifact and eventually led to dislodgment of the micro-electrode. When the pipette was filled with noradrenaline at these concentrations and placed at distances greater than 10–15 μm from the impaled cell, only small (< 4 mV) responses with slow (> 10 s) time courses were obtained, irrespective of the stimulus duration or frequency ($n = 5$). Responses similar to that shown in Fig. 2 were consistently elicited when the pipette was filled with 1 mM-noradrenaline and the pipette tip placed within a radius of approximately 30–100 μm of the cell surface.

The amplitude of the noradrenaline potential evoked by pressure (or ionophoretic) ejection was strikingly non-graded, regardless of the concentration of noradrenaline in the pipette or its position. Most often, responses similar to those obtained with the i.p.s.p.s were observed, in that a just-threshold stimulus (usually a single 2·5–5 ms pressure pulse) evoked a fast (1–3 s) hyperpolarizing potential of 10–22 mV amplitude while the maximum amplitude (28–35 mV) was achieved by the second or third stimulus at 5–20 Hz. As long as the pressure pipette was within the radius where it evoked a response, changing its position resulted in an alteration of the decay phase of the noradrenaline potential with little or no change in the amplitude or initial rise time.

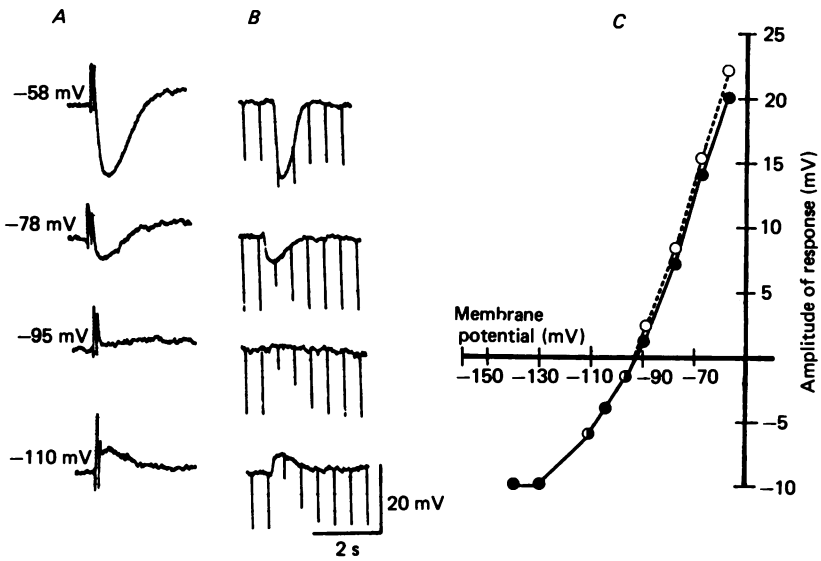

Fig. 3. Effect of hyperpolarizing the membrane on the amplitude of the i.p.s.p. evoked by 2 stimuli at 15 Hz (*A*) and the noradrenaline potential evoked by a 4 ms pressure ejection pulse (*B*). Amplitudes of both responses decreased as the membrane was hyperpolarized and reversed polarity at the same potential (*C*). The decrease in the amplitude of the electrotonic potentials (downward deflexions in the voltage traces of *B*) during the noradrenaline pressure-ejection potential indicate an increased membrane conductance. Records were obtained from the same cell. Calibrations refer to *A* and *B*.

In contrast to the results obtained with noradrenaline, similar experiments performed with KCl or acetylcholine in the pressure pipette showed that the depolarizing potentials elicited by these agents could be continuously graded in amplitude from <1 mV to 15–25 mV (i.e. threshold for action potential initiation) by altering the position of the pipette and/or the frequency and duration of the pressure pulses. Moreover, while the fast e.p.s.p. could be mimicked readily in amplitude and time course when acetylcholine was applied by ionophoresis, the fastest nicotinic acetylcholine potential evoked with pressure ejection was about 4–10 times slower than the fast e.p.s.p. No hyperpolarizing potential was ever recorded when acetylcholine was applied by ionophoresis or pressure ejection, although slow (0·5–5 s) depolarizations which were blocked by atropine (100 nM) were recorded in

five neurones. Slow muscarinic potentials in guinea-pig submucous plexus have been described previously by Mihara *et al.* (1983).

The time courses of i.p.s.p.s recorded in the present study were compared with the fastest, equal-amplitude, noradrenaline potentials evoked by pressure ejection. The time-to-peak (10–90 %) of the i.p.s.p. was 70–250 ms and its half-width was 420–950 ms ($n = 24$). The time-to-peak and half-width of the noradrenaline potentials were 80–250 ms and 500–1600 ms respectively ($n = 12$). No change in the amplitude or time course of the i.p.s.p. or of the noradrenaline potential was observed when the catecholamine uptake blocker, DMI (1–10 μM), was added to the bathing fluid ($n = 4$).

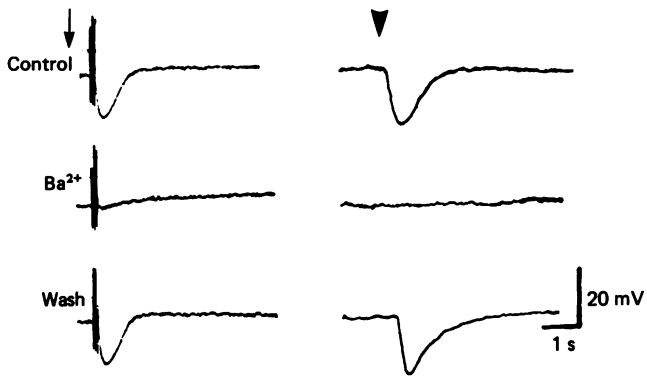

Fig. 4. Effect of Ba^{2+} (200 μM) on the i.p.s.p. (arrow) and the noradrenaline pressure-ejection potential (▼) recorded from the same neurone. Both responses were reversibly abolished by Ba^{2+}. In the presence of Ba^{2+} the amplitude of the fast e.p.s.p. was not altered. No change in the resting potential, input resistance or amplitude and duration of the directly evoked action potential was observed in this neurone during the application of Ba^{2+}.

Previous experiments have shown that the i.p.s.p. in submucous plexus neurones is due to an increased K^+ conductance (Surprenant, 1984) and that the ionophoretic noradrenaline potential is associated with the same conductance change and reversal potential as the i.p.s.p. (Hirst & Silinsky, 1975). Fig. 3 shows that the noradrenaline potential evoked by pressure ejection was also associated with an increased conductance and reversed at the same membrane potential (−93 mV) as the i.p.s.p. Similarly, in seven other neurones the reversal potentials for the i.p.s.p. and the noradrenaline pressure-ejection potential were identical in the same neurone, ranging from −89 mV to −102 mV in different cells. In all neurones examined the noradrenaline potential evoked by pressure ejection was associated with a large conductance increase ($n = 35$). A doubling (to 10 mM) of the external $[K^+]$ shifted the reversal potential of the noradrenaline pressure-ejection potential by 17–20 mV in the depolarizing direction, while halving the external $[K^+]$ produced an 18 mV hyperpolarizing change in this reversal potential ($n = 2$). These results are in accord with the previous, more detailed study on the ionic basis of the i.p.s.p., in which the K^+ dependence of the i.p.s.p. reversal potential showed a Nernst relationship with external K^+ between 1 and 10 mM (Surprenant, 1984).

Further evidence that a K^+ current is responsible for the i.p.s.p. as well as the noradrenaline potential evoked by pressure ejection was provided by experiments

with Ba^{2+} and quinine, both of which are known to be potent inhibitors of various K^+ currents (Hagiwara & Byerly, 1981; Latorre & Miller, 1983). Ba^{2+} (100–300 μM) reversibly depressed, or abolished, the i.p.s.p. and the noradrenaline pressure-ejection potential without altering the amplitude or time course of the fast e.p.s.p. or the directly evoked action potential ($n = 4$) (Fig. 4). These concentrations of barium did not alter the resting potential, input resistance or membrane time constant when

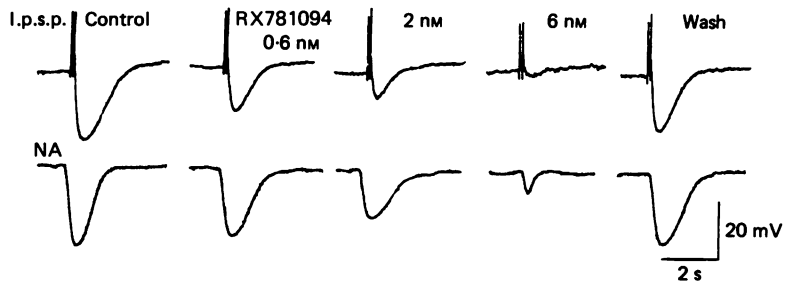

Fig. 5. Depression of the i.p.s.p. (upper row) and the noradrenaline (NA) pressure-ejection potential (lower row) recorded in one neurone by RX781094. I.p.s.p. evoked by 2 stimuli at 20 Hz, noradrenaline potential evoked by a 6 ms pressure-ejection pulse. Maximum effect of the antagonist was observed within 2–4 min after application. In this experiment normal solution was present for 15–20 min between applications of higher antagonist concentrations, by which time both responses had returned to control amplitude.

present for 5–10 min (longest times examined). Similar results were obtained with quinine (100 μM), although prolonged exposure (>5 min) to this concentration of quinine resulted in a widening of the directly evoked action potential ($n = 4$). In these experiments the maximum depression of the i.p.s.p. and the noradrenaline potential occurred before the prolongation of the evoked action potential became apparent.

Effects of 6-OHDA sympathectomy. Perfusion for 15–20 min with 6-OHDA resulted in a maintained 5–20 mV depolarization of the membrane which reversed within 4–15 min of wash-out ($n = 5$). Concomitant with the membrane depolarization was a 20–50 % depression of all of the synaptic potentials (fast and slow e.p.s.p.s as well as i.p.s.p.). The decrease in amplitude of the fast and slow e.p.s.p. did not progress in the continued presence of 6-OHDA and returned to control values within 15 min after removal of 6-OHDA. In contrast, the amplitude of the i.p.s.p. continued to decline during the initial 5–15 min of perfusion with 6-OHDA until a complete blockade was observed after approximately 15 min. I.p.s.p.s were not again recorded during nerve stimulation in three neurones in which the impalements were maintained for over 2 h after wash-out of 6-OHDA. The noradrenaline potential evoked by pressure ejection was depressed by 40–60 % while 6-OHDA was present but returned to within 10 % of control values within 20 min of wash-out ($n = 4$). No fluorescent nerve fibres could be detected in either the neuronal plexuses or the arterioles of the submucous plexus after this duration exposure to 6-OHDA ($n = 3$).

That 6-OHDA treatment did result in functional sympathectomy was further confirmed in experiments carried out on the arterioles of the submucous plexus. Excitatory junction potentials (e.j.p.s) recorded intracellularly from the arteriolar smooth muscle, and vessel constriction evoked by transmural nerve stimulation

(10 Hz for 1 s), were completely abolished after exposure and wash-out of 6-OHDA. However, the smooth muscle membrane depolarization and the vessel constriction evoked by pressure ejection (or superfusion) of noradrenaline was unaltered after wash-out of 6-OHDA. These experiments were carried out in the same preparation in which the synaptic and noradrenaline potentials were recorded from the plexus neurones ($n = 4$). E.j.p.s. in arterial smooth muscle have previously been shown to be of sympathetic origin (Surprenant, 1979; Holman & Surprenant, 1980; Cheung, 1982).

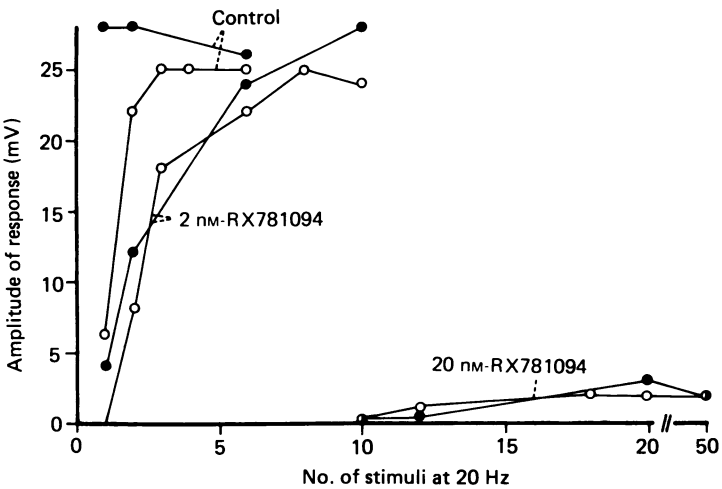

Fig. 6. Amplitude of the i.p.s.p. (○) and noradrenaline pressure-ejection potential (●) as a function of the number of stimuli delivered in control solution, in the presence of 2 nM-RX781094 and in the presence of 20 nM-RX781094. All values obtained from one impalement.

Effects of antagonists on i.p.s.p.s and noradrenaline potentials evoked by pressure ejection. The i.p.s.p. and the noradrenaline pressure-ejection potential were depressed by relatively low concentrations of the α_2-adrenoceptor antagonists phentolamine, yohimbine and RX781094. Fig. 5 shows the i.p.s.p. and the noradrenaline potential recorded from one neurone before and after addition of various concentrations of RX781094 (0·6–6·0 nM). RX781094 decreased the amplitudes of both responses by approximately the same amount.

It was possible to overcome the depression of the i.p.s.p. or the noradrenaline potential by increasing the number of stimuli used to evoke the response, provided the antagonist concentration was low. In the case of RX781094, increasing the number of pulses in the stimulus train reversed the inhibition of these responses when the antagonist concentration was $\leqslant 5$ nM; whereas at concentrations $\geqslant 20$ nM the depression of the responses was insurmountable ($n = 6$). An example is shown in Fig. 6 where the amplitudes of the i.p.s.p. and the noradrenaline potential are plotted as a function of the number of stimuli delivered in normal solution and in the presence of RX781094. Increasing the pressure pulse duration to as long as 2 s did not restore the noradrenaline potential when the antagonist was $\geqslant 20$ nM although prolonged

pressure pulses (>10 s) evoked slow hyperpolarizations whose amplitudes were 10–30% of control values ($n = 5$).

The effects of yohimbine, RX781094 and phentolamine on the i.p.s.p. evoked by two stimuli (at 20 Hz) and on the noradrenaline potential evoked by a single 4–6 ms pressure pulse were examined in a total of twenty-seven neurones. These stimulation parameters were chosen because they produced 70–100% of the maximum amplitude

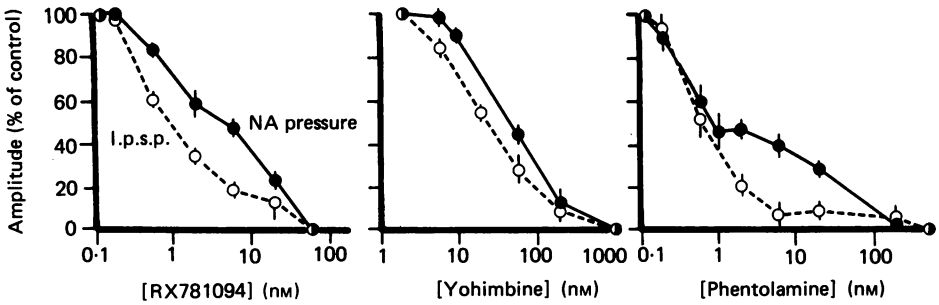

Fig. 7. Semilogarithmic plots of the amplitude of the i.p.s.p. (○) and the noradrenaline pressure-ejection potential (●) as a function of the antagonist concentration in the bath. In all experiments the i.p.s.p. was evoked by 2 stimuli at 20 Hz, the noradrenaline potential by a 4–6 ms pressure pulse. All points are the mean ± S.E. of mean; $n \geqslant 7$ for all points obtained in RX781094 and phentolamine, $n \geqslant 3$ for points in yohimbine.

in control solution. The results obtained from all experiments are plotted in Fig. 7. Values of the mean IC_{50} (the concentration of antagonist required to produce a 50% inhibition of the response) are listed in Table 1. It appears that the i.p.s.p. was slightly more sensitive to the antagonists than was the noradrenaline pressure-ejection potential. This may be related to the fact that several of the noradrenaline pressure-ejection responses were longer in duration (by 2–5 times) than the i.p.s.p.

Neither the i.p.s.p. nor the noradrenaline potential was altered by the addition of atropine (500 nM, $n = 7$), prazosin (1 μM, $n = 5$), propranolol (500 nM, $n = 7$) or naloxone (1 μM, $n = 5$).

Prolonged applications of noradrenaline and clonidine

Noradrenaline, when added to the superfusion fluid for periods of 2–10 min, produced a maintained, dose-dependent membrane hyperpolarization which was associated with an increased membrane conductance (Fig. 8). The maximum hyperpolarization observed in these experiments was 28–32 mV ($n = 14$). Similar types of hyperpolarizing responses and conductance increases were observed when the α_2-adrenoceptor agonist, clonidine, was applied by superfusion. The averaged dose–response curves for the hyperpolarizations produced in response to superfusion with clonidine or noradrenaline are plotted in Fig. 9.

Dose–hyperpolarization relations were also obtained for adrenaline and dopamine in a further four experiments. Adrenaline produced the same maximum hyperpolarization (30 mV) as did clonidine and noradrenaline; dopamine, in concentrations as high as 5 mM (in the presence of 10 μM-DMI), produced a maximum hyper-

polarization of 15–17 mV ($n = 3$). The rank order of potency of all agonists examined was clonidine > adrenaline > noradrenaline ≫ dopamine.

Ba^{2+} (300 μM) and quinine (100 μM) reversibly abolished the 12–16 mV hyperpolarization evoked in normal solution by 2 μM-noradrenaline ($n = 3$) and reduced the 26–30 mV hyperpolarization produced (in control solution) by 20 μM-noradrenaline

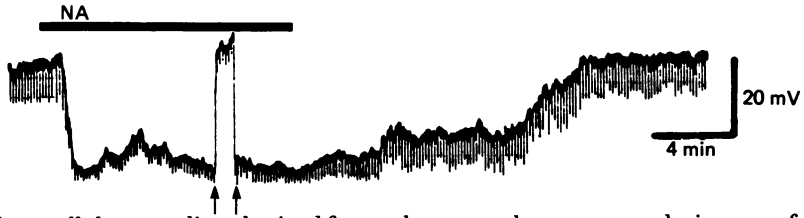

Fig. 8. Intracellular recording obtained from submucous plexus neurone during superfusion with 2 μM-noradrenaline (NA). Noradrenaline superfusion evoked a maintained hyperpolarization which was associated with a marked reduction in the amplitude of the electrotonic potentials. The fall in input resistance was not due to membrane rectification at hyperpolarized potentials since it was still apparent when the membrane was returned to the original resting potential by passing steady depolarizing current through the micro-electrode (at arrows). Noradrenaline was applied for the duration indicated by the bar.

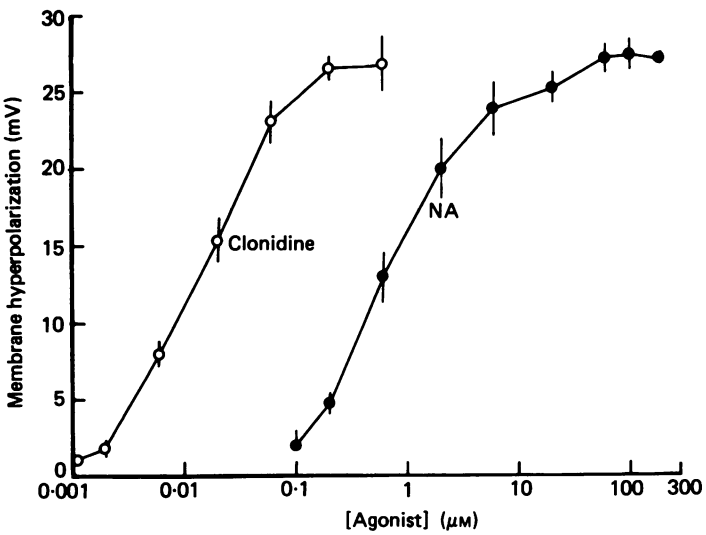

Fig. 9. Dose–response curves obtained from all experiments during superfusion with clonidine (○) and noradrenaline (●). Points are the mean ± S.E. of mean; $n \geqslant 10$.

to 8–14 mV ($n = 3$). When the membrane potential was held at values more negative than approximately −105 mV the noradrenaline superfusion response was symmetrically reversed in polarity.

Effects of α_2-adrenoceptor antagonists. In the presence of phentolamine, yohimbine or RX781094, higher concentrations of noradrenaline or clonidine were required to produce hyperpolarizations of amplitudes equivalent to those evoked in normal

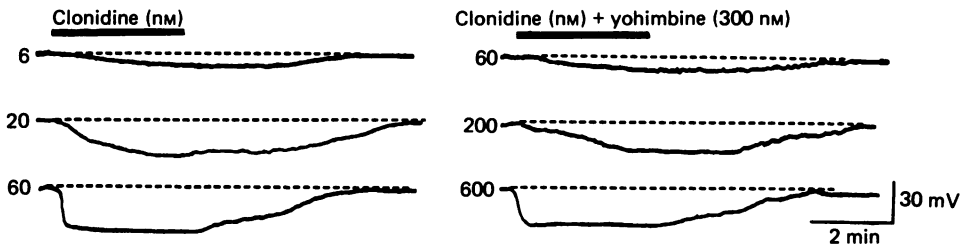

Fig. 10. Membrane hyperpolarizations recorded from one neurone during superfusion with 6, 20 and 60 nM-clonidine for the times indicated by the bar (left panel, top to bottom) and during superfusion with 60, 200 and 600 nM-clonidine in the presence of 300 nM-yohimbine (right panel, top to bottom).

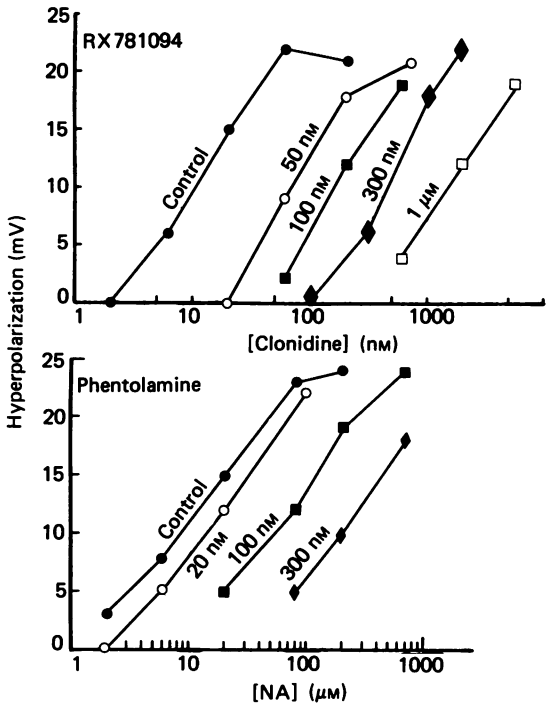

Fig. 11. Dose–hyperpolarization curves obtained before (control) and in the presence of increasing antagonist concentrations (as indicated). In the experiment illustrated in the upper graph, RX781094 was the α_2-antagonist; it produced a parallel, dose-dependent shift to the right in the clonidine response. Lower graph showed that phentolamine also shifted the noradrenaline response to the right in a parallel, dose-dependent manner. Results shown in upper graph were obtained from one neurone; those in lower graph obtained from a different neurone.

solution (Fig. 10); i.e. these antagonists shifted the dose–response curve to the right. The dissociation equilibrium constants (K_D) for these antagonists were determined on individual neurones by obtaining dose–response curves for noradrenaline before and after the addition of the antagonists. A total of nineteen neurones was examined in this manner. The maximum hyperpolarizations elicited by a 2–4 min superfusion

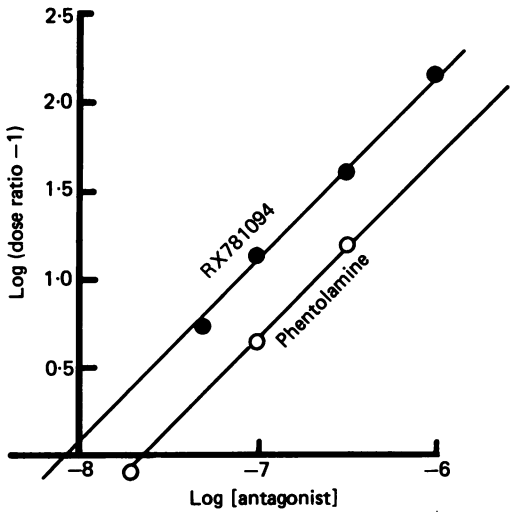

Fig. 12. Schild plots of the results shown in Fig. 11. In both cases the points form a straight line with a slope of 1. The x-intercept yields pA_2 values for RX781094 and phentolamine of 8·1 and 7·7 respectively.

TABLE 1. Effects of α_2-antagonists on three types of adrenergic responses recorded from submucous plexus neurones

	I.p.s.p.: IC_{50} (nM)	Noradrenaline pressure ejection: IC_{50} (nM)	Noradrenaline or clonidine perfusion: K_D (nM)	Noradrenaline or clonidine perfusion: Slope***
RX781094	1·9±0·7* (7)	4·5±1 (8)	4·7±0·8 (11)	1·01±0·04
	0·7–6·0**	1·0–10	3·2–12	0·86–1·18
Phentolamine	0·8±0·2 (8)	1·8±0·5 (9)	5·7±1·9 (4)	1·15±0·14
	0·4–2·5	0·4–5·0	2·0–12	0·9–1·6
Yohimbine	43±11 (3)	49±11 (4)	62±12 (4)	1·08±0·06
	20–65	20–100	35–100	0·9–1·25

* Refers to mean ± S.E. of mean; numbers in parentheses refer to number of preparations.
** Refers to range of values.
*** See text for meaning of slopes.

with three to five different agonist concentrations, first in the absence and then in the presence of two to four different antagonist concentrations, were recorded in each neurone. A period of 15–20 min was allowed between agonist applications, during which time no drug was present. Results obtained from two such experiments are shown in Fig. 11. RX781094, phentolamine and yohimbine produced parallel, dose-dependent shifts to the right of the noradrenaline and clonidine dose–response curves (Fig. 11).

The method of Arunlakshana & Schild (1959) was used to determine values for the dissociation equilibrium constant of the antagonists (Table 1). Fig. 12 plots the log (dose ratio − 1) *vs.* log antagonist concentration for the values determined from the graphs of Fig. 11. These Schild plots were straight lines with slopes not significantly

different from unity, which is compatible with a competitive interaction between agonist and antagonist at a single site.

The results obtained from all experiments in which the effects of α_2-adrenoceptor antagonists were examined (i.e. agonist superfusion, i.p.s.p. and noradrenaline pressure-ejection potential) are summarized in Table 1. The K_D values obtained in these experiments are very similar to values reported for α_2-adrenoceptors in several other tissues (e.g. Doxey, Roach & Smith, 1983; U'Prichard, Mitrius, Kahn & Perry, 1983).

DISCUSSION

The primary aims of these experiments were to elucidate the transmitter underlying the i.p.s.p. in submucous plexus neurones and to characterize its receptor activation by comparing the responses evoked by nerve stimulation, by brief, local application of noradrenaline and by steady superfusion with adrenoceptor agonists. Results obtained in the present study, together with those described previously by Hirst & Silinsky (1975), provide fairly conclusive evidence that noradrenaline, released from sympathetic nerves, is the primary or sole transmitter underlying the i.p.s.p. in this mammalian peripheral ganglion. That is, noradrenaline, applied by pressure ejection or ionophoresis, mimicked the i.p.s.p. in rise time and half-duration, amplitude, associated conductance increase, reversal potential as well as ionic dependence. 6-OHDA, which destroys sympathetic nerves *in vitro* (Aprigliano & Hermsmeyer, 1976; Cheung, 1982), irreversibly abolished the i.p.s.p. but had no lasting effect on the fast or slow e.p.s.p.s. The previous suggestion by Hirst & Silinsky (1975) that dopamine and noradrenaline may be equally likely candidates appears untenable in the light of more recent studies which have shown a virtual absence of dopamine within intestinal nerves (Diab, Dinerstein, Watanabe & Roth, 1976; Furness & Costa, 1980, 1982).

Is the i.p.s.p. due to activation of α_2-adrenoceptors? The present experiments demonstrated that the membrane hyperpolarization in response to steady superfusion with clonidine and noradrenaline was due to α_2-adrenoceptor activation. Values of K_D for phentolamine, RX781094 and yohimbine determined in this study under steady-state superfusion conditions are in agreement with values reported for α_2-adrenoceptors in a variety of other neuronal and non-neuronal tissues (Doxey, Smith & Walker, 1977; U'Prichard *et al.* 1983; Doxey *et al.* 1983). Thus, post-synaptic α_2-adrenoceptors must be present on submucous plexus neurones. Low concentrations (0·2–20 nM) of these same α_2-antagonists also decreased the i.p.s.p. and the noradrenaline potential evoked by brief pressure ejection pulses while α_1- and β-adrenoceptor blockers were without effect. The concentrations of RX781094, phentolamine and yohimbine which depressed the i.p.s.p. and the noradrenaline pressure-ejection potential by 50% were approximately equal to the antagonist concentrations which would occupy half the α_2-adrenoceptors under equilibrium conditions (i.e. K_D values determined from superfusion experiments). These findings suggest that α_2-adrenoceptor activation does underlie the i.p.s.p.

If the same receptors underlie both the response to steady-state superfusion of agonist and transient application of agonist, and if fractional occupancy by agonist is the only determinant of the hyperpolarization amplitude, then it would be expected

that a higher concentration of antagonist would be required to block the effect of a transient application of agonist than to block the effect of a prolonged application (Ehlert, Roeske & Yamamura, 1981; Motulsky & Mahan, 1984). Therefore, at first sight it was surprising to find that concentrations of α_2-antagonists close to their K_D caused at least 50 % inhibition of the response to *transient* application of noradrenaline (either by pressure pulse or by nerve stimulation) (Figs. 5 and 6). Although there is no direct information as to the time course of the presence of agonist close to the receptor, it may be assumed to be brief by comparison with the rate at which the antagonist dissociates from the receptor; the noradrenaline response (or i.p.s.p.) recovered with a half-time of about 480 s when the antagonist was washed out. We considered two possible reasons for the high sensitivity of the transient response to α_2-antagonists. First, the receptors involved in the two responses are not the same and they differ in their affinity for the antagonist. It is certainly possible that synaptically released transmitter acts only on local (subsynaptic) sites, and superfused transmitter acts on diffuse (extrasynaptic) sites; however, this kind of argument is probably not valid in the present circumstances because the response to pressure application of noradrenaline had a similarly high sensitivity to α_2-antagonists. In addition, the time course and reversal potential of the noradrenaline pressure-ejection potential and the i.p.s.p. were identical, which implies that the conductance changes underlying both responses were initiated at the same geometric location; this location appears to be at or close to the soma (Surprenant, 1984). As the tip of the pressure pipette was 50–100 μm from the neurone it can only be concluded that such application was diffuse. In other words, an explanation for the unexpectedly high sensitivity to the antagonist seems to be related to the time course of the agonist effect rather than a differential localization of the receptors involved.

The second explanation for the apparent difference between the blockade of brief and steady-state agonist applications may be that it is not simply the fractional receptor occupancy by the agonist which determines the response amplitude when the agonist is present briefly. Hyperpolarizations evoked by superfusion of agonist were graded with concentration; those evoked by transient application were not. When agonists were superfused the depression of the response by an antagonist could be surmounted by increasing the agonist concentration. Increasing the duration of pressure pulses applied to the noradrenaline pipette, or the number of stimuli applied to the presynaptic nerves, did not generally lead to a restoration of the transient response in the presence of antagonist. One interpretation of these findings is that the hyperpolarizing response can be initiated in these neurones either by a high fractional occupancy persisting for a short period of time, or by a low occupancy maintained for several seconds or minutes. This implies that there is a step between receptor occupancy and K^+ conductance increase which may require the accumulation of an intermediary substance to a certain critical threshold level.

The i.p.s.p., the noradrenaline pressure-ejection potential and the hyperpolarization produced by steady noradrenaline superfusion were all associated with an increased membrane conductance and a reversal potential near or equal to the K^+ equilibrium potential. These responses were abolished by low concentrations of the K^+ channel blockers Ba^{2+} and quinine. The present and previous study (Surprenant, 1984) showed that the reversal potentials for the i.p.s.p. and the noradrenaline potential

evoked by pressure ejection were dependent on the concentration of external K^+ in a manner predicted by the Nernst equation. These results indicate that the hyperpolarization evoked by nerve stimulation and by the application of noradrenaline appear to be due to an increased membrane conductance to K^+ ions.

Thus, it can be concluded that the K^+ conductance increase which underlies the i.p.s.p. in submucous plexus neurones is due to activation of post-synaptic α_2-adrenoceptors by noradrenaline released from sympathetic nerves.

The presence of post-synaptic α_2-adrenoceptors, which mediate small membrane hyperpolarizations upon exogenous application of catecholamines, has been demonstrated in autonomic ganglia (Brown & Caulfield, 1979; Ivanov & Skok, 1980; Cole & Shinnick-Gallagher, 1981). However, in spite of numerous attempts to demonstrate neuronally mediated post-synaptic adrenergic inhibition, i.p.s.p.s recorded from all peripheral ganglia other than submucous plexus have been shown to be due to the release of acetylcholine onto muscarinic receptors (Hartzell *et al.* 1977; Gallagher *et al.* 1982; Dodd & Horn, 1983). In contrast to the generally small amplitude muscarinic i.p.s.p.s observed even with high frequencies of stimulation (Dodd & Horn, 1983), adrenergic i.p.s.p.s of the submucous plexus rapidly (within one to four nerve pulses) and invariably attain amplitudes approaching the K^+ equilibrium potential. Adrenergic inhibitory synaptic potentials in the peripheral nervous system, which presently appear unique to submucous plexus neurones, may provide a powerful means of exerting inhibitory control over local neuronal circuitry.

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