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NON-CHOLINERGIC NEUROTRANSMISSION AND THE EFFECTS OF PEPTIDES ON THE URINARY BLADDER OF GUINEA-PIGS AND RABBITS

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SUMMARY

1. Supramaximal repetitive field stimulation with pulses of 50 μs produced contraction of strips of bladder from rabbits and guinea-pigs. Atropine reduced responses at all frequencies to about 60 % and the contraction was poorly maintained.

2. With the double sucrose-gap technique large excitatory junction potentials (e.j.p.s) were recorded with superimposed action potentials. These were not reduced by atropine or phentolamine.

3. Substance P (SP) produced contraction and increased the frequency of spontaneous action potentials recorded with micro-electrodes from bladder strips. Vasoactive intestinal peptide (VIP) produced relaxation and slowed action potentials in rabbit but had no effect in guinea-pig; neurotensin, somatostatin and leu-enkephalin were without action in either species.

4. When the tissue was kept in contact with SP, a second application after 10 min produced only a small contraction suggesting that SP receptors were densensitized. However, the electrical response to field stimulation was unchanged and the mechanical response was increased.

5. Chymotrypsin reduced mechanical responses to SP but had no effect on responses to field stimulation. The SP analogue, D-Pro^2, D-Phe^7, D-Trp^9-SP, had no effect on responses to SP or to field stimulation.

6. It is concluded that the bladder receives excitatory non-cholinergic innervation which is responsible for a large excitatory junction potential and contraction. Although SP can contract the detrusor muscle, it is unlikely that it is an excitatory transmitter or that any of the five peptides act as modulators of transmitter release.

INTRODUCTION

Stimulation of nerves in the wall of the bladder produces an increase in pressure due to contraction of smooth muscle layers. The response is blocked by tetrodotoxin, only partially resistant to atropine and unaffected by guanethidine, phentolamine or propanalol (Ambache & Zar, 1970; Dean & Downie, 1978). The bladder is therefore considered to contain non-cholinergic, non-adrenergic excitatory fibres. This is supported by observations on the junction potentials recorded with the double sucrose gap from rabbit bladder which showed that a late depolarization was

abolished by atropine but an initial depolarization with superimposed spike remained unchanged (Creed, Ishikawa & Ito, 1983).

There is known to be a wide distribution of such non-cholinergic innervation to visceral smooth muscles and a number of possible transmitters have been implicated including ATP and 5-hydroxytryptamine (Burnstock, 1972; Gershon, 1981). More recently there is strong evidence in the myenteric plexus and the smooth muscle cells of the small intestine to suggest that peptides may be transmitters (North, 1982). Experiments, carried out on the mechanical responses to peptides of the urinary bladder, have indicated that substance P (SP) could possibly be a transmitter but vasoactive intestinal peptide (VIP) is probably not (Sjögren, Andersson & Husted, 1982; MacKenzie & Burnstock, 1984). Few experiments have been carried out with other peptides. The aim of the present project was to investigate the contribution of non-cholinergic nerves to activity of the bladder and to use electrophysiological techniques to determine whether peptides are neurotransmitters. Experiments were carried out on strips of bladder from guinea-pigs and most were repeated on preparations from rabbits. A preliminary account of this work has been published (Creed & Callahan, 1985).

METHODS

Rabbits weighing 2–3 kg and guinea-pigs weighing 400–800 g were killed by a blow on the head, and bled. The urinary bladder was removed, and after opening it by two lateral incisions, the mucous membrane was removed. Dorsal longitudinal strips, 20 mm by 2 mm, were cut from the dome to the level of the ureter openings along the line of the smooth muscle bundles.

For mechanical recording strips were mounted in a 10 ml organ bath containing modified Krebs solution at 36 °C and gassed with 5 % CO_2 in O_2. Tension was measured with Grass force transducers (F.T.03) and displayed on a Grass polygraph. Supramaximal field stimulation was applied through two ring electrodes round the tissue with pulses of 50 μs duration. In order to test whether peptides influence the release or action of transmitters, responses to field stimulation in the presence and absence of drugs were compared by Student's t test.

The double sucrose-gap and micro-electrode techniques have already been described (Creed, Ishikawa & Ito, 1983). For the double sucrose gap 1 mm of the strip was in contact with Krebs solution in the central chamber at 35 °C. Mechanical activity, recorded with a force transducer, and electrical activity, recorded across one sucrose gap, were displayed on a cathode ray oscilloscope (Tektronix 5000) and put onto tape (Tandberg). Field stimulation was applied across the other gap from a ring electrode in the central chamber. For recording with micro-electrodes, strips were mounted in a bath at 36 °C with the serosal side uppermost. The tissue was superfused with Krebs solution made hypertonic by addition of 15 g sucrose to 100 ml Krebs solution to abolish movement. The micro-electrodes were filled with 3 M-KCl and activity photographed from a cathode ray oscilloscope.

The Krebs solution had the following composition (mM): NaCl, 120; KCl, 5·0; $CaCl_2$, 2·5; $NaHCO_3$, 25·0; $MgSO_4$, 1·0; NaH_2PO_4, 1·0 and glucose, 11·0; equilibrated with 5 % CO_2 in O_2. Drugs used were acetylcholine chloride (Hopkins and Wilkins), atropine sulphate, hexamethonium bromide, substance P (SP), vasoactive intestinal peptide (VIP), neurotensin, somatostatin and leu-enkephalin (all Sigma). Drugs were either added to the perfusing fluid or directly to the bath.

RESULTS

Responses to field stimulation

After setting up in the organ bath, the strips were allowed to equilibrate for 1 h. Some spontaneous activity was normally present throughout an experiment and the

effects of drugs or field stimulation on this and on the over-all tone was observed. Supramaximal field stimulation with pulses of 50 μs duration produced contraction of bladder strips. As the frequency of stimulation was increased from 1 to 50 Hz the contraction increased up to a maximum at 20 Hz (Fig. 1) when forces (mean $\pm$ S.D.) of $9{\cdot}0 \pm 3{\cdot}8$ g ($n = 12$) and $5{\cdot}35 \pm 2{\cdot}0$ g ($n = 20$) were exerted by guinea-pig and rabbit

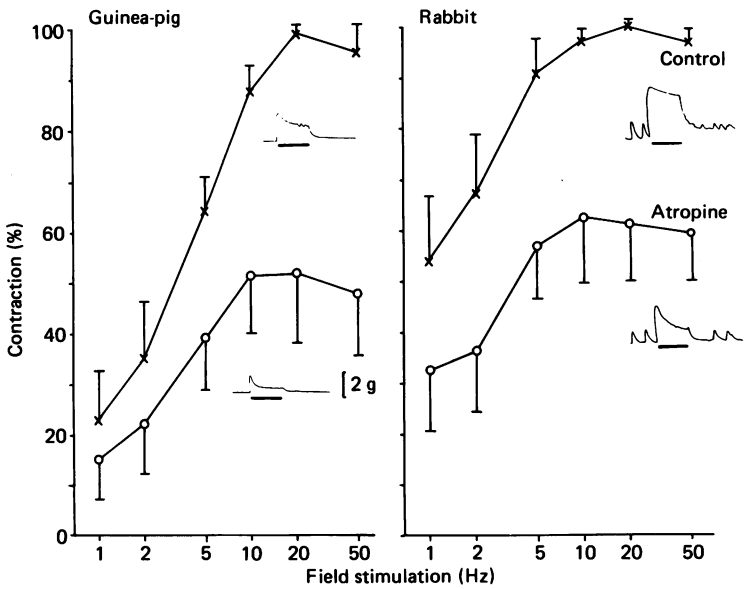

Fig. 1. The amplitude of responses to field stimulation at different frequencies of strips of urinary bladder taken from guinea-pigs and rabbits. For each preparation the largest response was taken as 100% and each point represents the mean ($\pm$S.D.) of twelve (guinea-pig) and twenty-two (rabbit) preparations. The upper curve ($\times$) was obtained in Krebs solution and the lower curve (O) after addition of atropine ($7{\cdot}5 \times 10^{-7}$ M). The insets are actual responses to stimulation at 10 Hz for 30 s and show the reduction in amplitude and poorly maintained responses in the presence of atropine.

strips respectively. At all frequencies the response was maintained throughout the period of stimulation in the rabbit but fell off slightly in the guinea-pig. In the presence of atropine ($7{\cdot}5 \times 10^{-7}$ M) the amplitude of responses was reduced. No further reduction occurred with higher doses of atropine. The reduction was the same at all frequencies for the rabbit with the response reduced to about 60% of control values at each frequency (Fig. 1). In the guinea-pig high frequencies were reduced more than low frequencies (to 50% at 50 Hz; to 62% at 1 Hz). In addition to the reduction in amplitude, atropine produced a marked change in shape, especially in the rabbit. Responses were no longer maintained during continued stimulation but decayed towards the unstimulated level within 10 s (Fig. 1 inset).

In order to investigate the electrical effects of field stimulation, the double sucrose gap was used. It was found that it was more difficult to record satisfactorily from the bladder strips of guinea-pig than rabbit, presumably because the bundles of smooth muscle cells were shorter in the smaller organ. However, in many preparations the responses to brief current pulses (50 μs) could be recorded and these were essentially similar to those already described for the rabbit (Creed, Ishikawa & Ito,

1983). In both species stimulation produced an initial excitatory junction potential (e.j.p.) with a superimposed action potential (Fig. 2). Compared with the rabbit, the action potential was less obvious in the guinea-pig but could be increased by application of two stimuli, separated by 10–50 μs. In the rabbit the e.j.p. was often followed by a later depolarization but this was not obvious in the guinea-pig. All responses were abolished by tetrodotoxin (10^{-7} M); atropine (5×10^{-7} M), which

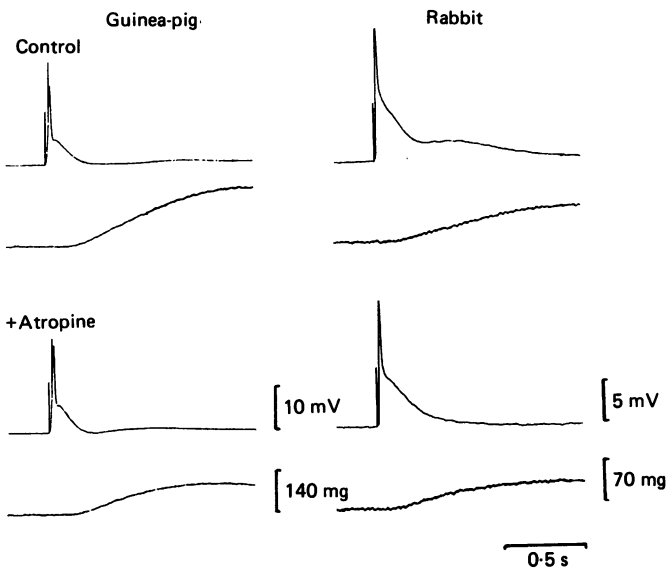

Fig. 2. The effects of atropine ($7{\cdot}5 \times 10^{-7}$ M) on electrical and mechanical responses to field stimulation of the bladder recorded in the double sucrose gap. For the rabbit a single stimulus of 50 μs duration was applied; for the guinea-pig two stimuli separated by 10 ms were applied. The excitatory junction potential and superimposed action potential were unchanged by atropine although the contraction was reduced.

reduced the contraction, abolished only the late depolarization in the rabbit whereas in the guinea-pig the e.j.p. was either unchanged or decayed more rapidly with no change in the peak amplitude; phentolamine ($1{\cdot}3 \times 10^{-6}$ M) had no effect in either species. The initial e.j.p. was therefore evoked by non-cholinergic, non-adrenergic excitatory neuro-transmission in both species.

Direct effects of peptides

The effects were investigated of five peptides, present in peripheral autonomic nerves, on mechanical and electrical activity of bladder and urethral strips (Table 1). They were added directly to the organ baths containing the strips. SP, at concentrations over 3×10^{-10} M increased the frequency of spontaneous activity of bladder strips from rabbit (Fig. 3), guinea-pig and dog and produced an increase in tone. Somatostatin (10^{-7} M) was without effect on any of the strips. The effects of the other peptides varied with the species. 10^{-7} M-VIP had no effect on strips of bladder from guinea-pig but at 10^{-9} M produced relaxation and reduced the amplitude of spontaneous activity of strips from rabbit (Fig. 3) or dog. This was particularly marked if activity had already been increased by application of acetylcholine or SP.

Leu-enkephalin and neurotensin at 10^{-7} M produced neither relaxation nor contraction of strips from rabbit or guinea-pig. However, they both increased the tone of strips from dog (Table 1). Urethral strips were far less sensitive than bladder strips. SP (10^{-7} M) produced a slight contraction of the urethra from all species and VIP a slight relaxation of strips from rabbit and dog, but no responses in guinea-pig. The other three peptides were without action.

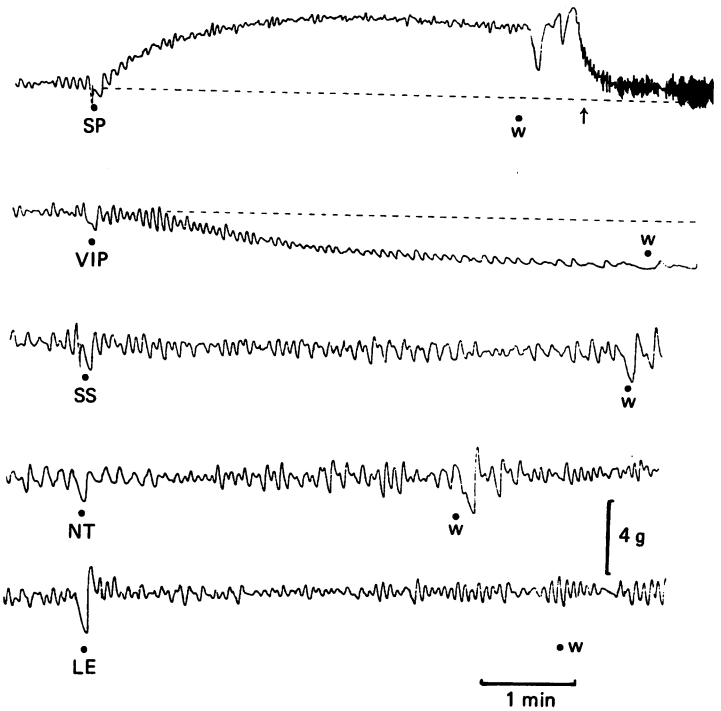

Fig. 3. The mechanical responses of strips of rabbit bladder to substance P (SP), vasoactive intestinal peptide (VIP), somatostatin (SS), neurotensin (NT) and leu-enkephalin (LE). Each peptide was added to give a final concentration of 10^{-8} M in the organ bath. The drugs were washed out at w. The paper speed was slowed 10 times at the arrow.

The contractions of the bladder to SP increased to a maximum within 30 s and were readily reversible when the drug was washed out. Some tachyphylaxis occurred but with 10 min between applications little reduction in the response to identical doses was seen. If the drug was not washed out the induced tone slowly decreased over 10 min and a second application produced only a small contraction (Fig. 4), suggesting that the continued presence of SP desensitized receptors. In the guinea-pig, detrusor strips from all parts of the bladder were equally sensitive to SP but strips from the dorsal part of the rabbit bladder were more sensitive than those taken from the ventral part. In both species, the threshold was 3×10^{-10} M, and maximum contractions had apparently not been reached at 10^{-6} M, which produced forces of 3·0–4·0 g. However, because of the tachyphylaxis it was not possible to obtain consistent responses to high doses or to estimate the ED_{50}. The mean force produced by 10^{-8} and 10^{-7} M-SP was $1{\cdot}2 \pm 0{\cdot}7$ g ($n = 14$) and $2{\cdot}2 \pm 0{\cdot}9$ g ($n = 13$) respectively for guinea-pig strips and $1{\cdot}8 \pm 0{\cdot}4$ g ($n = 9$) and $2{\cdot}3 \pm 0{\cdot}5$ g ($n = 6$) for rabbit dorsal strips.

TABLE 1. Responses of the lower urinary tract to peptides

	Bladder			Urethra		
	Guinea-pig	Rabbit	Dog	Guinea-pig	Rabbit	Dog
Substance P	++	++	++	0	+	+
VIP	0	−−	−−	0	−	−
Somatostatin	0	0	0	0	0	0
Neurotensin	0	0	++	0	0	0
Leu-enkephalin	0	0	+	0	0	0

Mechanical responses of muscle strips produced by peptides at 10^{-8} M. Mean contractions and relaxations greater than 1 g are shown as ++ and −− respectively. 0 indicates that there was no change in mechanical activity. Records were taken from four to twenty preparations.

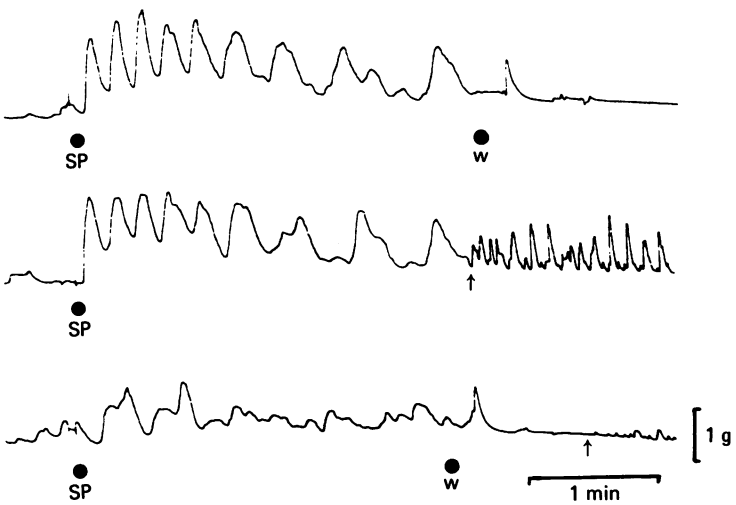

Fig. 4. Repeated applications of SP (10^{-8} M) to strips of guinea-pig bladder at 10 min intervals. When SP was washed out (w) the following response was similar. However, if SP remained in the bath the subsequent response was greatly reduced. The paper speed was slowed 10 times at the arrows.

By contrast maximum contractions to acetylcholine (ACh) were reproducible. In the guinea-pig a maximum contraction of $8{\cdot}7 \pm 1{\cdot}2$ g ($n = 8$) was produced at $5{\cdot}5 \times 10^{-3}$ M-ACh and the mean ED_{50} was $4{\cdot}05 \times 10^{-5}$ M. The sensitivity of the rabbit to ACh was higher with an ED_{50} of $6{\cdot}8 \times 10^{-6}$ M but the maximum force was similar ($8{\cdot}4 \pm 0{\cdot}8$ g, $n = 18$). Atropine ($7{\cdot}5 \times 10^{-7}$ M) shifted all response curves to the right so that doses of ACh to produce equivalent responses were increased by about a thousand times.

In strips taken from both rabbit and guinea-pig bladder, spontaneous electrical activity recorded with micro-electrodes consisted of regular spikes at 10–30/min. These were superimposed on a membrane potential of 35–40 mV. Addition of SP to the bath to give a final concentration of 10^{-8} M produced acceleration of spikes in most cells of both species, although there was little depolarization (Fig. 5). In the guinea-pig strips, VIP (10^{-8} M) produced no change whereas in the rabbit the frequency was usually decreased. Application of acetylcholine (10^{-8} M) also produced acceleration of spikes. In a few cells from both species, neither SP nor acetylcholine

produced a response. Atropine (10^{-7} M) prevented the acceleration due to acetylcholine but not to SP.

Effects of SP and other peptides on responses to field stimulation

If SP is an excitatory transmitter, prolonged contact would be expected to densensitize the receptors on the smooth muscles so that responses to nerve stimulation would be reduced. The effects of nerve stimulation in the presence and

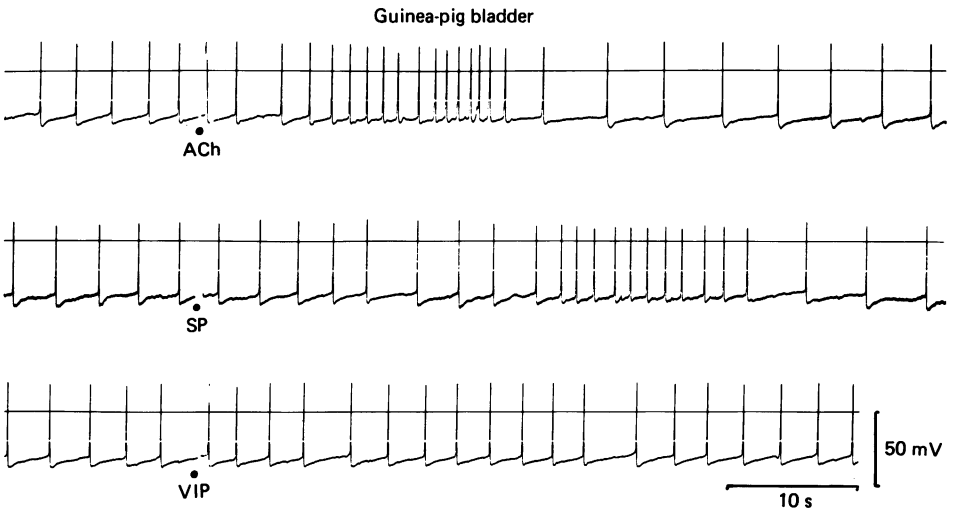

Fig. 5. The effects of SP, VIP and acetylcholine (ACh) on spontaneous action potentials recorded with micro-electrodes from guinea-pig bladder. Both SP and ACh increased the frequency whereas VIP was without action. The top trace is zero potential and the lower trace is the membrane potential.

absence of SP were therefore compared. Responses of guinea-pig bladder strips to pairs of stimuli were recorded with the double sucrose gap. SP was then added to the Krebs solution to give a concentration of 10^{-5} M and stimulation repeated 10 min later (Fig. 6). For the guinea-pig it was found that there was no significant change in the amplitude of the spike ($18{\cdot}4 \pm 3{\cdot}5$ mV in Krebs to $17{\cdot}9 \pm 2{\cdot}7$ mV in SP, $n = 5$) or in the e.j.p. ($5{\cdot}8 \pm 3{\cdot}7$ mV to $6{\cdot}3 \pm 3{\cdot}6$ mV, $n = 5$); in four of the five strips the mechanical response was actually increased on addition of SP with values ranging from 83 to 237 % of values recorded in Krebs. In two rabbit preparations there was a slight increase in the mechanical response to a single stimulus (113 %) and a small decrease in the spike (87 %) and e.j.p. (74 %) in the presence of SP at 10^{-5} M. In both species the mechanical response to SP itself was almost abolished after 10 min exposure to SP at 10^{-6} M, although the mechanical responses to repetitive stimulation were unchanged.

Alternatively the peptides might modify the transmitter release by the nerves. However, no consistent changes occurred in the amplitude of the electrical responses recorded with the double sucrose gap in the presence of any of the five peptides at a concentration of 10^{-5} M, although there was a tendency for mechanical responses to be increased by SP and reduced by VIP and somatostatin (Fig. 6). A similar trend was seen in the organ bath with mechanical responses to repetitive stimulation at

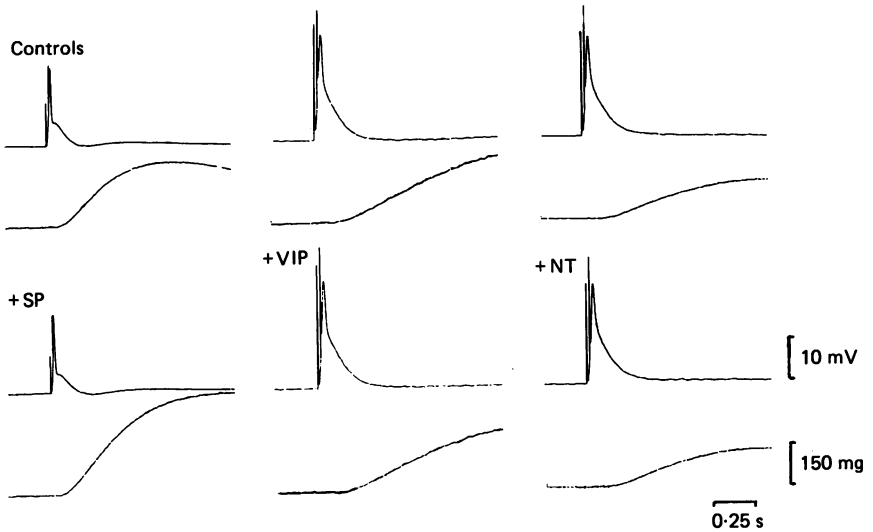

Fig. 6. The effects of SP, VIP and NT on the electrical and mechanical responses of guinea-pig bladder recorded with the double sucrose gap. The strips were stimulated every 2 min and the second record was obtained 10 min after addition of the peptide at 10^{-5} M. All responses were recorded in the presence of atropine ($7{\cdot}5 \times 10^{-7}$ M). None of the peptides altered the electrical response though the contraction was increased in SP and decreased in VIP.

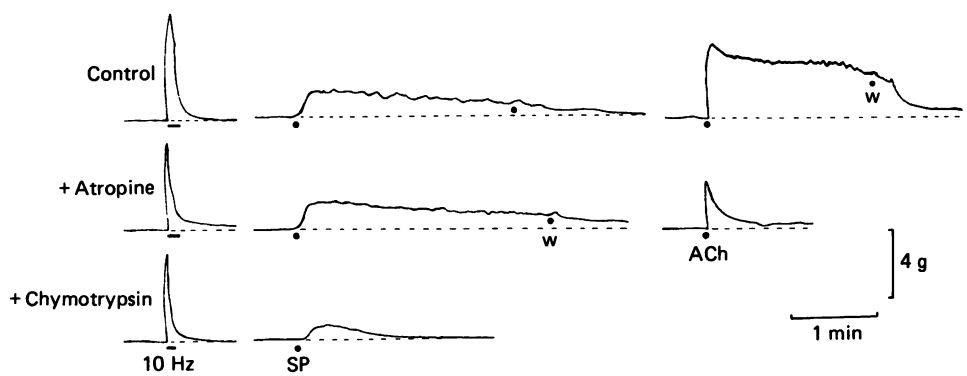

Fig. 7. Mechanical responses of guinea-pig bladder to field stimulation at 10 Hz for 10 s, SP (2×10^{-7} M) and ACh (5×10^{-4} M). After obtaining responses in Krebs solution, atropine ($7{\cdot}5 \times 10^{-7}$ M) was added to abolish the cholinergic response to field stimulation. Addition of chymotrypsin (20 μg/ml) reduced the response to SP but had no effect on the nerve-induced response.

different frequencies in either the presence or absence of atropine at $7{\cdot}5 \times 10^{-7}$ M. These results suggest that it is unlikely that one of these peptides is an excitatory transmitter or modifies its release.

Effects of SP antagonists on electrical and mechanical responses

The most useful test for the identification of a neurotransmitter is selective block of responses to nerve stimulation in parallel with responses to the transmitter. The enzyme, chymotrypsin, has been used in the myenteric plexus to prevent the action

of SP by deactivating it. In the guinea-pig bladder strip, SP at a concentration of 10^{-8} M produced a well-maintained contraction of $1{\cdot}1 \pm 0{\cdot}5$ g ($n = 4$). In the presence of 20 μg chymotrypsin/ml, this dose of SP produced a smaller contraction ($0{\cdot}6 \pm 0{\cdot}3$ g) which declined to the base line within 1 min (Fig. 7). Chymotrypsin at this concentration had no effect on the contraction evoked by acetylcholine or repetitive nerve stimulation, though higher concentrations produced non-specific reductions. The electrical and mechanical responses to double stimuli recorded in the double sucrose gap were also completely unaffected by 20 μg chymotrypsin/ml (Fig. 8).

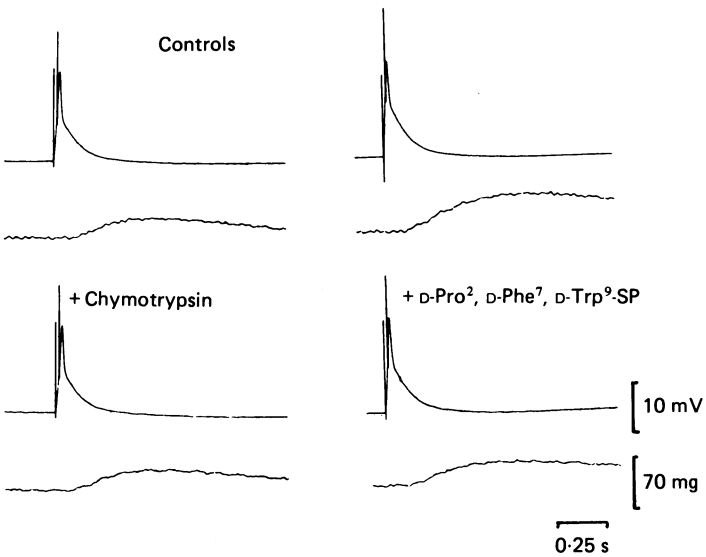

Fig. 8. The effects of chymotrypsin (20 μg/ml) and the SP analogue, D-Pro^2, D-Phe^7, D-Trp^9-SP (10^{-6} M) on the responses of the guinea-pig bladder. All responses were recorded in the presence of $7{\cdot}5 \times 10^{-7}$ M-atropine. Neither drug reduced the amplitude of the e.j.p. or action potential.

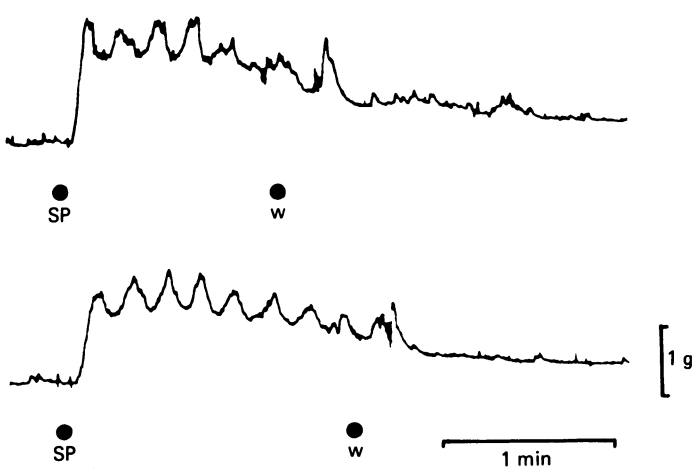

Fig. 9. Mechanical responses of guinea-pig bladder strips to SP (10^{-8} M) recorded in the absence (top) and presence of the SP analogue, D-Pro^2, D-Phe^7, D-Trp^9-SP (10^{-6} M).

Recently a number of analogues of SP have been synthesized which antagonize the actions of SP by combining with its receptors. One of these, D-Pro^2, D-Phe^7, D-Trp^9-SP, has been reported to block the action of SP on guinea-pig and rabbit bladders (Husted, Sjögren & Andersson, 1981). However, this was not supported in the present experiments. Reproducible contractions of bladder strips from guinea-pigs were evoked by 10^{-8} M-SP at 10 min intervals. Addition of the analogue at 10^{-6} M produced no reduction in the subsequent response to SP (Fig. 9). The analogue also failed to change the electrical or mechanical responses to field stimulation recorded with the double sucrose gap (Fig. 8). Similar results were obtained for rabbit strips.

DISCUSSION

In the rabbit it has already been shown from electrical recording with the double sucrose gap that nerve stimulation produces two distinct depolarizing responses, only the second of which is blocked by atropine (Creed, Ishikawa & Ito, 1983). The present experiments indicate that in the guinea-pig a second response was less obvious but was occasionally apparent in the falling phase of the initial e.j.p. as a step which was selectively abolished by atropine. These results suggest that in both species non-cholinergic transmission is responsible for an initial rapid e.j.p. and contraction. This provides an electrical basis for previous results that have shown that mechanical responses, induced by nerve stimulation, are only partially resistant to atropine (Ambache & Zar, 1970; Downie & Dean, 1977). It would be interesting to record electrical responses from human strips which are reported to have only cholinergic excitatory innervation (Sjögren, Andersson, Husted, Mattiasson & Moller-Madsen, 1982; Sibley, 1984).

Several peptides have been identified in nerves of the guinea-pig small intestine (Furness & Costa, 1980) and electrophysiological experiments have suggested that these may be transmitters (North, 1982). In the lower urinary tract, nerves containing peptides are more sparse but SP, somatostatin, enkephalin and VIP have been identified in nerves to the bladder of several species including cats and guinea-pigs (Alm, Alumets, Brodin, Håkanson, Nilsson, Sjöberg & Sundler, 1978; Hökfelt, Schultzberg, Elde, Nilsson, Terenius, Said & Goldstein, 1978) suggesting that here too they may be involved in neurotransmission.

There have been several reports that SP produces contraction of bladder strips including those from rabbit and guinea-pig (Sjögren, Andersson & Husted, 1982; MacKenzie & Burnstock, 1984). These results were confirmed in the present experiments and the electrical recordings with micro-electrodes suggest that SP acted by altering the membrane properties so as to increase the frequency of action potentials. This action was not due to release of acetylcholine, which also increases frequency (Creed, 1971) since the acceleration was not blocked by atropine. It is also unlikely to act through other nerves since the mechanical response in rabbit was not reduced in the presence of tetrodotoxin (Sjögren, Andersson & Husted, 1982).

The present results also confirm that VIP has no action in the guinea-pig bladder below 10^{-6} M (Finkbeiner, 1983). Although Johns (1979) and MacKenzie & Burnstock (1984) obtained a small contraction at concentrations over 10^{-6} M, the high concentration and long latency were considered to make it unlikely that VIP is an excitatory

transmitter. VIP, at concentrations over 10^{-9} M, caused relaxation of strips from rabbit (Levin & Wein, 1981) and from human and pig (Klarskov, Gerstenberg & Hald, 1984) as well as from the dog bladder. The reduction in frequency of action potentials, recorded from rabbit strips in the present experiments, probably also indicates a direct effect of VIP on the membrane.

There are few published reports of effects of other peptides. On guinea-pig bladder leu-enkephalin (10^{-4} M) had no effect (MacKenzie & Burnstock, 1984) and somatostatin produced a slowly rising contraction but only at concentrations over 10^{-7} M (Sjögren, Andersson & Husted, 1982). In the present experiments somatostatin, leu-enkephalin and neurotensin up to 10^{-6} M had no effect on bladder strips from either rabbit or guinea-pig but both neurotensin and leu-enkephalin increased tone in strips from dog. The smaller responses of urethral strips may reflect activity of those smooth muscle bundles that extend from the bladder into the urethra, though anatomical and electrophysiological investigations suggest that the muscle layers in the two regions have distinct properties (Gosling & Dixon, 1975; Callahan & Creed, 1981). It is possible, therefore, that SP could be an excitatory transmitter since it produces contraction of bladders from all species studied; VIP usually produces relaxation and could be an inhibitory transmitter whereas responses to the other peptides vary with the species.

Attempts to desensitize receptors with exogenous SP, however, suggested that this peptide is not an excitatory transmitter. With double sucrose-gap recording it was found that there was no decrease in the e.j.p. in strips from either rabbit or guinea-pig after exposure to 10^{-5} M-SP for 10 min. Mechanical recording in both species indicated that the presence of only 10^{-7} M greatly reduced responses to SP itself whereas nerve-induced responses were enhanced. In the guinea-pig Hourani (1984) also showed that 10^{-6} M inhibited mechanical responses to SP but potentiated responses to non-cholinergic nerve stimulation.

The present experiments with antagonists are inconclusive. Addition to the bath of the enzyme chymotrypsin, which deactivates SP, produced no change in the e.j.p. in either species. Similar concentrations did not alter the mechanical response to field stimulation but reduced the response to SP confirming previous reports on guinea-pig strips (MacKenzie & Burnstock, 1984). It is possible, however, that SP, released by nerves very close to the muscle cells, may not be destroyed sufficiently quickly by chymotrypsin. The synthetic antagonist D-Pro^2, D-Phe^7, D-Trp^9-SP did not block the e.j.p. but also failed to abolish the mechanical response to SP contradicting the report by Husted, Sjögren & Andersson (1981). Capsaicin has been reported to reduce bladder responses (Longhurst, Belis, O'Donnell, Galie & Westfall, 1984; Maggi, Santicioli & Meli, 1984) but this drug has several actions besides its ability to deplete cells of SP. The evidence therefore suggests that SP is unlikely to be an excitatory transmitter but it cannot be definitely ruled out until more specific antagonists become available.

Alternatively the peptides could be acting as modulators of transmitter release or action. Significant but small increases in amplitude of the nerve-induced mechanical responses in both species were observed in the presence of SP whereas somatostatin and VIP produced some reduction. The e.j.p., however, was unchanged. In the guinea-pig, potentiation was recorded for SP (Hourani, 1984) and no effect for VIP (Johns, 1979), Husted, Sjögren & Andersson (1981) failed to show potentiation in

either species with SP or somatostatin unless the strips were pre-treated with guanethidine, atropine and indomethacin. It is probable that the amplitude of mechanical responses is dependent on the tone of the strips, and it has already been shown that SP has a direct excitatory effect on the smooth muscle cells and VIP may have an inhibitory effect.

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