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A PHARMACOLOGICAL INVESTIGATION OF SYNTHETIC SUBSTANCE P ON THE ISOLATED GUINEA-PIG ILEUM

R. W. Bury and M. L. Mashford

Departments of Pharmacology and Medicine (St Vincent's Hospital), University of Melbourne, Parkville, Victoria, Australia

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SUMMARY

1. The pharmacological properties of synthetic substance P have been studied on the guinea-pig ileum and compared with those of acetylcholine and other agonists.

2. The effects of synthetic substance P in the presence of atropine, hexamethonium, mepyramine and certain of the drugs which antagonize serotonin are in close agreement with those reported for the naturally occurring peptide.

3. The spasmogenic action of substance P is not mediated by cholinergic mechanisms or release of prostaglandins, and does not appear to involve release of serotonin. The inability of tetrodotoxin to attenuate responses to substance P suggests that its spasmogenic action is not elicited through neural mechanisms. Thus, it is likely that substance P acts directly on the smooth muscle of the ileum.

4. Since substance P is present in the brain and can depolarize neurones, it may be a neurotransmitter. A screening of various centrally acting drugs, whose mechanisms of action are unclear, was undertaken to seek possible interactions with substance P. Pimozide was the most potent in depressing responses to substance P but none of the drugs caused the specific antagonism which would assist in elucidating a possible physiological role for substance P.

Key words: centrally acting drugs, guinea-pig ileum, pharmacology, substance P.

INTRODUCTION

Early work to find a specific antagonist to the actions of substance P has been unsuccessful. However, these studies employed crude and partially purified extracts (Lembeck & Zetler, 1971) in which the endecapeptide identified by Chang, Leeman & Niall (1971) as substance P was probably just one of the many peptides present. Contaminants in these preparations may have masked a relatively specific interaction of the tested compounds with substance P itself. The availability of synthetic substance P of known structure and purity has provided the opportunity to determine some of its pharmacological properties for comparison with

Correspondence: Dr M. L. Mashford, Department of Medicine, St Vincent's Hospital, Fitzroy, 3065 Victoria, Australia.

those reported for crude and partially purified extracts from brain and intestine (Lembeck & Zetler, 1962; Dix Christensen & Haley, 1966).

The discovery of agents interacting specifically with substance P (e.g. producing antagonism or potentiation) would assist in the elucidation of its possible functional roles and mechanism of action. Since evidence is accumulating that substance P may be a neurotransmitter in the central nervous system (Powell *et al.*, 1973; Konishi & Otsuka, 1974a,b; Takahashi *et al.*, 1974; Krnjevic & Morris, 1974; Phillis & Limacher, 1974a,b; Takahashi & Otsuka, 1975) it is possible that centrally acting drugs may exert some of their actions by interacting with this peptide in an analogous way to their known effects on established neurotransmitters.

Screening of such centrally acting drugs thus seemed a reasonable strategy in seeking drugs interacting specifically with substance P. The gut contains large amounts of substance P, much of which appears to be intraneuronal (Pearse & Polak, 1975; Nilsson *et al.*, 1975), and the guinea-pig ileum is especially sensitive to the spasmogenic action of substance P (Pernow, 1953), therefore, the use of this tissue should permit the detection of various types of possible interactions between drugs and substance P, such as antagonism, potentiation, release of peptide from endogenous stores, and decrease in its removal from receptors. This argument assumes that receptors in the gut and the central nervous system are identical; while this is not justified by direct evidence, there is at present no suggestion that receptors for substance P exhibit the type of radical differentiation seen with those for acetylcholine and biogenic amines.

METHODS

Guinea-pigs of either sex (300–550 g) were killed by cervical dislocation and two 4 cm segments of distal ileum were removed. These were suspended at a tension of approximately 1 g in 25 ml organ baths containing aerated Tyrode's solution at 32°C. Contractions were monitored isotonically by Harvard smooth muscle transducers and a Rikadenki recorder. At the beginning of each experiment, a full concentration-response curve for substance P was obtained. A small adjustment of the tension on the tissue was effected to achieve responses to substance P in a concentration of 3.0 nmol/l which were between 45 and 55% of the maximal response. Three consecutive responses to this concentration of substance P were obtained at 4-min intervals using 30 s contact time. A further response to the same dose was then recorded 3 min after the addition of the test drug. The same experimental design was employed for acetylcholine (11 nmol/l) and in those experiments in which they were used, for bradykinin (7.5 nmol/l) and histamine (26 nmol/l).

In the guinea-pig ileum preparation, the slopes and the maximal responses of the log concentration-response curves for all the above agonists were similar (Fig. 1), and the concentrations quoted above for each agonist produced contractions which were within 10% of the magnitude of the responses to 3 nmol/l of substance P. Preparations which failed to exhibit the required sensitivity were discarded.

In the appropriate experiments, nicotine in concentrations of 6.2–12 μmol/l caused submaximal contractions of similar magnitude to those elicited by the other agonists at the concentrations stated. The slope of the log concentration-response curve for nicotine was similar to that for acetylcholine. Similar experiments were performed using serotonin except that a time cycle of 8 min was used in order to prevent the development of tachyphylaxis. Because of the variability of sensitivity of ileum to serotonin, it was necessary to vary the concentra-

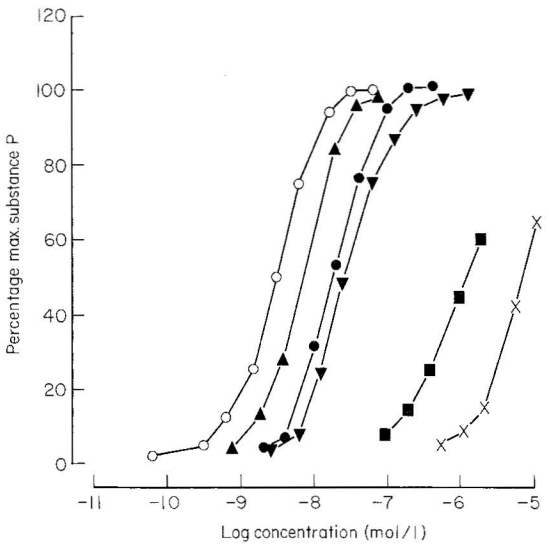

Fig. 1. Typical log concentration-response curves derived from one experiment, for the action of substance P (○—○), bradykinin (▲—▲), acetylcholine (●—●), histamine (▼—▼), serotonin (■—■) and nicotine (×—×) on the guinea-pig ileum. Responses are expressed as percentage of maximal response to substance P.

tion used for different pieces of gut, in the range of 1.0-2.5 μmol/l, to produce responses of similar magnitude to those produced by the other agonists. The log concentration-response curve for serotonin had a lower gradient than those for the other agonists (Fig. 1).

Synthetic substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) was obtained from Protein Research Foundation, Osaka, Japan. Bradykinin BRS 640 (Sandoz), acetylcholine chloride (Koch-Light), serotonin creatinine sulphate (Koch-Light) nicotine sulphate (H. W. Woods) and histamine acid phosphate (David G. Bull) were the other agonists used.

Other drugs used were: atropine sulphate (David G. Bull), mepyramine maleate (David G. Bull), hexamethonium bromide (Koch-Light), cyproheptadine hydrochloride (Merck, Sharp and Dohme), morphine sulphate (David G. Bull), phenoxybenzamine hydrochloride (Smith, Kline and French), bromolysergide (BOL 148; Sandoz), methysergide (UML 491; Sandoz), lysergide (LSD 25; Sandoz), indomethacin (Merck, Sharp and Dohme), tetrodotoxin (Sankyo), sodium pentobarbitone (May and Baker), sodium phenytoin (Parke-Davis), chlordiazepoxide hydrochloride (Roche), Δ 9-tetrahydrocannabinol (Δ 9-THC; N.I.M.H., Bethesda, Maryland, U.S.A.), fluphenazine hydrochloride (Squibb), amitryptyline hydrochloride (Merck, Sharp and Dohme), pimozide (Ethnor), leptazol (Knoll, A.G.), strychnine hydrochloride (Koch-Light), amantadine hydrochloride (Ciba-Geigy), and bismuth subgallate (Allen and Hanbury).

RESULTS

Effects of atropine, mepyramine and hexamethonium

The responses to synthetic substance P were unaffected by concentrations of atropine up to 5.8 μmol/l, which was 100 times in excess of that required to abolish responses to acetylcholine.

Mepyramine in concentrations up to 10 μmol/l had no effect on responses to substance P, but responses to acetylcholine were reduced to 55% (s.e.m. = 6%) of their control level; responses to histamine were abolished by 0.1 μmol/l of mepyramine.

Responses to substance P were not reduced by a concentration of hexamethonium bromide (0.11 mmol/l) which blocked the action of nicotine. Concentrations of hexamethonium greater than 1.1 mol/l potentiated responses to substance P, the greatest mean degree of potentiation being 139% (s.e.m. = 17%) with the highest concentration of hexamethonium used (5.5. mmol/l): this potentiation was non-specific since responses to acetylcholine were also potentiated (136%, s.e.m. = 23%).

Effects of drugs which influence responses to serotonin

The responses to substance P were reduced to a mean of 48% (s.e.m. = 8%) of control by a concentration of cyproheptadine (11 μmol/l) which was at least ten times greater than that sufficient to abolish responses to serotonin and acetylcholine.

Responses to substance P were unaffected by morphine in the concentrations 5.3 nmol/l–5.3 μmol/l. Morphine did not alter responses to acetylcholine, but those to serotonin were reduced to a mean of 10% (s.e.m. = 1%) of control.

Phenoxybenzamine (1.2 μmol/l) reduced the responses to substance P to a mean of 65% (s.e.m. = 8%) of control. This concentration depressed responses to acetylcholine to 40% (s.e.m. = 5%) and responses to serotonin to 19% (s.e.m. = 5%) of control.

Responses to substance P and acetylcholine were unaffected by concentrations of methysergide up to 0.85 μmol/l but these caused only 40–50% reduction of responses to serotonin. Higher concentration of methysergide (8.5 μmol/l) potentiated responses to substance P and acetylcholine by 122% (s.e.m. = 12%) and 123% (s.e.m. = 13%), respectively, and there was partial reversal of the antagonism to serotonin.

Lysergide (95 and 240 nmol/l) caused potentiation of responses to substance P and acetylcholine to 120% (s.e.m. = 7%) and 141% (s.e.m. = 13%) of their respective control values with the higher concentration. This concentration of lysergide reduced responses to serotonin to 57% (s.e.m. = 2%) of control.

Bromolysergide (BOL) caused a progressive but modest depression of responses to all the agonists with increasing concentration up to the highest used (7.2 μmol/l). This concentration reduced responses to substance P, acetylcholine and serotonin to 42% (s.e.m. = 4%), 46% (s.e.m. = 9%) and 28% (s.e.m. = 6%), respectively, of control values.

The presence of serotonin (5.0 μmol/l) in the bathing fluid reduced responses to substance P to 47% (s.e.m. = 8%) of control but acetylcholine responses were reduced to a similar degree (to 58%, s.e.m. = 10%). In contrast to the long-lasting tachyphylaxis to serotonin produced by high concentrations of the agonist in this tissue, the depression of responses to substance P and acetylcholine was readily reversible.

Effect of indomethacin

Responses of substance P were unaffected by indomethacin in concentrations up to 11 μmol/l, but were diminished to 41% (s.e.m. = 4%) of control values by 100 μmol/l. Responses to acetylcholine were reduced to 50% (s.e.m. = 6%) by the higher concentration.

Effect of tetrodotoxin

Responses to substance P were unaffected by tetrodotoxin (12.5 nmol/l–1.25 μmol/l). Responses to nicotine were abolished by 125 nmol/l of tetrodotoxin.

Interactions between substance P and some centrally acting drugs

Responses to substance P and acetylcholine were equally depressed by pentobarbitone (Fig. 2); this was significant with concentrations of pentobarbitone greater than 16 μmol/l.

Phenytoin (1.5–150 μmol/l), chlordiazepoxide (0.12–120 μmol/l) and Δ9-tetrahydrocannabinol (0.13–130 μmol/l) produced non-specific depression of responses to substance P and acetylcholine of a similar type to that produced by pentobarbitone.

Fluphenazine (Fig. 3b) was about twenty times more potent in antagonizing responses to substance P than was pentobarbitone. However, the depression was non-specific since responses to acetylcholine and bradykinin were equally diminished.

Responses to substance P were attenuated by amitriptyline in low concentrations (0.13 μmol/l) (Fig. 3a). Responses to bradykinin were reduced to a similar degree to those to sub-

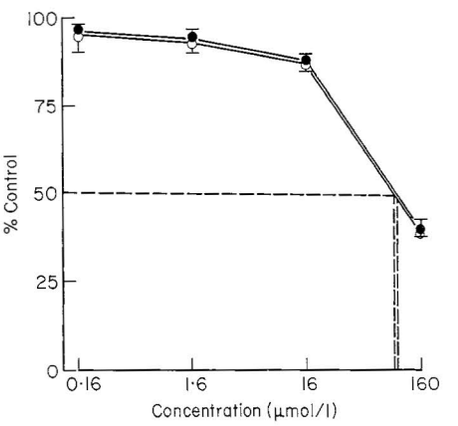

Fig. 2. The effect of increasing concentrations of pentobarbitone on contractions of guinea-pig ileum elicited by 3.0 nmol/l of substance P (o—o) and 11 nmol/l of acetylcholine (•—•), expressed as percentage of the control responses. Each point represents the mean of four experiments; vertical bars are standard errors of the mean. The two vertical dotted lines demonstrate the derivation of the concentrations of pentobarbitone required to reduce the response to substance P and to acetylcholine by 50%.

stance P, but responses to acetylcholine were antagonized more markedly. In depressing responses to substance P, amitriptyline was about fifty times more potent than pentobarbitone.

Pimozide was the most potent of the drugs tested in attenuating the responses to substance P. This effect was nonspecific since acetylcholine responses were depressed to the same degree throughout the concentration range (8.7 nmol/l–8.7 μmol/l). The highest concentration of pimozide used completely abolished the responses to substance P and acetylcholine.

Responses to both substance P and acetylcholine were depressed by strychnine to a similar extent over the concentration range 0.98–98 μmol/l. The highest concentration reduced responses to 30% (s.e.m. = 2%) of the control response.

Responses to substance P were little affected by leptazol in concentrations of 2.9–290 μmol/l; a moderate diminution of responses to acetylcholine to 45% (s.e.m. = 5%) of control was observed in the presence of the highest of these concentrations.

Amantadine caused little antagonism to substance P in concentrations up to 210 μmol/l.

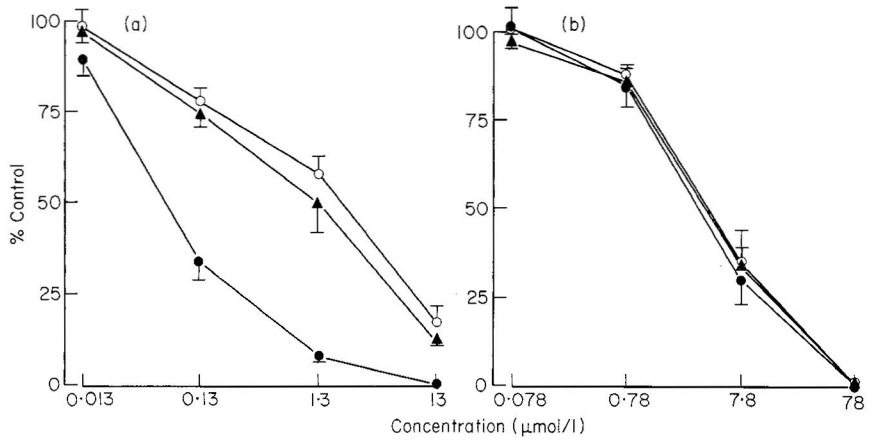

Fig. 3. The effect of increasing concentrations of (a) amitriptyline and (b) fluphenazine on contractions of the guinea-pig ileum elicited by 3.0 nmol/l of substance P (o—o), 11 nmol/l of acetylcholine (●—●), and 7.5 nmol/l of bradykinin (▲—▲), expressed as percentage of control responses. Each point is the mean of four experiments; vertical bars are standard errors of the mean.

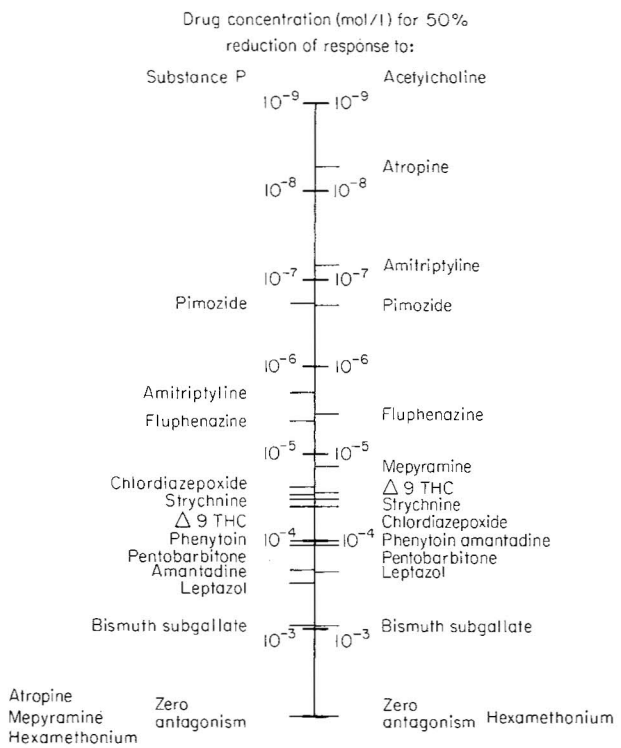

Fig. 4. An index of the relative potencies of various drugs as unspecific antagonists of responses of guinea-pig ileum to substance P and acetylcholine. The scale represents molar concentrations and the position of the drug is defined by the concentration reducing agonist responses by 50%. The method of determining these concentrations is described in the text and illustrated in Fig. 2.

Concentrations of bismuth subgallate up to 970 μmol/l had no effect on substance P responses.

The concentrations of these centrally acting drugs required to reduce the responses to substance P and acetylcholine to 50% of control are shown in Fig. 4. These have been calculated by interpolation of points on the 'antagonist' concentration-response curves (see Fig. 2).

DISCUSSION

Drug interactions with natural extracts of substance P have been studied extensively (Lembeck & Zetler, 1962; Dix Christensen & Haley, 1966) but no data of this type have previously been available for the pure peptide.

The spasmogenic effect of synthetic substance P on the guinea-pig ileum was unchanged in the presence of atropine and mepyramine, and low concentrations of hexamethonium caused a non-specific potentiation. These agree with earlier findings using impure substance P (von Euler & Gaddum, 1931; Pernow, 1951; Douglas *et al.*, 1951; Pernow, 1953).

The action of serotonin on the guinea-pig ileum is complex since this agonist stimulates different receptor sites in nerve and smooth muscle (Born, 1970) and there is no agent which causes complete and specific antagonism of its spasmogenic action: morphine, which prevents the component of action due to stimulation of intramural neural structures, has no effect on responses to synthetic substance P. Of the other drugs used, BOL, cyproheptadine and phenoxybenzamine cause some depression of responses to synthetic substance P, but in all cases responses to acetylcholine are depressed at least as much, and both are usually less affected than are responses to serotonin. Methysergide and lysergide both cause potentiation of responses to synthetic substance P, but very similar effects are seen with acetylcholine. The exposure of the ileum to high concentrations of serotonin results in a long-lasting tachyphylaxis (Rocha e Silva, Valle & Picarelli, 1953), but the diminution of responses to synthetic substance P and acetylcholine by high concentrations of serotonin (5 μmol/l) are readily reversible after washing the ileum. The results reported here for synthetic substance P are in agreement with those obtained with the naturally occurring material (Krivoy, 1957; Smith & Walaszek, 1962; Walaszek, Huggins & Smith, 1963). Thus none of these agents have specific effects on responses to the peptide, nor is there any evidence for participation of tryptaminergic mechanisms in its spasmogenic action on the preparation used.

It has been suggested that the mechanism of action of substance P in producing pain and reduction in venous outflow in the rabbit ear involve interaction with prostaglandins (Lembeck & Juan, 1974). Concentrations of indomethacin which are reported to inhibit prostaglandin synthesis in various tissues (Flower, 1974) had no effect on the responses to synthetic substance P. Thus it is unlikely that the contractions are mediated or facilitated by prostaglandins. High concentrations of indomethacin reduced the response to substance P and acetylcholine to a similar degree. This appears to be a direct, unspecific effect of indomethacin, and Lembeck & Juan (1974) found that high concentrations also inhibited responses to bradykinin of the guinea-pig ileum, although they noted that responses to prostaglandin were more markedly reduced.

Despite evidence that substance P can stimulate neurones, the results suggest that neural pathways are not involved in the mechanism of action of exogenous substance P in this tissue. Concentrations of tetrodotoxin which abolished the effect of nicotine had no effect on the responses to substance P and acetylcholine.

In view of the lack of specific interaction between synthetic substance P and those drugs

which modify the ability of the ileum to contract in response to various modes of stimulation (namely activation of cholinergic, histaminergic or tryptaminergic receptors, release of prostaglandins or stimulation of nerves), it is likely that substance P acts directly on the smooth muscle of the guinea-pig ileum.

None of the centrally acting drugs screened in the present study caused specific antagonism to substance P on the guinea-pig ileum, although several drugs caused unspecific depression. Pimozide, a relatively specific antagonist of dopamine in the central nervous system (Anden *et al.*, 1970), was the most potent in reducing responses to substance P. Amitriptyline and fluphenazine had quite potent depressant effects. Chlordiazepoxide, strychnine, Δ9-THC, phenytoin, pentobarbitone and leptazol were less potent. Because of the occurrence of high concentrations of substance P in the substantia nigra (Powell *et al.*, 1973), the interaction between the anti-parkinsonian agent amantadine and substance P was studied. However, only a minor attenuation of responses to substance P was observed in the presence of high concentrations of this drug.

Bismuth subgallate has been implicated in the development of a reversible neurological syndrome associated with dementia in certain patients (Australian Drug Evaluation Committee Report, 1974). The mechanism in producing this effect is unknown. It had no effect on substance P responses in the guinea-pig ileum.

Since the slopes of the dose-response curves of substance P and acetylcholine are parallel in the assay, and the magnitude of control responses were within closely defined limits, the summary of the results with various antagonists shown in Fig. 4 gives an indication of the relative potencies. This is, perhaps, a crude measure of antagonism since it does not distinguish between the action of competitive and non-competitive antagonists. A more rigorous approach would involve the construction of complete agonist concentration-response curves in the presence of various antagonist concentrations. This would enable the calculation of pA_2 values for competitive antagonists or pA_h values for non-competitive antagonists (Schild, 1957). Such a procedure would not be practicable as the first step is screening large numbers of drugs to detect specific interactions with the agonist under study.

These findings reported in this paper provide no evidence that interaction with substance P is involved in the mechanism of the central effects of the various drugs used, nor have they provided the specific antagonist which is necessary if any physiological role of substance P is to be elucidated.

Nevertheless, the profile of the interactions of these drugs with synthetic substance P forms a background upon which future research with the peptide may be based.

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