European Journal of Pharmacology, 87 (1983) 77–84
Elsevier Biomedical Press

TISSUE SELECTIVITY OF SUBSTANCE P ALKYL ESTERS: SUGGESTING MULTIPLE RECEPTORS

STEPHEN P. WATSON *, BENGT E.B. SANDBERG, MICHAEL R. HANLEY and LESLIE L. IVERSEN

MRC Neurochemical Pharmacology Unit, Medical Research Countil Centre, Medical School, Hills Road, Cambridge CB2 2QH, U.K.

Received 11 June 1982, revised MS received 7 September 1982, accepted 26 October 1982

S.P. WATSON, B.E.B. SANDBERG, M.R. HANLEY and L.L. IVERSEN, *Tissue selectivity of substance P alkyl esters: suggesting multiple receptors,* European J. Pharmacol. 87 (1983) 77–84.

Previous studies from this laboratory suggested that two subtypes of substance P receptor may exist, based on the observations that substance P and related peptides did not exhibit complete cross-desensitisation on guinea-pig ileum, and that two distinct rank orders of potency of tachykinins were observed in various test systems. The present study has added support to this hypothesis by extending the screening of tachykinins to further bioassays and by testing novel analogues. In particular, C-terminal alkyl esters of substance P were found to exhibit a high degree of selectivity to one putative receptor subtype. The synthesis of the alkyl esters by esterification of substance P free acid is described.

Substance P Multiple receptors Substance P alkyl esters Guinea-pig ileum Tachykinins

1. Introduction

Substance P was the first peptide described to have a dual brain-gut distribution (Von Euler and Gaddum, 1931). Since then considerable evidence has accumulated to suggest that it is a neurotransmitter or neuromodulator within the central and peripheral nervous systems (Bury and Mashford, 1977). Substance P belongs to a family of naturally occurring peptides, known as the tachykinins (table 1), which share a common C-terminal amino acid sequence, Phe-X-Gly-Leu-Met-NH_2, and potently contract a wide range of smooth muscle preparations (Bury and Mashford, 1977). Full biological activity appears to reside in this C-terminal

* To whom all correspondence should be addressed.

TABLE 1

Structures of selected tachykinins and their analogues.

	1	2	3	4	5	6	7	8	9	10	11
Substance P	Arg-	Pro-	Lys-	Pro-	Gln-	Gln-	Phe *-	Phe-	Gly *-	Leu *-	Met-NH_2 *
Physalaemin	Glp-	Ala-	Asp-	Pro-	Asn-	Lys-	Phe *-	Tyr-	Gly *-	Leu *-	Met-NH_2 *
Eledoisin	Glp-	Pro-	Ser-	Lys-	Asp-	Ala-	Phe *-	Ile-	Gly *-	Leu *-	Met-NH_2 *
Kassinin Asp-	Val-	Pro-	Lys-	Ser-	Asp-	Gln-	Phe *-	Val-	Gly *-	Leu *-	Met-NH_2 *
Phyllomedusin		Glp-	Asn-	Pro-	Asn-	Arg-	Phe *-	Ile-	Gly *-	Leu *-	Met-NH_2 *
Substance P methyl ester	Arg-	Pro-	Lys-	Pro-	Gln-	Gln-	Phe *-	Phe-	Gly *-	Leu *-	Met *-OCH_3
Eledoisin related peptide						Lys-	Phe *-	Ile-	Gly *-	Leu *-	Met-NH_2 *

* Conserved residues; Glp = pyroglutamic acid.

0014-2999/83/0000-0000/$03.00

region of the molecule (see reviews by Hanley and Iversen, 1980; Sandberg and Iversen, 1982).

The existence of multiple receptors for substance P may account for the marked variation in the relative potencies of the tachykinins on different systems (e.g. Falconieri Erspamer et al., 1980). Previous work in this laboratory has described two distinct patterns of rank order of potency of tachykinins, suggesting two sub-classes of substance P receptor (Lee et al., 1982). One type, termed 'SP-P' and exemplified by the guinea-pig ileum, has the rank order physalaemin $\geqslant$ substance P $\simeq$ eledoisin $\simeq$ kassinin, while a second type, termed 'SP-E' and exemplified by the rat vas deferens, has the rank order kassinin $\geqslant$ eledoisin $\gg$ substance P $\simeq$ physalaemin (Lee et al., 1982). In addition, Lee et al. (1982) noted that substance P and eledoisin did not exhibit complete cross-desensitisation on guinea-pig ileum, and recovered from desensitisation at different rates.

The present study was, therefore, undertaken to examine the relative potencies of tachykinins in a variety of tissue preparations, and tentatively to classify these in terms of SP-P or SP-E systems. In addition, the potencies of a series of C-terminal substance P alkyl esters on these systems were determined.

2. Materials and methods

2.1. General methods

The activity of peptides was assayed on isolated smooth muscle preparations. The tissues were suspended in 2 ml organ baths containing a modified Krebs-bicarbonate solution (composition in mM: NaCl 127, KCl 2.5, $CaCl_2$ 1.8, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 10), gassed with a 95% O_2–5% CO_2 mixture and maintained at a temperature of 37°C. Contractions were recorded isotonically under a resting tension of 600 mg. Tissues were left for 30–60 min before the start of experiments.

Potency estimates were made relative to substance P using a 2×2 assay, or if the peptide was in short supply or very weakly active a bracketing or threshold concentration assay was employed. When sufficient peptide was available, preliminary experiments were carried out to ascertain whether it produced the same maximum response as substance P.

For desensitisation experiments, matched doses (producing responses that were approximately 40–60% of the maximal response to substance P) of eledoisin and substance P were administered to the tissue alternately until constant responses were obtained. The tissue was then challenged with a desensitising dose of agonist, and the time course of recovery of response monitored by alternate administration of the two peptide standards. Atropine (10^{-6} M) was present throughout these experiments (see Lee et al., 1982).

The following smooth muscle preparations and time cycles were used. For the distal ileum of the guinea-pig, rat duodenum and longitudinal strips of the rat and guinea-pig bladder a 3 min time cycle with a 30 s contact time was used. For longitudinal strips of hamster and mouse bladder a 5–15 min time cycle with a 1–3 min contact time was needed. On the latter two preparations responses were slow both in onset and offset and tachyphylaxis was often apparent. In the guinea-pig vas deferens and rat vas deferens we measured the ability of the peptides to potentiate the contractions evoked by co-axial electrical stimulation. For the rat vas deferens the stimulation parameters were: $1.5 \times$ maximum voltage, 0.15 Hz, 0.4 ms; and for the guinea-pig vas deferens trains of 5 pulses were used ($1.5 \times$ maximum voltage; 20 Hz; 1 ms) at 10 s intervals. Drugs were administered to both preparations on a 5 min cycle with a 60 s contact time.

2.2. Preparation of solutions

Stock solutions of peptides were made up in distilled water and stored either as frozen or freeze-dried aliquots at −20°C. Dilutions were made on the day of the experiment in Krebs solution containing 0.1% bovine serum albumin to prevent the adsorption of peptides to glass. Peptide concentrations were verified using amino acid analysis where necessary.

2.3. Synthesis of substance P esters

2.3.1. General

Substance P methyl and ethyl esters were prepared either by esterfication of substance P free acid [11-desamido]substance P or by complete synthesis of the peptides by the solid phase technique followed by trans-esterfication of the peptidyl resin (Sandberg, unpublished observations). Substance P n-propyl ester described in these observations was synthesised only by the latter method. The methyl and ethyl esters synthesised by the two different techniques were equipotent in bioassays. The material synthesised from [11-desamido]SP was identical on TLC, HPLC and amino acid analysis with the material prepared by solid phase synthesis in our laboratory. The latter material gave on fast atom bombardment mass spectrometry (Barber et al., 1981) the following positive molecular ions ($M\text{-}H^+$): SP-OMe (1362), SP-OEt (1376) and SP-OPr (1390). Details of the solid phase synthesis and sequence of the purified products will be published elsewhere (Sandberg, unpublished observations).

2.3.2. Substance P methyl ester (SP-OMe)

Acetylchloride (1 ml) was added to 20 ml of ice-cold methanol and left for 15 min. A portion (60 μl) of this solution (i.e. 1 N HCl/MeOH) was added to a solution of substance P free acid (2.1 mg, 1.6 mol) in 600 μl methanol and the reaction was left for 30 h at room temperature. The sample was rotavaporated to dryness, dissolved in 1 ml methanol and rotavaporated. The residue, dried over KOH in a desiccator over-night was finally dissolved in 800 μl acetonitrile/10 mM ammonium acetate 1 : 3 (v/v) and applied to a reverse-phase C_{18} (0.39 × 30 cm) column in portions

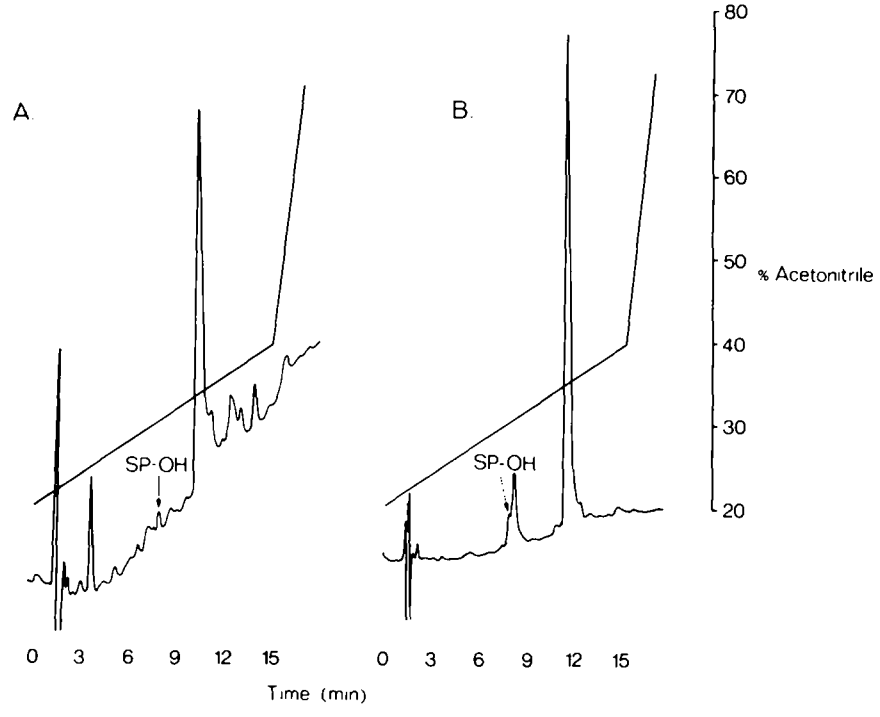

Fig. 1. (A) Reverse-phase high performance liquid chromatography of 5 μl (1 mg/ml) crude substance P methyl ester, SP-OMe, on a Bondapak C_{18} (0.39 × 30 cm) column applying a 15 min linear gradient from 20 : 80 to 40 : 60 acetonitrile/50 mM phosphoric acid (v/v) at 35°C and at a flow-rate of 2 ml/min. The absorbance at 220 nm was monitored, using a detector setting of 0.04 AUFS. (B) Reverse-phase high performance liquid chromatography of 5 μl (4 mg/ml) crude substance P ethyl ester, SP-OEt. The absorbance at 220 nm was monitored, using a detector setting of 0.16 AUFS.

of 330 μl (for analytical high performance liquid chromatography (HPLC) see fig. 1). The peptide was eluted isocratically with acetonitrile/10 mM ammonium acetate 28 : 72 (v/v) at 35°C and at a flow rate of 2 ml/min. The material corresponding to the main UV peak (254 nm) was collected, 10 ml of water was added, and the sample lyophilized. The final yield was 1.45 mg (69%) and the product was homogeneous on HPLC as well as on thin layer chromatography (TLC).

2.3.3. Substance P ethyl ester (SP-OEt)

Substance P free acid (2 mg, 1.5 mol) was suspended in 600 μl absolute ethanol and 60 μl 1 N HCl/ethanol (prepared from acetylchloride and absolute ethanol as above) was added. The peptide did not dissolve completely. After 30 h at room temperature, 200 μl of acetonitrile was added and the sample was left for another 24 h. The sample was finally rotavaporated to dryness, suspended in 1 ml absolute ethanol and rotavaporated. The residue was dissolved in 400 μl water and lyophilized. Acetonitrile/10 mM ammonium acetate 1 : 3 (v/v) 400 μl was added (for analytical HPLC see table 1) and 210 μl was applied to a reverse-phase C_{18} (0.39 × 30 cm) column. The peptide was eluted isocratically with acetonitrile/10 mM ammonium acetate 31 : 69 (v/v) at 35°C and at a flow rate of 2 ml/min. The material corresponding to the main UV peak (254 nm) was collected, 10 ml water was added, and the sample lyophilized. *Yield*: 1.05 mg (51%) of the desired peptide shown to be homogeneous on TLC and HPLC.

2.4. Materials

Substance P, substance P-(5-11), physalaemin, eledoisin, kassinin, eledoisin related peptide and substance P free acid were purchased from Peninsula Lab. Inc., San Carlos, California. Phyllomedusin was kindly donated by Dr. R. de Castiglione. The peptides were analysed by HPLC and

TABLE 2

Relative potencies of tachykinins and analogues (SP = 1.0). Potencies of tachykinins and analogues on various smooth muscle preparations are expressed relative to substance P (= 1.0) and represent the mean of at least 3 determinations. Absolute potencies can be evaluated through the use of table 3. Standard errors were less than 40%, and usually within 10% of the mean.

	SP-P				SP-E			
	Guinea-pig ileum [a]	Guinea-pig vas deferens	Guinea-pig bladder	Rat bladder	Rat vas deferens	Rat duodenum	Mouse bladder	Hamster bladder
Substance P	1	1	1	1	1	1	1	1
Physalaemin	2.43 [b]	0.80	1.3	1.81	0.60 [b]	1.8	1.07	0.70
Eledoisin	0.90 [b]	0.60	0.94	0.96	86 [b]	13.9	119	105
Kassinin	1.10 [b]	0.34	0.75	0.73	181 [b]	11.1	163	372
Phyllomedusin	1.4 [b]	1.9	0.91	1.25	7 [b]	0.90	3.1	ND [d]
Substance P methyl ester	0.95	0.52	0.95	0.61	0.01 [c]	0.017	< 0.002	0.007 [c]
Substance P ethyl ester	0.26	0.27	0.35	0.19	0.026 [c]	0.006	< 0.007	0.007 [c]
Substance P propyl ester	0.063	0.13	0.15	0.15	< 0.005	0.006	< 0.007	0.009 [c]
Substance P free acid	0.0009	0.002	0.001	0.002	0.09 [b]	0.0016	< 0.007	0.095
Eledoisin related peptide	0.232	0.213	0.10	0.16	17.5	2.6	5.2	7.8
Substance P-(5-11)	0.3 [b]	0.40	0.41	0.38	0.37	0.40	0.38	1.43

[a] In presence of 10^{-6} M atropine. [b] From Lee et al. (1982). [c] Threshold concentration assay. [d] ND not determined.

found to be at least 90% pure, with the exception of substance P-(5-11) which was contaminated by the corresponding pyroglutamyl and methionine sulphoxide derivatives. Atropine sulphate was purchased from Sigma Chemical Co., Poole.

All HPLC runs were carried out on a DuPont Model 850 equipped with a thermostatic column compartment model 851 and a UV detector Model 852. Ammonium acetate for HPLC was recrystallized from hot-methanol/ether. TLC was performed on precoated silica plates (Merck DC Fertigplatten Kiselgel, 60 F_{254}) using the system ethyl acetate/pyridine/water/acetic acid (5 : 5 : 3 : 1 v/v). For amino acid analysis, peptides were hydrolyzed in sealed tubes for 19 h at 100°C in 5.7 N HCl containing 2% thioglycolic acid and analysed on a Beckman model 119 CL.

3. Results

3.1. Potencies of tachykinins and analogues

The potencies of the tachykinins relative to substance P on the smooth muscle preparations described in the methods are shown in table 2. These potencies have been used to classify the muscles tentatively in terms of SP-P and SP-E systems according to the scheme suggested by Lee et al. (1982). Thus, guinea-pig ileum, guinea-pig vas deferens, guinea-pig bladder and rat bladder exhibited the SP-P rank order of tachykinin potencies, and rat vas deferens, rat duodenum, mouse bladder and hamster bladder exhibited the rank order consistent with the SP-E sub-class. There was close agreement for the relative potencies of tachykinins between different muscles within the same sub-class, with only the rat duodenum exhibiting potencies intermediate between SP-P and SP-E groups. These tachykinin potencies are in good agreement with those in the literature (Falconieri Erspamer et al., 1973; 1980).

TABLE 3

Absolute potencies of substance P in different bioassays.

Preparation	EC_{50} substance P (M)
SP-P	
Guinea-pig ileum	1.4×10^{-9}
Guinea-pig vas deferens	3.5×10^{-8}
Guinea-pig bladder	2.3×10^{-7}
Rat bladder	2.4×10^{-8}
SP-E	
Rat vas deferens	8.0×10^{-6}
Rat duodenum	7.0×10^{-7}
Mouse bladder	2.1×10^{-5}
Hamster bladder	2.1×10^{-5}

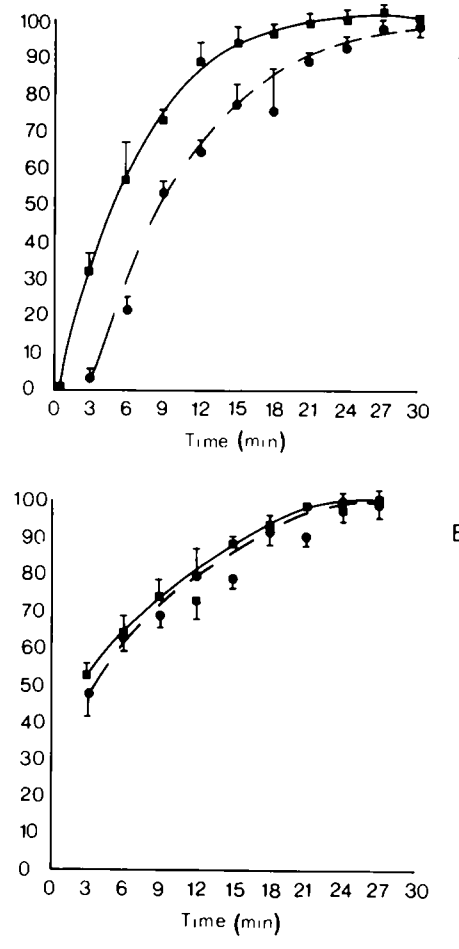

Fig. 2. (A) Rate of recovery of responses to sub-maximal doses of substance P (■———■) and eledoisin (●------●) on guinea-pig ileum following desensitisation induced by substance P methyl ester (1.85×10^{-7} M, 2 min). Lines have been fitted by eye. Results are means ± S.E.M. from 7 experiments. (B) Rate of recovery of responses to sub-maximal doses of substance P (————) and eledoisin (●------●) on rat duodenum following desensitisation induced by substance P (1.86×10^{-5}, 2 min). Lines have been fitted by eye. results are means ± S.E.M. from 6 experiments. *Ordinates*: % pre-desensitisation response.

Relative to substance P, table 2 also shows that the C-terminal methyl, ethyl and propyl esters of substance P were highly selective to SP-P systems, while eledoisin related peptide was selective to SP-E systems. Substance P-(5-11) and phyllomedusin were approximately equipotent with substance P on all systems (table 2). Again, good agreement for the relative potencies of these analogues between different muscles within the same sub-class was apparent.

SP-P and SP-E systems could also be differentiated by using the EC_{50} (concentration required to produce 50% of the maximum response) of substance P (table 3). Substance P was considerably more potent on SP-P systems, with EC_{50} values in the range 1–300 nM compared with 0.7–20 μM on SP-E systems. In terms of absolute potencies as well as relative potencies alkyl esters of substance P were selective to SP-P systems, but eledoisin and kassinin cannot be considered selective ligands for SP-E sites as they possess similar absolute potencies on both SP-P and SP-E preparations.

3.2. Desensitisation experiments

The half-lives of recovery of responses to submaximal doses of eledoisin and substance P following desensitisation on guinea-pig ileum induced by substance P methyl ester (185 nM; 2 min) were 9 and 5 min respectively (fig. 2). In contrast, eledoisin and substance P responses recovered at similar rates following desensitisation induced by substance P on rat duodenum (fig. 2) and rat bladder (data not shown).

4. Discussion

The present results have allowed us to describe further SP-P and SP-E systems based on the differing rank orders of potency of tachykinin analogues suggested by Lee et al. (1982). Examination of related analogues showed that the C-terminal heptapeptide substance P-(5-11) exhibits an approximately constant potency relative to substance P in all systems, while eledoisin-related peptide has a preference for SP-E preparations. These results indicate that the C-terminal region of these peptides, thought to be responsible for receptor recognition and activation (see review by Hanley and Iversen, 1980), is also capable of differentiating between the two putative receptor subtypes.

The replacement of the C-terminal amide moiety of substance P by a series of alkyl esters conferred selectivity to SP-P systems. This was most apparent for the methyl ester which, although approximately equipotent with substance P and eledoisin on SP-P systems, was 100–1000 times less potent than substance P on SP-E systems. Increasing the size of the ester substitution and thereby the hydrophobicity of the C-terminal region resulted in a gradual drop in potency on SP-P systems but little change in potency on SP-E systems, hence a decrease in selectivity was observed. The synthesis of these alkyl esters by a simple one step esterification of substance P free acid has been described; this method can easily be replicated by any laboratory with facilities for purification of products. Previous reports confirm that substance P methyl ester is approximately equipotent with substance P in guinea pig ileum (SP-P) (Cascieri et al., 1981) and rat salivation (Liang and Cascieri, 1981).

Differing rank orders of potency of agonists cannot be considered as proof of the existence of receptor subtypes because marked variations can exist between tissues in factors such as metabolism, rate of access to the receptor, rate of onset of desensitisation etc (see Kenakin, 1982), all of which can interfere with the potency estimates obtained. However, the consistency of relative potency estimates between different muscles within the SP-P or SP-E groups argues in favour of receptor sub-types. For example, the potency range of eledoisin relative to substance P on the SP-P systems described was 0.60–0.96 compared with 13.9–119 on the SP-E systems. Furthermore, the EC_{50} values of substance P are consistently lower on SP-P than SP-E systems, and this similarly lends support to the concept of receptor sub-types. Finally, the possibility that alkyl esters of substance P appear more potent on SP-P than SP-E systems because of a more rapid rate of hydrolysis in the latter tissues is not consistent with the present results as the likely product of hydrolysis, substance P-free acid, is *more* potent than the alkyl

esters in rat vas deferens and hamster bladder (SP-E systems).

Obvious problems are likely to arise when studying tissues containing both putative receptor sub-types. Thus a system containing approximately equal numbers of 'SP-E' and 'SP-P' receptors will appear as a SP-P system. One approach to this problem was adopted by Lee et al. (1982) by monitoring the recovery of response to matched doses of substance P and eledoisin on the guinea-pig ileum following desensitisation. They observed two distinct rates of recovery and suggested that this was indicative of two receptor sub-classes on this tissue. This was interpreted as further support for SP-P and SP-E receptor sub-classes. However, if this is so then desensitisation induced by substance P methyl ester would not be expected to interfere to a large extent with the response to eledoisin on guinea-pig ileum. This was clearly not the case, and argues against the existence of the 'SP-E' receptor subtype on guinea-pig ileum. The possibility either of the existence of a third sub-type of substance P receptor, or that differing rates of recovery from desensitisation are not necessarily due to the presence of more than one receptor must be considered. It is interesting to note that on rat bladder and rat duodenum substance P and eledoisin recover from desensitisation with similar half-lives.

Two subtypes of substance P receptor have also been proposed by Teichberg et al. (1981), based on differing rank orders of potency of substance P and its C-terminal fragments in various tissues. Substance P was reported to be approximately equipotent with substance P-(5-11) on guinea-pig ileum and rat ileum, but more than one order of magnitude less potent on guinea-pig bladder and bovine pupillary sphincter. The present results, however, do not appear to support this classification of substance P receptors, as substance P-(5-11) exhibited an approximately constant potency relative to substance P on all systems.

Finally, an important question is whether these putative receptor sub-types exist within the central nervous system. The presence of both SP-P and SP-E sites seems possible. Thus, eledoisin has been reported to be 17 and 15 times more potent than substance P in eliciting scratching behaviour in mice (Share and Rackham, 1981) and in its antidipsinogenic activity in rats (De Caro et al., 1980) respectively, but 0.3 times as potent in competing for [^{3}H]substance P binding to rat brain membranes (Hanley et al., 1980). As SP-E sites exhibit a relatively low affinity for substance P they are unlikely to be detected in binding studies utilising [^{3}H]substance P as the ligand. The existence of more than one subtype of substance P receptor in the central nervous system has also proposed by Frederickson et al. (1978) and by Oehme et al. (1980) in order to explain the observations that in low doses intra-ventricular administered substance P is analgesic, but that in higher doses it is hyperalgesic.

In summary, the present study has added further support for the existence of SP-P and SP-E receptor sub-types through the description of further test systems and demonstrating that substance P alkyl esters are highly selective to SP-P systems. However, the existence of receptor subtypes must be tentative as alternative explanations for the observed potency differences cannot be excluded, and so proof of their existence will only be obtained through the adoption of an alternative approach, e.g. the development of a selective antagonist.

Acknowledgements

Sincere thanks to Chris Rayner for excellent technical assistance, Adam Doble for numerous useful discussions and Mary Wynn for expert secretarial help.

References

Barber, M., R.S. Bardoli, R.D. Sedgwick and A.N. Tyler, 1981, Fast atom bombardment of solids as an ion source in mass spectrometry, Nature 293, 270.

Bury, R.W. and M.L. Mashford, 1977, Substance P: its pharmacology and physiological roles, Aust. J. Exp. Biol. Med. Sci. 55, 671.

Cascieri, M.A., M.M. Goldenberg and T. Liang, 1981, Biological activity of substance P methyl ester, Mol. Pharmacol. 20, 457.

De Caro, G., M. Massi and L.G. Micossi, 1980, Modifications of drinking behaviour and of arterial blood pressure induced by tachykinins in rats and pigeons, Psychopharmacology 68, 243.

Falconieri Erspamer, G.F., V. Erspamer and D. Piccinelli, 1980, Parallel bioassay of physalaemin and kassinin, a tachykinin dodecapeptide from the skin of the African frog *Kassina senegalensis*, Naunyn-Schmiedeb. Arch. Pharmacol. 311, 61.

Falconieri Erspamer, G.F., L. Negri and D. Piccinelli, 1973, The use of preparations of urinary bladder smooth muscle for bioassay of and discrimination between polypeptides, Naunyn-Schmiedeb. Arch. Pharmacol. 279, 61.

Frederickson, R.C.A., V. Burgis, C.F. Harrell and J.D. Edwards, 1978, Dual actions of substance P on nociception: possible role of endogenous opioids, Science 199, 1359.

Hanley, M.R. and L.L. Iversen, 1980, Substance P receptors, in: Neurotransmitter Receptors, Part 1, Series B, Vol. 9, eds. S.J. Enna and H.I. Yamamura, Chapman and Hall, London, p. 71.

Hanley, M.R., B.E.B. Sandberg, C.M. Lee, L.L. Iversen, D.E. Brundish and R. Wade, Specific binding of ^{3}H substance P to rat brain membranes, Nature 286, 810.

Kenakin, T.P., 1982, Organ selectivity of drugs. Alternatives to receptor selectivity, Trends Pharmacological Sci. 3, 153.

Lee, C.-M., L.L. Iversen, M.R. Hanley and B.E.B. Sandberg, 1982, The possible existence of multiple receptors for substance P, Naunyn-Schmiedeb. Arch. Pharmacol. 318, 281.

Liang, T. and M.A. Cascieri, 1981, Substance P receptor on parotid cell membranes, J. Neurosci. 1, 1133.

Oehme, P., H. Hilse, E. Morgenstern and E. Gres, 1980, Substance P: does it produce analgesia or hyperalgesia?, Science 208, 305.

Sandberg, B.E.B. and L.L. Iversen, 1982, 'Substance P' Perspective, J. Med. Chem. 25, 1009.

Share, N.N. and A. Rackman, 1981, Intracerebral substance P in mice: behavioural effects and narcotic agents, Brain Res. 211, 379.

Teichberg, V.I., S. Cohen and S. Blumberg, 1981, Distinct classes of substance P receptors revealed by a comparison of the activities of substance P and some of its segments, Regulatory Peptides 1, 327.

Von Euler, U.S. and J.H. Gaddum, 1931, An unidentified depressor substance in certain tissues, J. Physiol. 72, 74.