MOLECULAR PHARMACOLOGY, 20:457–459

Biological Activity of Substance P Methyl Ester

MARGARET A. CASCIERI,[1] MARVIN M. GOLDENBERG,[2] AND TEHMING LIANG[1]

Departments of Biochemical Endocrinology and Immunology, Merck Institute for Therapeutic Research, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065

Received May 11, 1981; Accepted July 3, 1981

SUMMARY

The COOH-terminal amide of substance P was replaced with a methyl ester, and the potency of this substance P methyl ester was compared with that of substance P and substance P free acid in stimulating salivation in rats and in stimulating guinea pig ileum contraction *in vitro*. Compared with substance P, substance P methyl ester was found to be 30% as potent in stimulating salivation and equipotent in ileum contraction. Substance P free acid was only 0.1% and 0.9% as potent as substance P in stimulating salivation and ileum contraction, respectively. The results suggest that the hydrophobicity of the COOH-terminal portion of substance P may be important for the molecule in its interaction with the substance P receptor.

The substance P molecule (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) can be divided into two domains according to physical and chemical properties and biological activities. The NH_2-terminal portion of the molecule contains arginine and lysine residues and therefore is positively charged at physiological pH. The remainder of the molecule is hydrophobic. With the exception of its chemotactic activity (1) and its neuroblastoma growth-stimulating activity (2), which have been attributed to the NH_2-terminal tetrapeptide sequence, all other biological activities, including salivation (3–5), phosphatidylinositol turnover in the salivary glands (3), vasodilation (6), intestinal contraction (6–8), and depolarization of motoneurons (9), can be demonstrated with its COOH-terminal hexapeptide.

The COOH-terminal of substance P is amidated. Deamidation of the COOH-terminal amide decreases the biological potency by two to four orders of magnitude (3, 5, 10, 11). The COOH-terminal of substance P free acid is negatively charged under physiological conditions. It is not known whether the significantly lower biological potency of substance P free acid is due to the presence of this negative charge on the free acid or whether the amide group itself is required for potent biological activity. To distinguish between these two possibilities, we evaluated the ability of substance P methyl ester to stimulate salivation in rats and guinea pig ileum contraction. We have previously reported that substance P methyl ester can inhibit the binding of an ^{125}I-labeled substance P derivative to the substance P receptor on rat parotid cells (12).

Substance P methyl ester was custom-synthesized for us by Bachem (Torrance, Calif.); substance P was purchased from Beckman Instruments Inc. (Palo Alto, Calif.), and substance P free acid was purchased from Bachem. High-pressure liquid chromatography (absorbance at 210 nm) indicated that the purity of all three peptides was greater than 98%.

The salivation assay was a modification (5) of the procedure of Leeman and Hammerschlag (13). Peptides were dissolved in 0.1 N acetic acid and were diluted in 0.15 N NaCl solution to the desired concentration immediately before injection. Sprague-Dawley male rats (body weight 300–350 g) were anesthetized with ether. The peptide solution (0.1 ml) was injected via a tail vein and saliva was immediately collected from the buccal cavity with a Pasteur pipette over a period of 2 min. The volume of saliva was measured by pipette. Three to five rats were used at each dosage level. Basal release of saliva was determined by injection of the vehicle alone.

The guinea pig ileum contraction assay was similar to that previously reported (14) and modified as follows: A 2-cm strip of guinea pig ileum was attached to a transducer and was bathed in 10 ml of Krebs-Ringer bicarbonate buffer aerated with 95% O_2-5% CO_2. Peptides were dissolved and diluted in water to the desired concentration immediately before use. The peptide solution (0.25 ml) was added to the tissue bath and the contraction of the tissue was recorded with a chart recorder. Between doses, the tissue was rinsed with 10 ml of Krebs-Ringer bicarbonate buffer seven times and then it was allowed 15 min for recovery.

Figure 1 shows the stimulation of salivation in anesthetized rats by various dosages of substance P, its free acid, and its methyl ester. Substance P methyl ester produced maximal stimulation; however, the free acid at the highest dosage tested produced only 30% of the maximal stimulation produced by substance P. The values of ED_{50} and the relative potencies of these com-

[1] Department of Biochemical Endocrinology.
[2] Department of Immunology.

0026-895X/81/1030457-03$02.00/0

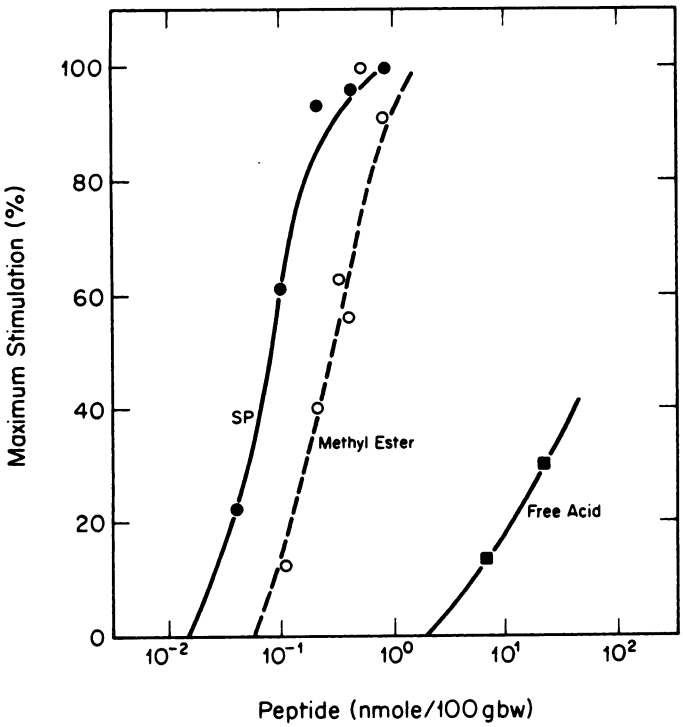

FIG. 1. *Stimulation of salivation in anesthetized rats by substance P (SP), substance P methyl ester, and substance P free acid*

Data are presented as percentage of the maximal substance P response in individual experiments, and each *point* represents the average of three separate experiments. The maximal stimulation by substance P was 425 ± 58 μl of saliva/rat (mean ± S.D.) and the basal saliva secretion was 3 ± 2 μl/rat.

pounds are listed in Table 1. Thus, substance P methyl ester and substance P free acid were 30% and 0.1%, respectively, as potent as substance P in stimulating salivation.

Figure 2 shows the titration of the stimulation of guinea pig ileum contraction by these three peptides. The values of ED_{50} and the relative potencies of these compounds also are listed in Table 1. The methyl ester was 110% as potent as substance P and the free acid had 0.9% the potency of substance P in stimulating contraction of guinea pig ileum.

Hydrophobicity in the COOH-terminal portion of substance P appears to be an important characteristic for the molecule to bind to and activate the substance P receptor. This is indicated by our present observation that both substance P and its methyl ester have a much higher potency than substance P free acid in stimulating salivation and intestinal contraction. We also previously observed that they are more potent than the free acid in displacing the binding of a ^{125}I-labeled substance P derivative to the substance P receptor on rat parotid cells (12). Replacement of the COOH-terminal amide with a methyl ester maintains the relative hydrophobicity of the peptide and only slightly increases the bulk of the COOH terminus. The presence of the negative charge on substance P free acid may hinder the interaction of this peptide with the substance P receptor. In addition, the free acid may be more susceptible than the amide to degradation by carboxypeptidases.

TABLE 1

Relative potency of substance P (SP), SP methyl ester, and SP free acid in the stimulation of salivation in rats and of guinea pig ileum contraction

The doses required to give 50% maximal stimulation (ED_{50}) were calculated after regression analysis of the dose response data. The relative potencies were calculated using the parallel line bioassay technique (15). The 95% confidence limits of the relative potencies are given in parentheses. The relative potency of substance P free acid in the salivation assay is only estimated from the ratio of the values of ED_{50}, since the regression line for the limited number of data points for the free acid was not parallel to the line generated by substance P.

Peptide	Salivation		Ileum contraction	
	ED_{50}	Relative potency	ED_{50}	Relative potency
	nmoles/100 g body wt	%	*M*	%
SP	0.076	100	1×10^{-8}	100
SP methyl ester	0.26	30 (23.5–37.8)	9×10^{-9}	110 (87.1–154.2)
SP free acid	91	~0.1	1×10^{-6}	0.9 (0.6–1.3)

The potency of the methyl ester is equal to that of substance P in the guinea pig ileum assay, but it is significantly less potent than substance P in stimulating salivation. It remains to be determined whether this difference is due to a greater sensitivity of substance P methyl ester to circulating peptidases or to a different substance P receptor population in the guinea pig ileum and the rat salivary glands. As previously reported (5, 10,

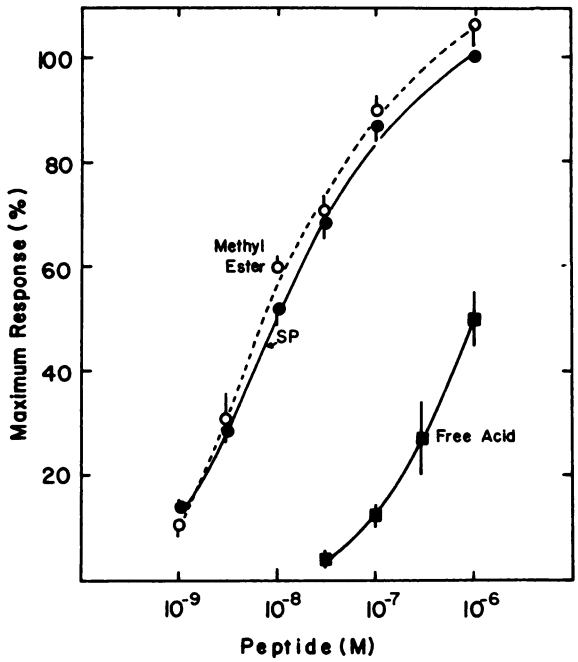

FIG. 2. *Stimulation of guinea pig ileum contraction by substance P (SP), substance P methyl ester, and substance P free acid*

Data are presented as percentage of maximal contraction stimulated by substance P. Each *point* represents the average of data from 12 strips of guinea pig ileum for substance P and the average of data from 4 strips for the methyl ester and the free acid. The *bars* indicate standard errors.

11), the free acid is a weak agonist. Although the free acid at the dosages that we tested did not produce maximal stimulation, it has been reported to be a full agonist in stimulating salivation (3), turnover of phosphatidylinositol in the salivary glands (3), and contractions in the guinea pig ileum (11).

Although many substance P analogues have been prepared and their biological activities evaluated (16), to our knowledge, substance P methyl ester has not been previously reported. A better understanding of the structural requirements for receptor binding and for biological activity should help in the design of substance P agonists and antagonists.

ACKNOWLEDGMENTS

We are grateful to E. M. Subers for assistance in the guinea pig ileum assay, to Drs. E. H. Cordes and D. F. Veber for their review of the manuscript, and to Ms. Madeline Spencer for typing the manuscript.

REFERENCES

1. Bar-Shavit, Z., R. Goldman, Y. Stabinsky, P. Gottlieb, M. Fridkin, V. I. Teichberg, and S. Blumberg. Enhancement of phagocytosis—a newly found activity of substance P residing in its N-terminal tetrapeptide sequence. *Biochem. Biophys. Res. Commun.* **94**:1445–1451 (1980).
2. Narumi, S., and Y. Maki. Stimulatory effects of substance P on neurite extension and cyclic AMP levels in cultured neuroblastoma cells. *J. Neurochem.* **30**:1321–1326 (1978).
3. Hanley, M. R., C. M. Lee, L. M. Jones, and R. H. Michell. Similar effects of substance P and related peptides on salivation and on phosphatidylinositol turnover in rat salivary glands. *Mol. Pharmacol.* **18**:78–83 (1980).
4. Leeman, S. E., E. A. Mroz, and R. E. Carraway. Substance P and neurotensin, in *Peptides in Neurobiology* (H. Gainer, ed.). Plenum Press, New York, 99–144 (1977).
5. Liang, T., and M. A. Cascieri. Substance P stimulation of amylase release by isolated parotid cells and inhibition of substance P induction of salivation by vasoactive peptides. *Mol. Cell. Endocrinol.* **15**:151–162 (1979).
6. Bury, R. W., and M. L. Mashford. Biological activity of C-terminal partial sequences of substance P. *J. Med. Chem.* **19**:854–856 (1976).
7. Yajima, H., K. Kitagawa, and T. Segawa. Structure-activity correlations in substance P. *Chem. Pharm. Bull. (Tokyo)* **21**:2500–2506 (1973).
8. Yanaihara, N., C. Yanaihara, M. Hirohashi, H. Sato, Y. Iizuka, T. Hashimoto, and M. Sakagami. Substance P analogs: synthesis, and biological and immunological properties, in *Substance P* (U. S. von Euler and B. Pernow, eds.). Raven Press, New York, 27–33 (1977).
9. Otsuka, M., and S. Konishi. Electrophysiological and neurochemical evidence for substance P as a transmitter of primary sensory neurons, in *Substance P* (U. S. von Euler and B. Pernow, eds.). Raven Press, New York, 207–214 (1977).
10. Mroz, E. A., and S. E. Leeman. Substance P. Vitam. Horm. **35**:209–281 (1977).
11. Couture, R., A. Fournier, J. Magnan, S. St-Pierre, and D. Regoli. Structure-activity studies on substance P. *Can. J. Physiol. Pharmacol.* **57**:1427–1436 (1979).
12. Liang, T., and M. A. Cascieri. Specific binding of an immunoreactive and biologically active ^{125}I-labeled N(1)acylated substance P derivative to parotid cells. *Biochem. Biophys. Res. Commun.* **96**:1793–1799 (1980).
13. Leeman, S. E., and R. Hammerschlag. Stimulation of salivary secretion by a factor extracted from hypothalamic tissue. Endocrinology **81**:803–810 (1967).
14. Goldenberg, M. M. Tachyphylaxis to the inhibitory action of atropine on the cholinergic response to nicotine, in vitro. *Arch. Int. Pharmacodyn.* **180**:264–280 (1969).
15. Finney, D. J. Parallel line bioassay technique, in *Statistical Method in Biological Assay*. Charles Griffin & Company, London, 59–104 (1978).
16. Hanley, M. R., and L. L. Iversen. Substance P receptors, in *Neurotransmitter Receptors* (S. J. Enna and H. I. Yamamura, eds.), Part 1. Chapman and Hall, London, 71–103 (1980).

Send reprint requests to: Dr. Tehming Liang, Merck Institute for Therapeutic Research, Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, N. J. 07065.