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COMPARISON OF SPANTIDE II AND CP-96,345 FOR BLOCKADE OF TACHYKININ-EVOKED CONTRACTIONS OF SMOOTH MUSCLE

Rolf Håkanson,* Zunyi Wang* and Karl Folkers+

*Farmakologiska Institutionen, Lunds Universitet, 223 62 LUND, Sweden,
+Institute for Biomedical Research, The University of Texas at Austin, Texas

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CP-96,345, a quinuclidine, is a potent inhibitor of substance P for the NK_1 receptor of bovine brain, but has reduced potency for the corresponding receptor of the rat and mouse, and none for NK_2 or NK_3 receptors. A related quinuclidine showed similar but lower potency than CP-96,345 for NK_1. CP-96,345 was more potent than the spantide I of 1984, D-Arg^1,Pro^2,Lys^3,Pro^4,Gln^5,Gln^6,D-Trp^7,Phe^8,D-Trp^9,Leu^{10}, Leu^{11},NH_2.

Our continued designs for antagonists of substance P led to spantide II in 1990 which is: D-NicLys^1,Pro^2,3-Pal^3,Pro^4,D-Cl_2Phe^5,Asn^6,D-Trp^7,Phe^8, D-Trp^9,Leu^{10},Nle^{11}-NH_2. The pA_2 values of spantide II and CP-96,345 for guinea pig taenia coli were 7.6 and 6.8, respectively. The pIC_{50} values for blockade of tachykinin-mediated neurotransmission in the rabbit iris sphincter were 6.1 and 5.4, respectively. Spantide II was nearly 10 times more potent than CP-96,345 in these two assays.

INTRODUCTION.- There is considerable evidence that supporting a role for tachykinins are neurotransmitters (1). Tachykinins in sensory C-fibers have an important role in the transmission of painful stimuli from the periphery (2), and in evoking symptoms associated with neurogenic inflammation (3). The effects of tachykinins are mediated via at least three different subpopulations of receptors, NK_1 receptors that favour substance P (SP), NK_2 receptors that favour neurokinin A (NKA), and NK_3 receptors that favour neurokinin B (NKB) (4).

The elucidation of the functional significance of tachykinins will greatly benefit from access to potent and specific antagonists. Over the years, many non-selective antagonists of tachykinin receptors have been described (5-11). These antagonists have been full-length tachykinin analogues or truncated C-terminal analogues of six to eight residues (5-11). The recently described Spantide II is an example of a undecapeptide tachykinin antagonist with high potency and little histamine-mobilizing effect (5).

Snider et al. (12) described a non-peptide antagonist for the NK_1-receptor for substance P which was designated CP-96,345. An accompanying

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paper by McLlean et al. (13) compared inhibition constants, K_1, of CP-96,345 and spantide I which were 0.20 $\pm$ 0.02 and 751 nM, respectively, i.e., CP-96,345 is more potent than spantide I, introduced in 1984 (14). Since then, more potent peptide inhibitors of SP have been achieved including spantide II of 1990 (15).

We have compared spantide II and CP-96,345 and report the results from using the isolated rabbit iris sphincter muscle and guinea pig taenia coli. After completing this comparison, we read the account of Rouissi et al. (16) on a quinuclidine (II), which is closely related to CP-96,345. Quinuclidine II is less active than CP-96,345 for a NK_1 receptor and is almost inactive for the NK_2 and NK_3 receptors. Both quinuclidines are selective for NK_1.

SUBSTANCES AND METHODS.- Spantide II is D-NicLys^1,Pro^2,3-Pal^3,Pro^4, D-Cl$_2$Phe^5,Asn^6, D-Trp^7,Phe^8,D-Trp^9,Leu^{10},Nle^{11}-NH$_2$ (15). CP-96,345 was a gift from AB Astra, Pain Control, Sodertalje, Sweden. SP was purchased from Peninsula Laboratories, Inc., in California. Atropine was from Alcon, TX, and guanethidine from CIBA-Geigy, Basel, Switzerland. CP-96,345 and SP were dissolved in 0.01 N acetic acid. Spantide II was dissolved in 0.5 N acetic acid (10^{-3}M solution).

Isolated Rabbit Iris Sphincter.- Adult pigmented rabbits of either sex, weighing 1.5 - 3.0 kg, were killed by a blow to the neck and exsanguinated. The sphincter muscle of the rabbit iris was excized, opened and mounted (14). A gas mixture of 7% CO_2 in O_2 at a pH 7.2 - 7.3 at 37°C was bubbled through the modified Krebs solution (14). Mechanical activity was recorded isometrically using a Grass FT03 force displacement transducer and a Grass model 7 polygraph. Before the start of each experiment, the preparation was allowed to equilibriate for 90 min., and stretched with a force of 1.5 mN (subsequently maintained during the resting condition). Electrical stimulation with square wave pulses(20 Hz, 25 V, 0.3 ms duration, pulse train 10 s) was supplied by a means of platinum electrodes connected to a Grass S4C stimulator. Because repeated electrical stimulation led to a gradual exhaustion of the contractile response, the two iris halves, mounted in separate baths, were stimulated in parallel, one being exposed to increasing concentrations of the tachykinin antagonists, the other was a control preparation without antagonists. In each experiment, the supression of the electrically evoked contraction in the drug-exposed preparation was directly compared with the equivalent response in the control preparation (set as 100%), and expressed as per cent of control. Concentration-response curves were constructed and the pIC_{50} values were calculated (i.e. the negative logarithm of the molar concentration of the antagonist producing 50% inhibition of the electrically evoked contraction).

Isolated Guinea Pig Taenia Coli.- A preparation of the guinea pig taenia coli consisted of longitudinal smooth muscle with the attached myenteric plexus (8), and was placed in a modified Krebs solution which was kept at 4° C for about 1 h. and then mounted vertically on a Perspex holder in a 8-ml organ bath maintained at 33° C. The bath was oxygenated to a pH of 7.2 - 7.3. One end of the preparation was attached to a rigid support and the other to a lever connected by a spring to a Grass FT03 force displacement transducer. The mechanical activity of the preparation was recorded continuously on a Grass model 7 polygraph. The load on the muscle was set at 0.2 g. Concentration-response curves were constructed by adding SP in a cumulative manner. The contractile response was expressed in relation to that evoked by 10^{-5} M carbachol added at the end of the experiment. All EC_{50} values were calculated by linear regression analysis to the steep part of each concentration-response curve. Three to four concentration-response curves for SP were obtained from each preparation, one with SP alone and the others with different concentrations of the antagonist present. The pA_2 values and the slopes of the Schild plots were calculated (15-16). The slopes should not be different from -1 if the antagonism is competitive in nature. Student's test was used for statistical analysis and a p value of 0.05 or less was considered significant.

RESULTS.- Neither spantide II nor CP-96,345 (in the concentrations tested) influenced the basal tone of either the rabbit iris or the guinea pig taenia coli. Both CP-96,345 and spantide II supressed the noncholinergic, nonadrenergic contractile response of the iris sphincter muscle evoked by electrical nerve stimulation. The results are in Table 1. SP contracts the taenia coli of the guinea pig. In the presence of CP-96,345, the concentration-response curve for SP was shifted to the right, suggesting competitive inhibition. The slope of the Schild plot was not

Table 1. Comparison of CP-96,345 and spantide II

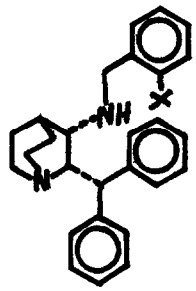
Antagonist	Inhibition of Tachykinin-Evoked Responses		
	Endogenous SP (iris sphincter)	Exogenous SP (taenia coli)	
	pIC_{50}	pA_2	slope
CP-96,345	5.4 +/- 0.2 (5)	6.8 +/- 0.3 (7)	-0.98
spantide II	6.1 +/- 0.1 (9)*	7.6 +/- 0.2 (19)*	-0.94

Numbers in the brackets are the number of experiments.
*$p < 0.05$.

different from -1 (Table 1). Also, spantide II exerted a competitive antagonism to SP in this system (5). Data on the comparison of spantide II and CP-96,345, expressed as pIC_{50} and pA_2, respectively, are in Table 1.

DISCUSSION.- Spantide I of 1984 was the first full-length tachykinin antagonist to be subjected to a detailed pharmacological analysis (14). Since the introduction of Spantide I, subsequent designs resulted in Spantide II (15) which was found to be more potent as an antagonist and less active on mast cells than Spantide I (5-11).

Snider et al. (12), McLlean et al. (13) and Rouissi et al. (16) reported two quinuclidines, I and II, to be inhibitors of the NK_1 receptor of substance P, but these substances showed less binding to the NK_2 and NK_3 and other neurotransmitter receptors. CP-96,345 was quite potent for the NK_1 receptor of bovine brain, but was less potent for this receptor of the rat and mouse. CP-96,345 was viewed as a tool for research on physiological activities of substance P.



I = OCH_3 (CP-96,345)
II = Cl

CP-96,345, exclusive of pi-electrons for binding in the receptor, has two presumed functionalities for binding in the receptor, the secondary and tertiary amino groups.

The organic structure of the undecapeptide, spantide II, shows at least 18 functionalities, including backbone functionalities, for binding in the receptor or at least 10 functionalities for such binding exclusive of backbone participation. Therefore, the minimal functionalities for binding of CP-96,345 to the receptor when compared to the muliple functionalities of spantide II for binding allows consideration of CP-96,345 with that of truncated antagonists of an undecapeptide. A truncated analog of an undecapeptide has fewer functionalities than that of the undecapeptide. This structural comparison of CP-96,345 with spantide II for receptor binding may possibly explain why CP-96,345 has activity for only one of the three NK receptors, and then for only the NK_1 receptor of limited mammalian species. Nevertheless, the selective limitation of binding of CP-96,345 for the NK_1 receptor is important and useful to elucidate the specificity of antagonists and receptors.

We compared inhibitory activities of spantide II and CP-96,345 with the following results. Spantide II had a pA_2 value of 7.6 for the taenia coli of the guinea pig in comparison with 6.8 for CP-96,345. Spantide II had a pIC_{50} value of 6.1 in comparison with 5.4 for CP-96,345 for blockade of the tachykinin-mediated neurotransmission in the iris sphincter muscle of the rabbit.

Consequently, spantide II is nearly 10-fold more potent than CP-96,345 in two assay systems. It is expected that undecapeptide analogs which are more potent than spantide II will be designed. It appears, based on present data, that the selectivity of CP-96,345 for only the NK_1 receptor detracts from the potential clinical usefulness of this quinuclidine. Other non-peptide molecules, designed in the future, could become clinically useful in this field.

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