PHARMACOLOGIC CHARACTERIZATION OF BRONCHOSPASM INDUCED BY SUBSTANCE P (SP) AND SP FRAGMENTS IN GUINEA-PIG

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ABSTRACT

Using pharmacologic approach, neurokinin receptor-mediated bronchoconstrictor responses in anesthetized guinea-pigs was characterized. Thus, the bronchospastic effects of substance P (SP) and SP fragments (all administrated intravenously) before and after giving vehicle or selective neurokinin receptor antagonists were compared. Ranking order of potency of SP or SP fragments for induction of bronchoconstriction was: SP^{4-11} >> SP^{5-11} = SP^{3-11} = SP^{2-11} > SP = SP^{6-11} (the number of amino acid in the sequence of SP fragments are shown by superscript). The neurokinin 1 (NK1) receptor antagonists (CP 96,345 or CP 99,994, 3 mg kg^{-1}, iv) did not change baseline values of pulmonary flow resistance (R_L) and dynamic pulmonary elastance (E_L) and did not eliminate bronchopulmonary responses to these peptides but decreased changes in R_L and E_L in response to SP and SP fragments. The neurokinin 2 (NK2) receptor antagonist SR 48,968 (1 mg kg^{-1}, iv) failed to induce a rightward shift in dose-response curves to SP or SP fragments except to SP^{4-11}. Combinations of NK1 and NK2 receptor antagonists shifted dose-response curves to SP and SP fragments more than that of NK1 receptor antagonists alone. These findings reveal that SP-induced bronchoconstriction is mediated by its C-terminal sequence and this response is mainly via NK1 receptors. Moreover, bronchopulmonary responses to SP and its C-terminal fragments are complex and there may be interactions between NK1 and NK2 receptors in the lungs.

Key words: Substance P, Substance P fragments, Neurokinin receptor antagonist, Bronchospasm, Guinea-pig

INTRODUCTION

Substance P (SP) is a neuropeptide considered to function as a neurotransmitter or neuromodulator in the central and peripheral nervous system (1-2). It is well characterized in terms of sites and mechanisms of biosynthesis, distribution, sites of the release and biological actions (2). SP belongs to a family of closely-related peptides known as the tachykinins which are widely distributed in the airways and lungs of several species, including humans and guinea-pigs (3-6). Given parenterally, they induce a variety of responses including contraction of bronchial smooth muscle, mucus secretion, vasodilation, extravasation of plasma proteins and recruitment of inflammatory cells, which are known as neurogenic inflammation (7-9). It has been suggested that release of endogenous tachykinins, SP and neurokinin A (NKA), from pulmonary afferent C-fibres contributes to the bronchial obstruction in asthma (7-10).

With few, but important exceptions (e.g. mast cell degranulation and behavioral effects), the biological effects of tachykinins are mediated via their structurally similar carboxyl terminal sequences:.Phe-X-Gly-Leu-Met-COOH (4, 11-13). These effects are via specific neurokinin receptors termed, NK1 (SP-preferring), NK2 (NKA-preferring) and NK3 (Neurokinin B-preferring) receptors (4, 14-15). The use of these selective neurokinin receptor agonists and antagonists has sterenghted the existence of these three distinct receptors (14, 16). It has been shown by *in vitro* and *in vivo* experiments that non-selective or selective neurokinin receptor antagonists reduced or abolished tachykinins-induced bronchospasm (17).

As with other peptide transmitters, the physio-

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logical effects of tachykinins are limited by their enzymatic degradation at or near the site of their release. They are all subject to cleavage by neutral endopeptidase (NEP); SP is also subject to cleavage by angiotensin-converting enzyme (ACE) and aminopeptidase (18-20). ACE hydrolyzes SP at Gly^{9}-Leu^{10} and Phe^{8}-Gly^{9}. NEP cleaves SP preferentially at the amino-terminal side of the hydrophobic amino acid residues Phe^{7} or Phe^{8} and Leu^{10}. Some of these metabolites are subjected to further cleavage by these enzymes (20). The predominant metabolites of SP are SP^{1-9} and SP^{10-11} (the number of amino acid in the sequence of SP fragments are shown by superscript) (21-22). By i*n vitro* and *in vivo* experiments it has been demonstrated that NEP or ACE inhibitors augment SP and NKA biological effects (17, 23-24). It has been shown that the products of NEP and ACE hydrolysis of SP at Gly^{9}-Leu^{10} are inactive in anesthetized, mechanically ventilated guinea-pigs (23). However, SP^{3-11} and SP^{5-11} resulting from cleavage of SP by dipeptidyl (amino) peptidase IV are more potent bronchoconstrictor than SP (23). This study was designed to characterize neurokinin-mediated responses in anesthetized, paralyzed and mechanically ventilated guinea-pigs by comparing the actions of CP 96,345 or CP 99,994, selective NK1 receptor antagonists (4, 25), and SR 48,968 (26), a selective NK2 receptor antagonist, against SP- or SP fragments-induced bronchoconstriction. As some of the SP fragments which were used in this study are metabolites of SP (20), this information will provide a basis for further pharmacological stu-dies in *in vivo* models involving release of SP.

MATERIALS AND METHODS

Materials

Drugs used were: substance P (Biochem Pharma Inc., Montreal, PQ), succinylcholine chloride, SP^{9-11}, SP^{8-11}, SP^{7-11}, SP^{6-11}, SP^{5-11}, SP^{4-11}, SP^{3-11}, SP^{2-11}, SP^{1-4}, SP^{1-7} and SP^{1-9} (Sigma, St. Louis, MO, USA), Sodium Pentobarbital (Euthanyl^{TM}, M.T.C Pharmaceuticals, Markham, ON); Methionine, Phe-Phe, Phe-Gly, Gly-Leu and Leu-Met (Bachem Bioscience Inc., Philadelphia, PA USA). CP 96,345 was a gift of Pfizer Inc., Groton, CO. and SR 48,968 was a gift from Sanofi Recherche, Montpelier, France.

Animals

Groups (n = 5) of SPF-quality, female Hartley-strain guinea-pigs (weight range: 350-450 g) were obtained from Charles River Inc., St. Constant, Quebec. They were transported in filter-top boxes and housed in laminar flow units (Bioclean^{TM}, Hazleton) on grids over trays of rock salt and were fed guinea-pig chow supplemented with apples. Water was allowed *ad lib*.

Measurement of airways' responsiveness

Groups (n = 5) of SPF-quality, female, Hartley-strain guinea-pigs (weight range: 350-450 g) were used. They were anesthetized with Sodium Pentobarbital (40-50 mg kg^{-1}, ip, additional doses of 5 mg kg^{-1}, iv, as required). Their tracheas were cannulated (PE240) and artificial respiration was applied (tidal volume = 9 ml/kg, pump speed = 20 strokes min^{-1}) with a rodent ventilator (Ugo Basile, Varese, Italy). A jugular vein was cannulated (PE50) for iv administration of drugs. Succinylcholine (0.03 mg kg^{-1}, iv) was given to paralyse animals and prevent spontaneous respiratory movements. Pulmonary flow resistance (R_L) and dynamic pulmonary elastance (E_L) were measured continuously, breath-by-breath, by the use of a computerized system (27). Agonists were given in ascending order of dose and after each dose animals were inflated and allowed to rest to get back to the baseline values of R_L and E_L. Dose-response (peak response as percent change from baseline values of R_L and E_L) curves to SP, SP^{9-11}, SP^{8-11}, SP^{7-11}, SP^{6-11}, SP^{5-11}, SP^{4-11}, SP^{3-11}, SP^{2-11}, SP^{1-4}, SP^{1-7} and SP^{1-9} were established and the effects of selective NK1 or NK2 receptor antagonist were determined. The amino acid sequence of these peptides are shown in Table 1. The relative bronchoconstrictor activities of SP and SP fragments were estimated by fitting data using linear regression. Table 2 shows the bronchoconstrictor activities of SP and SP fragments. Antagonists were given intravenously 15 min before administration of SP or SP fragments. The solutions of SR 48,968 (4 mg) in ethanol (95%, 0.1 ml), SP^{6-11} in dimethylsulfoxide, SP^{1-7} and SP^{4-11} in 0.05% HOAc were prepared. Other drugs were dissolved in distilled water. After dissolving, all drugs were diluted to final concentration with normal saline.

Statistical analysis

Dose-response curves were plotted as mean ± SEM. Data were analyzed using Sigmastat^{TM} and SAS program. Mann-Whitney rank sum tests, Student's *t* tests and one-way ANOVA

were used to examine differences among responses; linear least square regression analyses were used to determine linearity and relative potency of peptides. The ED_{700} [doses of peptides (nmol kg^{-1}) required to cause 700% increase in R_L from baseline values] were used to test significant differences in potency of peptides. Significance was assumed at the 5% level.

RESULTS

Bronchopulmonary responses to SP and SP fragments

N-terminal fragments of SP and C-terminal fragment of SP containing less than 4 amino acid had no bronchospastic effect at doses which were administrated (up to 3.5 μmol kg^{-1}). Intravenous administration of SP (1.49-5.94 nmol kg^{-1}), SP^{2-11} (1.68-6.71 nmol kg^{-1}), SP^{3-11} (1.83-7.31 nmol kg^{-1}), SP^{4-11} (0.041-0.828 nmol kg^{-1}), SP^{5-11} (1.15-11.50 nmol kg^{-1}) and SP^{6-11} (1.35-10.80 nmol kg^{-1}) produced dose-dependent increases in R_L and E_L (for R_L: R^2 = 0.9594-0.9983; for E_L: R^2 = 0.8997-0.9742) (figs. 1-2). On plotting maximal (peak) responses to individual doses, the bronchoconstrictor activities of SP and SP fragments were estimated by fitting data using linear regression. ED_{700} (R_L) and ED_{250} (E_L)(Concentration required to cause 700% increase in R_L or 250% increase in E_L, respectively) were determined from the dose-response curves and used to compare the relative bronchoconstrictor activity of these peptides. Data are summerized in table 2. The order of potency of SP and SP fragments was: $SP^{8-11} < SP^{7-11} < SP^{6-11} = SP < SP^{5-11} = SP^{3-11} = SP^{2-11} << SP^{4-11}$ (figs. 1-2 and table 2).

Effects of neurokinin receptor antagonists on bronchopulmonary responses induced by SP and SP fragments

Involvement of neurokinin receptors in mediating the bronchoconstrictor activity of SP and SP fragments were determined using the potent and selective NK1 and NK2 receptor antagonists. The selective NK1 receptor antagonists, CP 96,345 or CP 99,994 (3.0 mg kg^{-1}, iv), and the selective NK2 receptor antagonist, SR 48,968 (1.0 mg kg^{-1}, iv), did not alter baseline values of R_L (before = 0.27 ± 0.03; after = 0.27 ± 0.03; n = 38, $p > 0.05$) and E_L (before = 1.75 ± 0.29; after = 1.74 ± 0.31; n = 38, $p > 0.05$). The selective NK1 receptor antagonists caused a significant rightward shift of the dose-response curves to SP and SP fragments ($p < 0.05$) (figs. 3-8). By contrast, the selective NK2 receptor antagonist had no significant effect on bronchospastic effects of SP as well as its C-terminal fragments except for SP^{4-11} ($p > 0.05$) (figs. 3-8). However, a combination of the NK1 and NK2 receptor antagonists induced significantly greater rightward shift in the SP or SP fragments dose-response curve (figs. 3-8). Results are quantitated and summarized in table 3. As in the presence of antagonists large quantities of peptides were required to get ED_{700}, ED_{300} (R_L) (dose of agonist required to cause 300% increase in R_L) was used to compare the effect of neurokinin receptor antagonists on SP- and SP fragments-induced bronchospasm. The ratios of dose of agonists required for induction of equivalent change in R_L in the absence and the presence of selective neurokinin antagonist were computed (table 3). In the presence of NK1 receptor antagonist, about 3-9 folds and in the presence of both NK1 and NK2 receptor antagonists about 38-59 folds peptide was required to get similar responses (table 3). Data for E_L, which were similar to those for R_L, are not shown.

DISCUSSION

This study confirms and extends previous observations that intravenous administration of SP or C-terminal fragments of SP induce bronchoconstriction in anesthetized guinea-pigs (23). N-terminal fragments of SP had no bronchospastic effect which confirms involvement of C-terminal of SP in inducing bronchospasm further. SP^{6-11} was as potent bronchoconstrictor as SP but C-terminal fragment of SP containing 3-5 amino acids showed no specific effects. These findings suggest that at least 6 amino acid in the C-terminal of SP is required for induction of a specific bronchospastic activity. Removal of up to 4 amino acid from N-terminal of SP increased the bronchoconstrictor activities of these peptides as SP^{2-11}, SP^{3-11}, SP^{4-11} and SP^{5-11} were more potent bronchoconstrictor than SP. However, further removal of 2 or more amino acids from C-terminal abolished broncho-constrictor activities of these peptides. The bronchospastic effect of C-terminal fragments, SP^{7-11} and SP^{8-11}, at high concentration could be explained by interaction with non-specific sites since other basic amino acid residues like tuftsin,

mastoparan and cationic protein

Table 1 Amino acid sequence of SP and SP fragments

Amino acid sequence												Abbreviation
1	2	3	4	5	6	7	8	9	10	11		
Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met	$CONH_2$	SP
								Gly	Leu	Met	$CONH_2$	SP^{9-11}
							Phe	Gly	Leu	Met	$CONH_2$	SP^{8-11}
						Phe	Phe	Gly	Leu	Met	$CONH_2$	SP^{7-11}
					Gln	Phe	Phe	Gly	Leu	Met	$CONH_2$	SP^{6-11}
				Gln	Gln	Phe	Phe	Gly	Leu	Met	$CONH_2$	SP^{5-11}
			Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met	$CONH_2$	SP^{4-11}
		Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met	$CONH_2$	SP^{3-11}
	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met	$CONH_2$	SP^{2-11}
Arg	Pro	Lys	Pro	$CONH_2$								SP^{1-4}
Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	$CONH_2$			SP^{1-9}

The amino acid sequences of SP and SP fragment are shown from their amino terminal. For SP, the first amino acid in its amino terminal is shown by number1. The carboxy terminal of SP and SP fragments which have been used in these experiment were amidated.

Table 2 The relative bronchoconstrictor activities of SP and SP fragments

Agonist	n	ED_{700} (R_L) (nmol kg^{-1})	Relative potency	ED_{250} (E_L) (nmol kg^{-1})	Relative potency
SP	5	5.81 ± 0.94	1	4.23 ± 0.51	1
SP^{2-11}	3	3.94 ± 0.63	1.48	2.42 ± 0.43	1.75
SP^{3-11}	3	3.80 ± 0.67	1.53	2.38 ± 0.52	1.77
SP^{4-11}	3	0.15 ± 0.03	38.73	0.47 ± 0.09	9
SP^{5-11}	3	3.85 ± 0.44	1.62	2.66 ± 0.61	1.59
SP^{6-11}	3	3.58 ± 0.74	1	4.05 ± 0.76	1.04
SP^{7-11}	3	> 2500	$< 2.3 \times 10^{-3}$	> 2500	$< 1.7 \times 10^{-3}$
SP^{8-11}	3	> 2500	$< 2.3 \times 10^{-3}$	> 2500	$< 1.7 \times 10^{-3}$
SP^{9-11}	3	> 5000	$< 1.2 \times 10^{-4}$	> 5000	$< 85 \times 10^{-4}$
SP^{10-11}	3	> 5000	$< 1.2 \times 10^{-4}$	> 5000	$< 85 \times 10^{-4}$
SP^{11}	3	> 5000	$< 1.2 \times 10^{-4}$	> 5000	$< 85 \times 10^{-4}$
SP^{7-8}	3	> 5000	$< 1.2 \times 10^{-4}$	> 5000	$< 85 \times 10^{-4}$
SP^{8-9}	3	> 5000	$< 1.2 \times 10^{-4}$	> 5000	$< 85 \times 10^{-4}$
SP^{9-10}	3	> 5000	$< 1.2 \times 10^{-4}$	> 5000	$< 85 \times 10^{-4}$

Values are means ± STD; n, number of experiments; ED_{700} (R_L), doses of peptides (nmol kg^{-1}) required to cause 700% increase in pulmonary flow resistance (R_L) from baseline values. ED_{250} (E_L), doses of peptides (nmol kg^{-1}) required to cause 250% increase in dynamic pulmonary elastance (E_L) from baseline values.

Table 3 Antagonism of bronchospastic effects of SP and SP fragments by selective neurokinin antagonists in anesthetized guinea-pigs

Neurokinin antagonists		Control	CP 96,345 or CP 99,994	Ratio	SR 48,968	Ratio	CP 96,345 or CP 99,994 + SR 48,968	Ratio
Tratment	n	ED300 (nmol kg^{-1})	ED300 (nmol kg^{-1})		ED300 (nmol kg^{-1})		ED300 (nmol kg^{-1})	
SP	5	2.99 ± 0.91	> 20	-	3.74 ± 0.94	1.25	> 40	-
SP^{2-11}	3	3.95 ± 0.91	17.35 ± 2.21	4.39	4.12 ± 0.89	1.04	173.25 ± 14.93	43.86
SP^{3-11}	3	2.53 ± 0.48	17.72 ± 2.00	7.00	2.58 ± 0.11	1.02	109.12 ± 15.76	43.13
SP^{4-11}	3	0.15 ± 0.03	1.39 ± 0.31	9.27	0.12 ± 0.03	0.80	5.63 ± 0.85	37.53
SP^{5-11}	3	3.86 ± 0.44	34.26 ± 5.31	8.88	4.02 ± 0.53	1.04	221.32 ± 19.43	57.34
SP^{6-11}	3	5.83 ± 0.74	16.83 ± 3.22	2.89	5.99 ± 0.82	1.03	345.73 ± 62.71	59.30

Values are means ± STD; n, number of experiments; ED_{300}, doses of peptides (nmol kg^{-1}) required to cause 300% increase in pulmonary flow resistance (R_L) from baseline values.

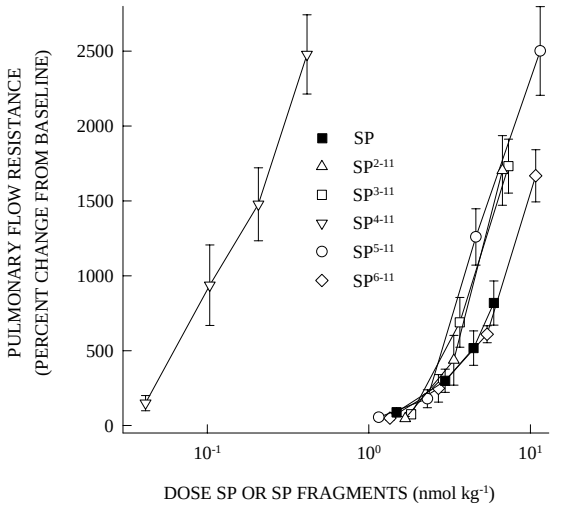

Fig. 1. Changes in pulmonary flow resistance in response to various doses of SP and SP fragments (all administrated intravenously) in anesthetized, paralyzed guinea pigs. Results, expressed as percent changes from baseline values, are mean ± SEM of 5 experiments.

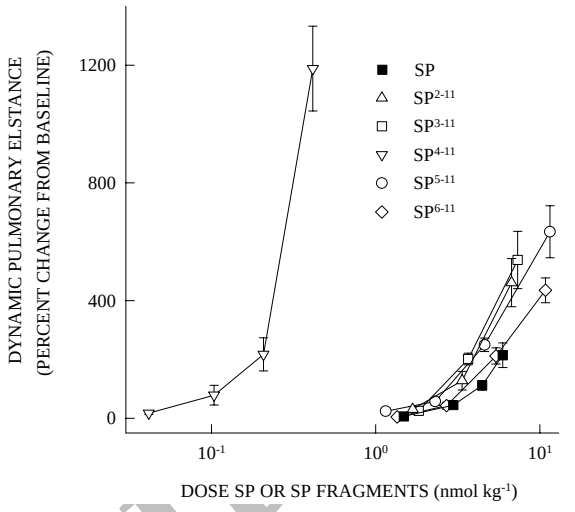

Fig. 2. Changes in dynamic pulmonary elastance (E_L) in response to various doses of SP and SP fragments (all administrated intravenously) in anesthetized, paralyzed guinea pigs. Results, expressed as percent changes from baseline values, are mean ± SEM of 5 experiments.

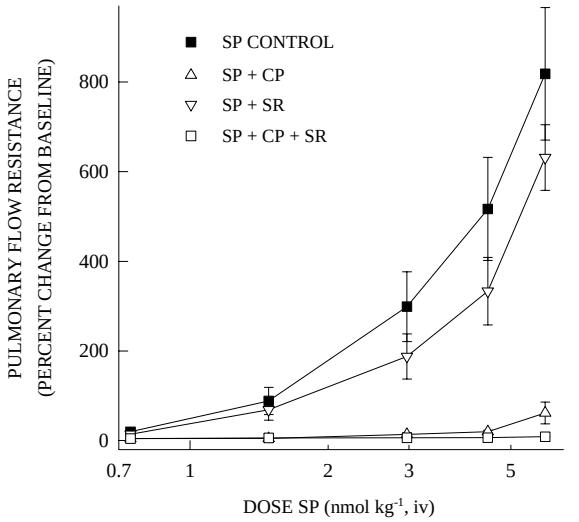

Fig. 3. The effects of iv administration of 3 mg kg^{-1} CP 96,435 (selective NK1 receptor antagonist), 1 mg kg^{-1} SR 48,968 (selective NK2 receptor antagonist) and combination of 3 mg kg^{-1} CP 96,435 and 1 mg kg^{-1} SR 48,968 on the changes in pulmonary flow resistance to various doses of SP iv. Results, expressed as percent changes from baseline values, are mean ± SEM of 5 experiments.

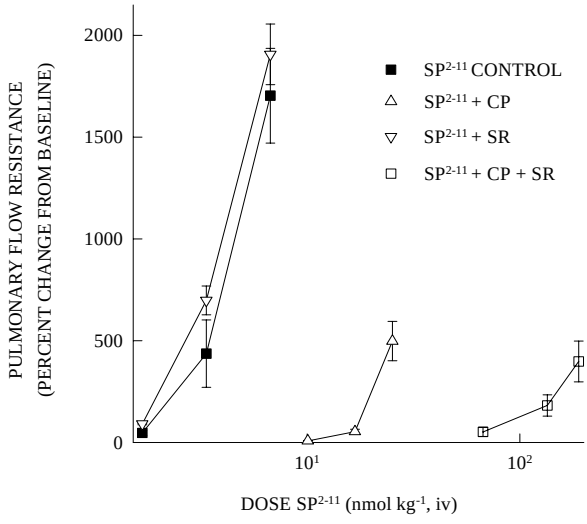

Fig. 4. The effects of iv administration of 3 mg kg^{-1} CP 99,994 (selective NK1 receptor antagonist), 1 mg kg^{-1} SR 48,968 (selective NK2 receptor antagonist) and combination of 3 mg kg^{-1} CP 99,994 and 1 mg kg^{-1} SR 48,968 on the changes in pulmonary flow resistance to various doses of SP^{2-11} iv. Results, expressed as percent changes from baseline values, are mean ± SEM of 3 experiments.

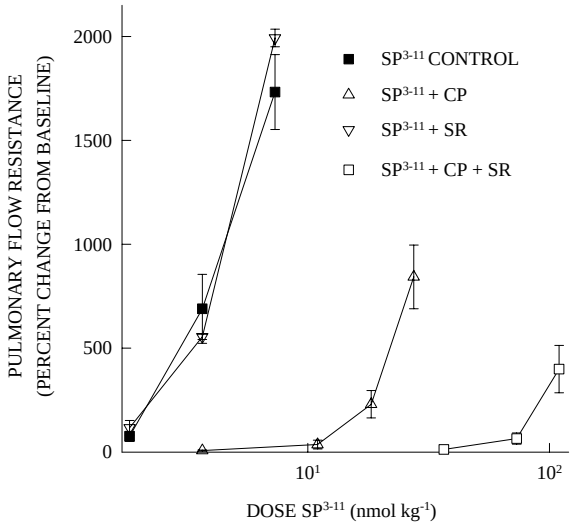

Fig. 5. The effects of iv administration of 3 mg kg^{-1} CP 99,994 (selective NK1 receptor antagonist), 1 mg kg^{-1} SR 48,968 (selective NK2 receptor antagonist) and combination of 3 mg kg^{-1} CP 99,994 and 1 mg kg^{-1} SR 48,968 on the changes in pulmonary flow resistance to various doses of SP^{3-11} iv. Results, expressed as percent changes from baseline values, are mean ± SEM of 3 experiments.

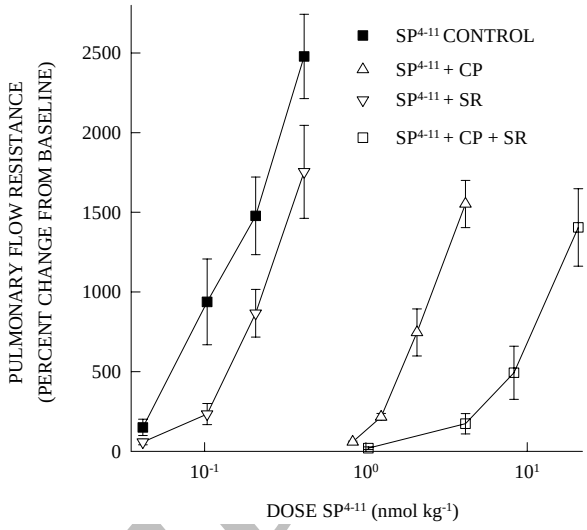

Fig. 6. The effects of iv administration of 3 mg kg^{-1} CP 96,435 (selective NK1 receptor antagonist), 1 mg kg^{-1} SR 48,968 (selective NK2 receptor antagonist) and combination of 3 mg kg^{-1} CP 96,435 and 1 mg kg^{-1} SR 48,968 on the changes in pulmonary flow resistance to various doses of SP^{4-11} iv. Results, expressed as percent changes from baseline values, are mean ± SEM of 3 experiments.

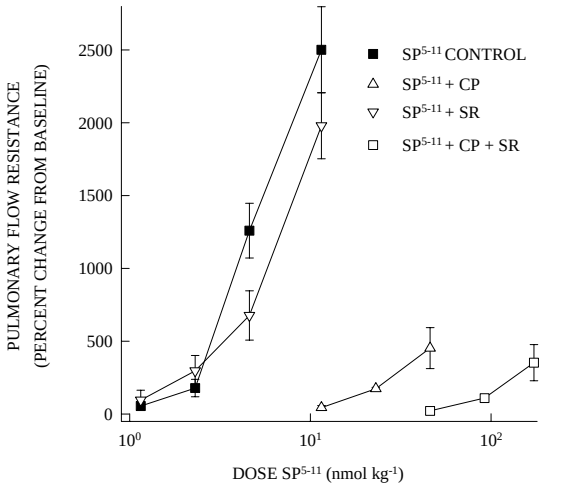

Fig. 7. The effects of iv administration of 3 mg kg^{-1} CP 96,435 (selective NK1 receptor antagonist), 1 mg kg^{-1} SR 48,968 (selective NK2 receptor antagonist) and combination of 3 mg kg^{-1} CP 96,435 and 1 mg kg^{-1} SR 48,968 on the changes in pulmonary flow resistance to various doses of SP^{5-11} iv. Results, expressed as percent changes from baseline values, are mean ± SEM of 3 experiments.

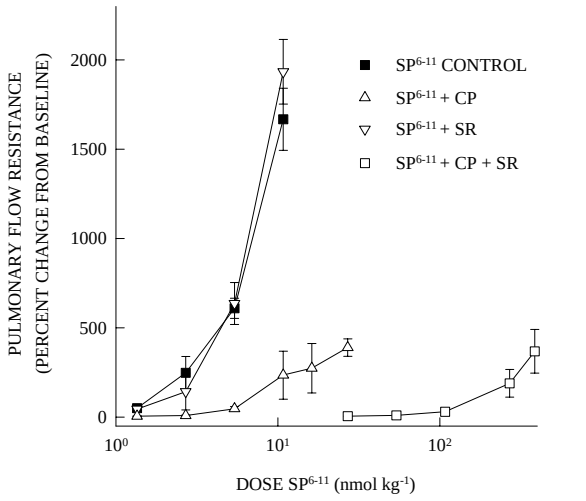

Fig. 8. The effects of iv administration of 3 mg kg^{-1} CP 96,435 (selective NK1 receptor antagonist), 1 mg kg^{-1} SR 48,968 (selective NK2 receptor antagonist) and combination of 3 mg kg^{-1} CP 96,435 and 1 mg kg^{-1} SR 48,968 on the changes in pulmonary flow resistance to various doses of SP^{6-11} iv. Results, expressed as percent changes from baseline values, are mean ± SEM of 3 experiments.

showed similar physiologic effects, possibly via direct activation of G-protein by basic peptides (28-29). The greater bronchospastic effects of SP^{2-11}, SP^{3-11}, SP^{4-11} and SP^{5-11} than SP may be due to lower susceptibility of these fragments to enzymatic degradation. SP^{4-11} was the most potent bronchoconstrictor fragment. The presence of proline at position 4 may account for this observation since proline can cause bending of the peptide leading to better fitting into the receptor. However, further addition of amino acids (lysine, proline and arginine) attenuated the effect of this peptide.

The selective NK1 receptor antagonists CP 96,345 or CP 99,994 and the selective NK2 receptor antagonist SR 48,968 did not change the baseline values of R_L and E_L indicating that their effects are not via nonspecific effects on baseline airway calibre or airway smooth muscle contractility. CP 96,345 or CP 99,994 greatly reduced responses to SP and SP fragments. By contrast, SR 48,968, at a dose that blocked responses to NKA and the selective NK2 receptor agonist [â-Ala^8]-NKA 4-10, significantly reduced responses to only SP^{4-11}. These findings suggest that SP and its C-terminal fragments induce bronchoconstriction mainly via NK1 receptors. As selective NK2 receptor antagonist reduced bronchospasm induced by SP^{4-11}, the greater bronchoconstrictor activity of SP^{4-11} may, at least in part, be explained by its effect on NK2 receptors. A combination of NK1 and NK2 receptor antagonists caused grater rightward shift of the dose-response curves to SP and SP fragments suggesting that: 1- bronchoconstriction induced by SP or SP fragments is mediated via both NK1 and NK2 receptors in guinea-pig, 2- pulmonary responses to these peptides are complex, and 3- there may be interactions between NK1 and NK2 receptors in and outside the lungs. This conclusion is supported by other findings (30-31) that were shown in guinea-pig both the NK1 and NK2 receptor must be involved in bronchospasm induced by these agonists. Also, it has been reported that capsaicin's bronchospastic effects were reduced by capsaicin-induced release of tachykinins that had stimulatory actions on NK2 receptors in sympathetic ganglia (32). This may explain ineffectiveness of selective NK2 receptor antagonist on bronchospasm induced by these peptides while in combination with selective NK1 receptor antagonists it induced significance decrease in this response.

In conclusion, SP-induce bronchoconstriction is mediated by its C-terminal sequence. Bronchospastic responses to SP and C-terminal fragments of SP in guinea-pigs are mediated mainly via NK1 receptors but, NK2 receptors are also involved in these responses. Bronchopulmonary responses to SP and its C-terminal fragments are complex and there may be interaction between NK1 and NK2 receptors in lung.

REFERENCES

1. Herpfer, I., Lieb, K. (2003) Substance p and substance p receptor antagonists in the pathogenesis and treatment of affective disorders. World J. Biol. Psychiatry. 4(2): 56-63.
2. Snijdelaar, D.G., Driksen, R., Slappendel, R., Crul B.J. (2000) Substance P. Eur. J. Pain 4: 121-135.
3. Maggio, J.E. (1985) "Kassinin" in mammals: the newest tachykinins. Peptides. 6 (Suppl 3): 237-43.
4. Maggi, C.A. (1993) Tachykinin receptors and airway pathophysiology. Eur. Respir. J. 6(5): 735-42.
5. Guard, S., Watson, S.P. (1991) Tachykinin receptor types: classification and membrane signalling mechanisms. Neurochem. Int.18: 149-165.
6. Maggi, C.A., Patacchini, R, Rovero, P., Giachetti, A. (1993) Tachykinin receptors and tachykinin receptor antagonists. J. Auton. Pharmacol. 13(1): 23-93.
7. Lundberg, J.M., Saria, A. (1987) Polypeptide-containing neurons in airway smooth muscle. Annu. Rev. Physiol. 49: 557-72.
8. Maggi, C.A. (1990) Tachykinin receptors in the airways and lung: what should be block? Pharmacol. Res. 22(5): 527-40.
9. Barnes, P.J. (1986) Asthma as an axon reflex. Lancet. 1(8475): 242-5.
10. Joos, G.F., De Swert, K.O., Schelfhout, V., Pauwels, R.A. (2003) The role of neural inflammation in

asthma and chronic obstructive pulmonary disease Ann. N. Y. Acad. Sci. 992: 218-30.
11. Bremer, A.A., Leeman, S.E., Boyd, N.D. (2000) The common C-terminal sequences of substance P and neurokinin A contact the same region of the NK-1 receptor. FEBS Lett. 486(1): 43-8.
12. Gerard, N.P., Bao, L, Xiao-Ping, H, Gerard, C. (1993) Molecular aspects of the tachykinin receptors. Regul. Pept. 43(1-2): 21-35.
13. Hasenohrl, R.U., Souza-Silva, M.A., Nikolaus, S., Tomaz, C., Brandao, M.L., Schwarting, R.K., Huston, J.P. (2000) Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. Neuropeptides. 34(5): 272-80.
14. 14. Guard, S., Watson, S.P. (1991) Tachykinin receptor types: classification and membrane signalling mechanisms. Neurochem. Int.18: 149-165.
15. Hokfelt, T., Pernow, B., Wahren, J. (2001) Substance P: a pioneer amongst neuropeptides. J. Intern. Med. 249(1): 27-40.
16. Nakanishi, S. (1991). Mammalian tachykinin receptors. Annu. Rev. Neurosci. 14: 123-136.
17. Foulen, D.M., Champion, E., Masson, P., Rodger, I.W., Jones, T.R. (1993) NK1 and NK2 receptors mediate tachykinin and resiniferatoxin-induced bronchospasm in guinea-pigs. Am. Rev. Respir. Dis. 48: 915-918.
18. Hallberg, M., Nyberg, F. (2003) Neuropeptide conversion to bioactive fragments - an important pathway in neuromodulation. Curr. Protein Pept. Sci. 4(1): 31-44.
19. Csuhai, E., Little, S.S., Hersh, L.B. (1995) Inactivation of neuropeptides. Prog. Brain Res. 104: 131-42.
20. Lilly, C.M., Drazen, J.M., Shore, S.A. (1993). Peptidase modulation of airway effects of neuropeptides. P.S.E.B.M. 203: 338-341.
21. Masata, R., Kenny, A.J. Turner, A.J. (1984). The metabolism of neuropeptides: the hydrolysis of peptides, including enkephalins, tachykinins and their analogues, by endopeptidase-24.11. Biochem. J. 223: 433-440.
22. Hall, M.E. Stewart, J.M. (1990). The substance P fragment SP(7-11) stimulates motor behaivior and nigral dopamine release. Pharmacol. Biochem. Behaivor. 41: 75-78.
23. Shore, S.A., Drazen, J.M. (1988). Airway response to SP and SP fragments in the guinea-pig. Pulm. Pharmacol. 1: 113-118.
24. Shore, S.A., Drazen, J.M. (1991) Relative bronchoconstrictor activity of neurokinin A and neurokinin A fragments in guinea-pigs. J. Appl. Physiol. 71: 452-457.
25. McLean, S., Ganong, A., Seymour, P.A., Snider, R.M., Desai, M.C., Rosen, T., Bryce, D.K., Longo, K.P., Reynolds, L.S., Robinson, G., et al. (1993) Pharmacology of CP-99,994; a nonpeptide antagonist of the tachykinin neurokinin-1 receptor. J. Pharmacol. Exp. Ther. 267(1): 472-4799.
26. Emonds-Alt, X., Vilain, P., Goulaouic, P., Proietto, V., Van Broeck, D., Advenier, C., Naline, E., Neliat, G., Le Fur, G., Breliere, J.C. (1992) A potent and selective non-peptide antagonist of the neurokinin A (NK2) receptor. Life Sci. 50(15): PL101-106.
27. Jafarian, A., Suresh, M.R., Kreutz, F.T., Biggs, D.F. (1995) Passive immunization with an anti-substance P antibody prevents substance P- and neurokinin A-induced bronchospasm in anesthetized guinea-pigs. Life Sci. 57(2): 143-153.
28. Mousli, M., Bronner, C., Bube, J.L., Tschirhart, E., Gies, J.P., Landry, Y. (1989) J. Pharmacol. Exp. Ther. 250: 329-335.
29. Mousli, M., Bueb, J.L., Bronner, C., Rouot, B., Landry, Y. G protein activation: a receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides. Trends Pharmacol. Sci. (1990) 11(9): 358-362.
30. Lundberg, J.M., Saria, A., Brodin, E., Roseli, S., Folkers, K. (1983) A substance P antagonist inhibits vagally-induced increase in vascular permability and bronchial smooth muscle contraction in guinea-pig. Proc. Natl. Acad. Sci. U.S.A. 80: 1120-1124.
31. Maggi, C.A., Giuliani, S., Ballati, L., Lecci, A., Manzini, S., Patacchini, R., Renzetti, A.R., Rovero, P., Quartara, L., Giachetti, A. (1991) J. Pharmacol. Exp. Ther. 257: 1172-1178.
32. Ballati, L., Evangelista, S., Maggi, C.A., Manzini, S. (1992) Effects of selective tachykinin receptor antagonists on capsaicin- and tachykinin-induced bronchospasm in anaesthetized guinea-pigs. Eur. J. Pharmacol. 214(2-3): 215-21.