J. Pharm. Pharmacol. 1993; 45: 1037–1041
Received December 8, 1992

SR 48968, A Novel Non-peptide Tachykinin NK-2-Receptor Antagonist, Selectively Inhibits the Non-Cholinergically Mediated Neurogenic Contraction of Guinea-pig Isolated Bronchial Muscle

YUICHIRO KAMIKAWA AND YASUO SHIMO

Department of Pharmacology, Dokkyo University School of Medicine, Mibu, Tochigi 321-02, Japan

Abstract—We have examined the actions of SR 48968 ((*S*)-*N*-methyl-*N*-[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide), a novel non-peptide tachykinin NK-2-receptor antagonist, on the response evoked by electrical field stimulation or by acetylcholine and neurokinin A on guinea-pig isolated airway smooth muscle. Electrical field stimulation (1–32 Hz, 0·3 ms, 30 V for 20 s) evoked a biphasic response in a frequency-dependent manner, consisting of a cholinergically-mediated fast contraction followed by a non-adrenergically-mediated relaxation in tracheal muscle and by a non-cholinergically-mediated slow contraction in bronchial muscle. SR 48968 (0·01–1 μM) caused a concentration-dependent inhibition of non-cholinergically mediated contraction of bronchial muscle, without significant influence on cholinergically and non-adrenergically-mediated responses. Submaximal contractions of tracheal and bronchial muscles evoked by exogenous neurokinin A (10–300 nM) were markedly inhibited by SR 48968 (0·1–1 μM), but those by exogenous acetylcholine (1–3 μM) were slightly inhibited by the antagonist. The results indicate that in guinea-pig isolated bronchial muscle, SR 48968 selectively inhibited non-cholinergically mediated neurogenic contraction via antagonism of NK-2 receptors.

Mammalian airway smooth muscle is controlled by cholinergic, adrenergic, non-adrenergic and non-cholinergic nerves but their distributions are different in proximal and distal airway and between species (Richardson 1979; Kamikawa 1993). In the guinea-pig airway, excitatory cholinergic and inhibitory non-adrenergic nerves are predominant in tracheal muscle, whereas excitatory cholinergic and non-cholinergic nerves are predominant in bronchial muscle (Coburn & Tomita 1973; Kamikawa & Shimo 1976, 1989; Andersson & Grundström 1983, 1987). There is much evidence supporting the possibility that vasoactive intestinal peptide or nitric oxide act as neurotransmitters of inhibitory non-adrenergic nerves while substance P or neurokinin A function in excitatory non-cholinergic nerves (Matsuzaki et al 1980; Lundberg et al 1983; Håkanson et al 1983; Leander et al 1984; Kamikawa & Shimo 1989; Li & Rand 1991). Tachykinins can activate different types of tachykinin receptors; NK-1 receptors for substance P, NK-2 receptors for neurokinin A and NK-3 receptors for neurokinin B and in the guinea-pig bronchi, NK-2 receptors are predominant subtypes for producing contraction (Frossard & Advenier 1991). None of the tachykinin antagonists previously examined possesses notable receptor selectivity and most have partial agonist activities (Håkanson et al 1983; Leander et al 1984; Kamikawa & Shimo 1989). Recently, we have reported that catecholamines, purines, opioid agonists and antitussive drugs can inhibit excitatory non-cholinergic neurotransmission of the guinea-pig bronchi via the prejunctional mechanisms (Kamikawa & Shimo 1989, 1990a,b, 1991).

SR 48968, (*S*)-*N*-methyl-*N*-[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide, is a newly developed non-peptide tachykinin antagonist with highly selective and competitive antagonism for NK-2 receptors (Advenier et al 1992; Emonds-Alt et al 1992). In the present study we have investigated the effect of SR 48968 on electrically-induced neurogenic responses of the guinea-pig airways.

Correspondence: Y. Kamikawa, Department of Pharmacology, Dokkyo University School of Medicine, Mibu, Tochigi 321-02, Japan.

Materials and Methods

Male guinea-pigs, 300–600 g, were anaesthetized with isoflurane, bled from the cervical artery, and the tracheobronchial tree was excised. After cutting longitudinally at the cartilaginous portion, a transverse strip, 2–3 mm wide, was excised from the cervical trachea, thoracic trachea, left main bronchus and right hilus bronchus. Each strip was immersed in a 10-mL organ bath filled with modified Krebs bicarbonate solution of the following composition (mM): NaCl 120, KCl 4·7, $CaCl_2$ 2·5, $MgCl_2$ 1·2, $NaHCO_3$ 25, KH_2PO_4 1·2, disodium edetate 0·03, ascorbic acid 0·12 and glucose 14 (pH 7·4). The Krebs solution always contained 20 μM choline chloride and was bubbled with 5% carbon dioxide in oxygen, and maintained at 37°C.

The preparation was suspended under an initial tension of 0·5 g and 60 min was allowed to elapse before experiments were started. Responses of airway smooth muscles were recorded by means of an isometric transducer (Nihon Kohden TB-651T) and a Nihon Kohden polygraph (RJG-4124). After the 60-min equilibration period, the preparation was contracted maximally with a single concentration of carbachol 10 μM. Following wash-out the preparation was allowed to equilibrate for a further 30 min. Electrical field stimulation was with rectangular pulses of 1–32 Hz frequency, 0·3 ms duration and 30 V for 20 s through bipolar platinum electrodes which were 10 mm apart and connected to a Nihon Kohden stimulator (SEN-1101). When the strip

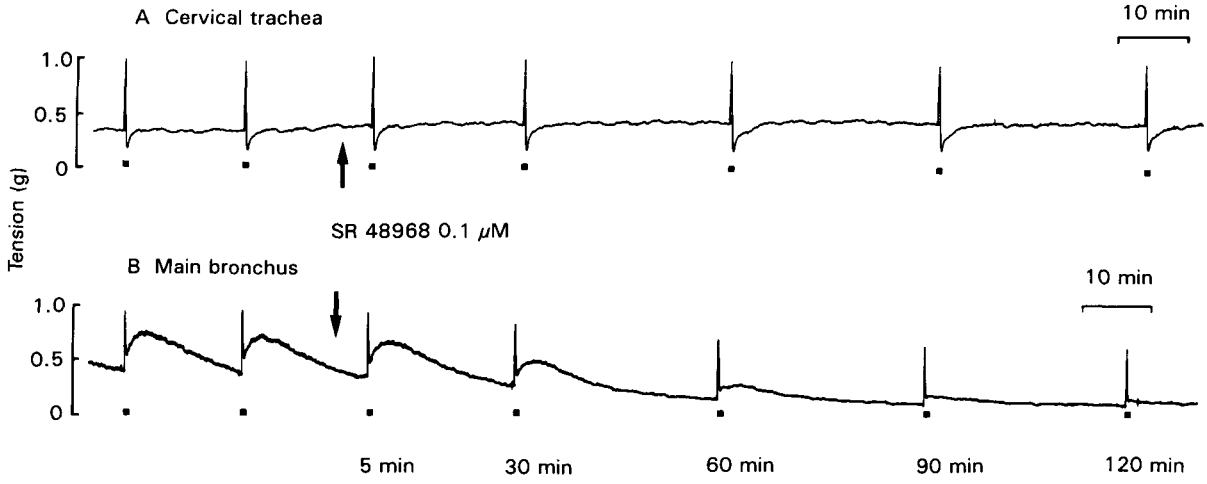

FIG. 1. Representative trace of biphasic responses to electrical field stimulation (10 Hz, 0·3 ms, 30 V for 20 s at ■ every 20 or 30 min) of guinea-pig isolated cervical trachea (A) and main bronchus (B) before and after administration of SR 48968 (0·1 μM at arrow).

was electrically stimulated, a biphasic response was obtained at every stimulus frequency. In cervical and thoracic tracheas, the response was composed of a cholinergically-mediated fast contraction followed by a non-adrenergically-mediated relaxation, but in main and hilus bronchi, a cholinergically-mediated fast contraction was followed by a non-cholinergically-mediated slow contraction (Coburn & Tomita 1973; Kamikawa & Shimo 1976, 1989; Andersson & Grundström 1983). To avoid the decrease of responsiveness to repetitive stimuli of the same preparation, all experiments were performed in parallel with only one frequency- or concentration-response curve for each preparation. Data are expressed as the mean ± s.e.m. Each experimental group consisted of 6–14 preparations taken from different animals. Student's *t*-test for unpaired observations was used for statistical evaluation of the data. $P < 0.05$ was considered significant.

Drugs used were carbachol (Sigma, St Louis, MO), acetylcholine chloride (Dai-ichi, Tokyo), neurokinin A (Peptide Institute, Osaka), isoflurane (Abbott, North Chicago, IL) and SR 48968 (Sanofi Recherche, Montpellier, France). To prepare the drug solutions, all drugs were dissolved in and diluted with saline. The molar concentrations of drugs in this paper refer to the final bath concentrations.

Results

Influence of incubation time

In preliminary experiments, the time course of the effect of SR 48968 on electrically-induced neurogenic responses was examined in the isolated cervical trachea and main bronchus. When the preparations were electrically stimulated at 10 Hz frequency, 0·3 ms duration and 30 V for 20 s every 20 to 30 min, reproducible biphasic responses were obtained (Fig. 1). A biphasic response of cervical trachea consisting of an initial fast contraction followed by a relaxation was unaffected by the addition of SR 48968 (0·1 μM) throughout 2–3 h experimental periods (Fig. 1A). On the other hand, SR 48968 (0·1 μM) selectively and time-dependently inhibited electrically-induced slow contraction, but not initial fast contraction, of main bronchus, and abolished it at 120 min after the drug addition (Fig. 1B). Hence, in the following experiments the tissues were pre-incubated with SR 48968 for 120 min.

Effect on neurogenic responses. In cervical trachea, initial contractions and following relaxations to electrical field stimulations (1–32 Hz) were slightly inhibited by the pre-treatment with SR 48968 (0·1–1 μM) for 120 min, but the differences were not statistically significant. Neurogenic biphasic responses of thoracic trachea were also unaffected by SR 48968 pretreatment (0·1–1 μM). In main bronchus, electrically-induced fast contractions were significantly inhibited by pretreatment with 0·1 μM SR 48968, but the inhibition was not observed with 1 μM. However, non-cholinergically-mediated slow contractions to high-frequency stimulations (8–32 Hz) were significantly inhibited by 0·01 μM SR 48968 pre-treatment, and 0·1 μM SR 48968 abolished the response throughout all frequency ranges examined. In hilus bronchus, cholinergically-mediated fast contractions to electrical stimulation were not affected by SR 48968 pretreatment (0·001–1 μM). Non-cholinergically-mediated slow contractions were not significantly modified with 0·01 μM SR 48968, but were selectively abolished by 0·1 or 1 μM SR 48968 pre-treatment (Fig. 2B). After wash-out, the neurogenic contraction was not fully restored to the original level even after 2 h.

Effect on contractions to acetylcholine or neurokinin A. Exogenously applied acetylcholine (1–30 μM) or neurokinin A (10–300 nM) produced a concentration-dependent contraction of all tracheal and bronchial strips examined. The height of these contractions were comparable with those of electrically-induced fast or slow contractions, respectively. Submaximal contractions to neurokinin A (10–300 nM) were markedly inhibited or abolished by pre-treatment with SR 48968 (0·1–1 μM) for 120 min in all airway preparations (Figs 3, 4). On the other hand, submaximal contractions to lower concentrations of acetylcholine (1–3 μM) were slightly but significantly inhibited by pre-treatment with SR 48968 (0·1–1 μM) in tracheal and bronchial strips, except hilus bronchus (Figs 3, 4). However, contractions to higher concentrations

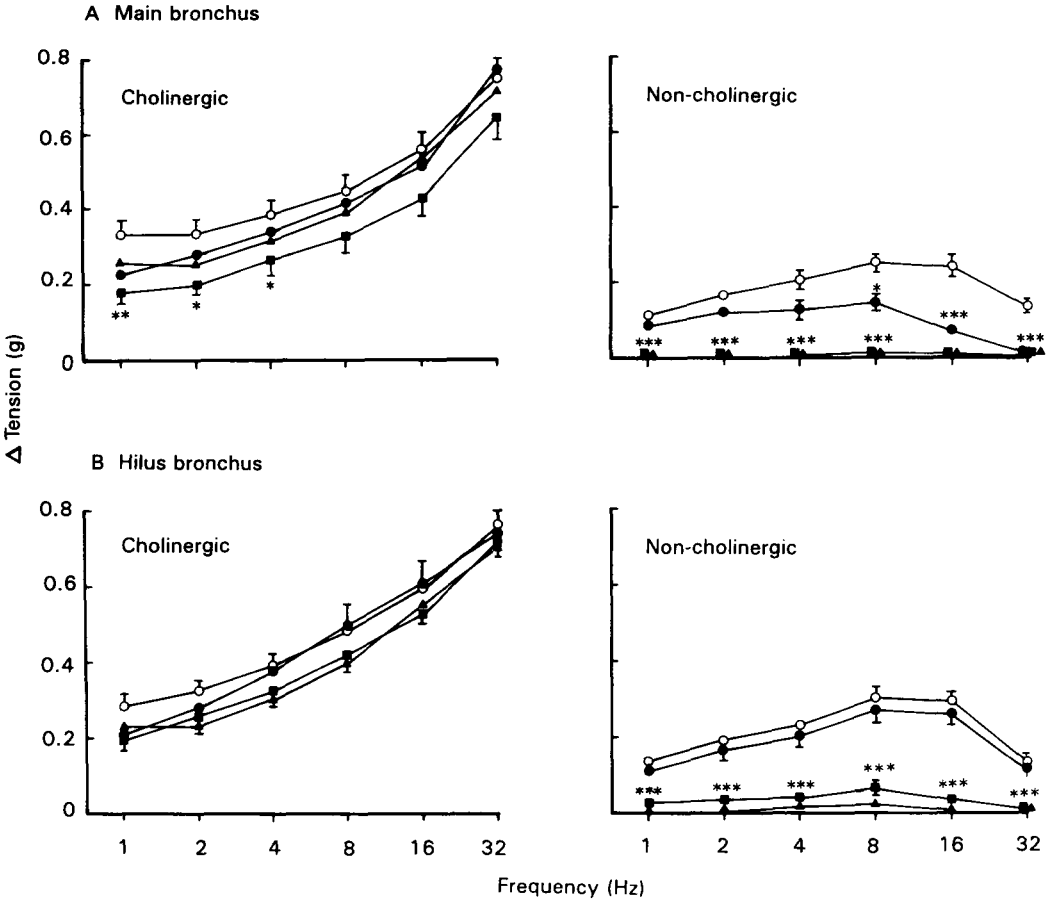

FIG. 2. Frequency-response relationship for biphasic contraction to electrical field stimulation (1-32 Hz, 0·3 ms, 30 V for 20 s) of guinea-pig isolated main bronchus (A) and hilus bronchus (B) in the absence (○, n = 14) or presence of 0·01 μM (●, n = 7), 0·1 μM (■, n = 8) and 1 μM (▲, n = 10) of SR 48968 for 2 h. Left panels, cholinergically-mediated fast contraction: right panels, non-cholinergically-mediated slow contraction. Ordinates, tension development from resting tone. Each point represents mean ± s.e.m. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

of acetylcholine (10–30 μM) were not modified with SR 48968.

Discussion

SR 48968 is the first non-peptide antagonist for NK-2 tachykinin receptors. The original reports by Emonds-Alt et al (1992) and Advenier et al (1992) showed that SR 48968 selectively and competitively antagonized the NK-2 receptor-mediated contractions of human and guinea-pig bronchi with pA_2 values of 9·4 and 10·5, respectively. Previous studies showed that neurokinin A was more potent than substance P or neurokinin B in contracting the guinea-pig bronchi and non-cholinergic bronchoconstriction was reduced by several peptide-analogue antagonists (Maggi et al 1991; Frossard & Advenier 1991). These findings indicated that both NK-1 and NK-2 receptors mediate the non-cholinergic contraction in guinea-pig bronchi but the relative contribution of NK-2 receptors is greater than that of NK-1 receptors. In the present experiments, low concentrations of SR 48968 selectively and potently inhibited the non-cholinergically-mediated slow contractions of the guinea-pig bronchi without inhibition of the cholinergically and non-adrenergically-mediated neurogenic responses. Since the same concentrations of SR 48968 also inhibited submaximal contractions of bronchial muscles to exogenously applied neurokinin A, the inhibitory action of SR 48968 on neurogenic contraction seems to be mediated by the antagonism of NK-2 receptors located in bronchial smooth muscles. The inhibitory action of SR 48968 was slightly different between main and hilus bronchi. In main bronchus, 0·01 μM SR 48968 significantly inhibited the non-cholinergically-mediated slow contractions evoked by high-frequency stimulations (8–32 Hz), but not by low-frequency stimulations (1–4 Hz). The same concentration of SR 48968 did not modify the response of hilus bronchus throughout every stimulus frequency; 0·1 μM SR 48968 abolished the electrically-induced slow contractions of main bronchus, but in hilus bronchus, 1 μM SR 48968 was required to abolish the response. The cause of these regional differences in the inhibitory action of SR 48968 is not clear, but differences in distributions of tachykinin-containing nerve fibres or tachykinin receptors might be involved (Ghatei et al 1982; Håkanson et al 1983; Andersson & Grundström 1983, 1987; Lundberg et al 1984; Castairs & Barnes 1986; Renzetti et al 1992; Kummer et al 1992). The present results provide further evidence supporting the theory that tachykinins play a role as excitatory neurotransmitters in the airway and that NK-2 receptors mediate a major part of the non-cholinergic

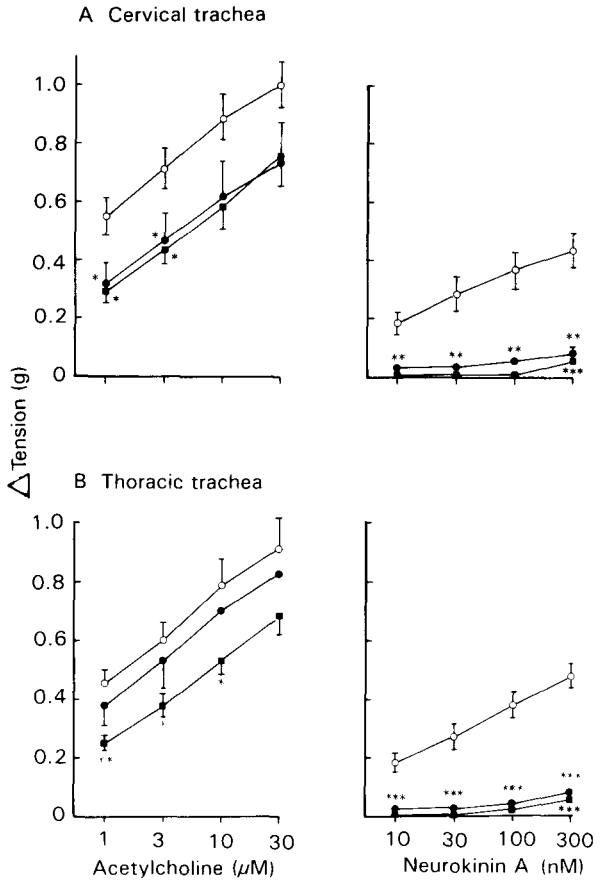

FIG. 3. Cumulative log concentration-response curves for submaximal contractions to acetylcholine and neurokinin A of guinea-pig isolated cervical trachea (A) and thoracic trachea (B) in the absence (○, n = 11) or presence of 0·1 μM (●, n = 8) and 1 μM (■, n = 7) of SR 48968 for 2 h. The ordinates show the developed tension by acetylcholine or neurokinin A. Each point represents mean ± s.e.m. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

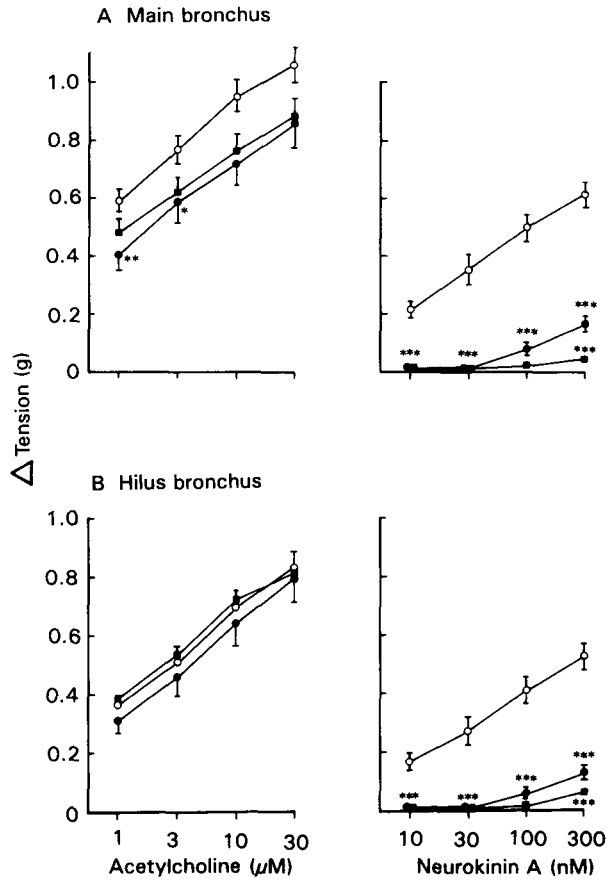

FIG. 4. Cumulative log concentration-response curves for submaximal contractions to acetylcholine and neurokinin A, of guinea-pig isolated main bronchus (A) and hilus bronchus (B) in the absence (○, n = 14) or presence of 0·1 μM (●, n = 8) and 1 μM (■, n = 10) of SR 48968 for 2 h. The ordinates show the developed tension by acetylcholine or neurokinin A. Each point represents mean ± s.e.m. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

excitatory transmission (Lundberg et al 1983; Leander et al 1984; Maggi et al 1991; Renzetti et al 1992). The inhibitory potency of SR 48968 on non-cholinergic responses observed in this study was greater than that of other NK-2 receptor antagonists (Maggi et al 1991), and the inhibitory effect of SR 48968 was slow (2 h) to reach equilibrium. A similar slow pharmacological equilibrium was also observed in reports by Emonds-Alt et al (1992) and Advenier et al (1992). Although the exact mechanism for these slow actions is not yet clear, an interaction between NK-2 receptors and non-peptide antagonists may occur with a very low rate constant for association. Recently, Martin et al (1992) also reported that low concentrations (10^{-11}–10^{-8} M) of SR 48968 selectively inhibited the electrically-induced, non-cholinergically-mediated contractions of the guinea-pig isolated main bronchus. Although they did not examine the frequency-response curve or regional difference of the inhibitory action, a maximal inhibition (83%) of 10^{-8} M SR 48968 against non-cholinergic contractions evoked by electrical stimulation at 16 Hz, was comparable with that (77%) observed in the present experiment (Fig. 2A).

SR 48968 did not significantly modify the cholinergically-mediated fast contractions of tracheal and bronchial muscles, except in the main bronchus where the contractions evoked by low-stimulus frequencies were significantly inhibited by pre-treatment with 0·1 μM, but not with 1 μM, SR 48968. In contrast, submaximal contractions to low concentrations of acetylcholine (1–3 μM) were slightly but significantly inhibited by SR 48968 (0·1–1 μM) in cervical and thoracic tracheas and main bronchus, but not in hilus bronchus. The cause of different influences of SR 48968 on neurogenic and acetylcholine-induced contractions is not clear. As appeared from large values of s.e.m. in concentration-response curves for acetylcholine (Figs 3, 4), the height of contractions to exogenous acetylcholine varied from preparation to preparation, whereas that of neurogenic contractions was relatively consistent (Fig. 2). The variation of responsiveness to exogenous acetylcholine, but not to neuronal stimuli, may be due to uneven distributions of junctional and extrajunctional muscarinic receptors or of cholinesterase activity (Mak & Barnes 1990; Adler et al 1991; Haddad et al 1991). However, nonspecific spasmolytic effects of SR 48968 might be negligible, because SR 48968 modified neither basal tone nor contractions to high concentrations of acetylcholine in any airway regions. Advenier et al (1992) also reported that 10^{-5} M SR 48968 did not shift the

concentration-response curves for acetylcholine, KCl, histamine and prostaglandin $F_{2\alpha}$.

In conclusion, a novel non-peptide tachykinin antagonist, SR 48968, selectively inhibited non-cholinergically mediated and neurokinin-A-induced contractions of the guinea-pig airways via antagonism to NK-2 receptors in airway smooth muscle.

Acknowledgements

We are grateful to Sanofi Recherche, Montpellier, France for a gift of SR 48968. This study was supported by the Human Science Foundation, Tokyo.

References

Adler, M., Reutter, S. A., Moore, D. H., Filbert, M. G. (1991) Regulation of acetylcholine hydrolysis in canine tracheal smooth muscle. Eur. J. Pharmacol. 205: 73–79

Advenier, C., Roussi, N., Nguyen, Q. T., Emonds-Alt, X., Breliere, J.-C., Neliat, G., Naline, E., Regoli, D. (1992) Neurokinin A (NK_2) receptor revisited with SR 48968, a potent non-peptide antagonist. Biochem. Biophys. Res. Commun. 184: 1418–1424

Andersson, R. G. G., Grundström, N. (1983) The excitatory non-cholinergic, non-adrenergic nervous system of the guinea-pig airways. Eur. J. Resp. Dis. 64 (Suppl. 131): 141–157

Andersson, R. G. G., Grundström, N. (1987) Innervation of airway smooth muscle. Efferent mechanisms. Pharmacol. Ther. 32: 107–130

Castairs, J. R., Barnes, P. J. (1986) Autoradiographic mapping of substance P receptors in lung. Eur. J. Pharmacol. 127: 295–296

Coburn, R. F., Tomita, T. (1973) Evidence for nonadrenergic inhibitory nerves in the guinea pig trachealis muscle. Am. J. Physiol. 244: 1072–1080

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 (NK_2) receptor. Life Sci. 50: PL-101-PL-106

Frossard, N., Advenier, C. (1991) Tachykinin receptors and the airways. Life Sci. 49: 1941–1953

Ghatei, M. A., Sheppard, M. N., O'Shaughnessy, D. J., Adrian, T. E., McGregor, G. P., Polak, J. M., Bloom, S. R. (1982) Regulatory peptides in the mammalian respiratory tract. Endocrinology 111: 1248–1254

Haddad, E.-B., Landry, Y., Gies, J.-P. (1991) Muscarinic receptor subtypes in guinea pig airways. Am. J. Physiol. 261: L327–L333

Håkanson, R., Sundler, F., Moghimzadeh, E., Leander, S. (1983) Peptide-containing nerve fibres in the airways: distribution and functional implications. Eur. J. Resp. Dis. 64 (Suppl. 131): 115–140

Kamikawa, Y. (1993) Neurogenic control of airway smooth muscle function. J. Pharmacol. Toxicol. Methods In press

Kamikawa, Y., Shimo, Y. (1976) Pharmacological differences of non-adrenergic inhibitory response and of ATP-induced relaxation in guinea-pig tracheal strip-chains. J. Pharm. Pharmacol. 28: 854–855

Kamikawa, Y., Shimo, Y. (1989) Adenosine selectively inhibits noncholinergic transmission in guinea pig bronchi. J. Appl. Physiol. 66: 2084–2091

Kamikawa, Y., Shimo, Y. (1990a) Inhibitory effects of catecholamines on cholinergically and non-cholinergically mediated contractions of guinea-pig isolated bronchial muscle. J. Pharm. Pharmacol. 42: 131–134

Kamikawa, Y., Shimo, Y. (1990b) Morphine and opioid peptides selectively inhibit the non-cholinergically mediated neurogenic contraction of guinea-pig isolated bronchial muscle. J. Pharm. Pharmacol. 42: 214–216

Kamikawa, Y., Shimo, Y. (1991) Inhibitory effects of non-narcotic antitussive drugs on cholinergically and non-cholinergically mediated neurogenic contractions of guinea-pig isolated bronchial muscle. J. Pharm. Pharmacol. 43: 242–246

Kummer, W., Fischer, A., Kurkowski, R., Heym, C. (1992) The sensory and sympathetic innervation of guinea-pig lung and trachea as studied by retrograde neuronal tracing and double-labelling immunohistochemistry. Neuroscience 49: 715–737

Leander, S., Grundström, N., Andersson, R. G. G., Håkanson, R. (1984) Neuronally mediated non-cholinergic contraction of guinea-pig bronchial muscle is inhibited by a substance P antagonist. Agents Actions 14: 315–318

Li, G. G., Rand, M. J. (1991) Evidence that part of the NANC relaxant response of guinea-pig trachea to electrical field stimulation is mediated by nitric oxide. Br. J. Pharmacol. 102: 91–94

Lundberg, J. M., Saria, A., Brodin, E., Rosell, S., Folkers, K. (1983) A substance P antagonist inhibits vagally induced increase in vascular permeability and bronchial smooth muscle contraction in the guinea pigs. Proc. Natl. Acad. Sci. USA. 80: 1120–1124

Lundberg, J. M., Hökfelt, T., Martling, C.-R., Saria, A., Cuello, C. (1984) Substance P-immunoreactive nerves in the lower respiratory tract of various mammalians including man. Cell Tissue Res. 235: 251–161.

Maggi, C. A., Patacchini, R., Rovero, P., Scanticioli, P. (1991) Tachykinin receptors and noncholinergic bronchoconstriction in the guinea-pig isolated bronchi. Am. Rev. Resp. Dis. 144: 363–367

Mak, J. C. W., Barnes, P. J. (1990) Autoradiographic visualization of muscarinic receptor subtypes in human and guinea pig lung. Am. Rev. Resp. Dis. 141: 1559–1568

Martin, C. A. E., Naline, E., Emonds-Alt, X., Advenier, C. (1992) Influence of (±)-CP-96345 and SR 48968 on electrical field stimulation of the isolated guinea-pig main bronchus. Eur. J. Pharmacol. 224: 137–143

Matsuzaki, Y., Hamasaki, Y., Said, S. I. (1980) Vasoactive intestinal peptide: a possible transmitter of non-adrenergic relaxation of guinea pig airways. Science 210: 1252–1253

Renzetti, L. M., Shenvi, A., Buckner, C. K. (1992) Nonadrenergic, noncholinergic contractile responses of the guinea pig hilar bronchus involve the preferential activation of tachykinin neurokinin$_2$ receptors. J. Pharmacol. Exp. Ther. 262: 957–963

Richardson, J. B. (1979) Nerve supply to the lungs. Am. Rev. Resp. Dis. 119: 785–802