British Journal of Pharmacology (1995) 115, 1215-1220

Endothelium-dependent contraction in intrapulmonary arteries: mediation by endothelial NK_1 receptors and TXA_2

[1]Hiroaki Shirahase, Mamoru Kanda, Kazuyoshi Kurahashi, Shohei Nakamura, Hachiro Usui & *Yoshiharu Shimizu

Pharmacology Division, Radioisotope Research Center, Kyoto University, Kyoto 606 and *Department of Community Health, School of Medicine, Tokai University, Kanagawa 259-11, Japan

1 We have examined whether three natural tachykinins, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) induce an endothelium-dependent contraction (EDC) in the rabbit isolated intrapulmonary artery.

2 Removal of the endothelium almost abolished the contraction induced by SP (10^{-8} M) while it did not attenuate the contraction induced by SP (10^{-7} M), NKA (10^{-9}–10^{-7} M) or NKB (10^{-8} and 10^{-7} M).

3 The EDC induced by SP (10^{-8} M) was abolished by NK_1 antagonists (FK-888, CP-96345, CP-99994 and SR-140333) but not by an NK_2 antagonist (SR-48968).

4 The EDC induced by SP was attenuated by cyclo-oxygenase inhibitors (aspirin and indomethacin), thromboxane A_2 (TXA_2) synthetase inhibitors (OKY-046, KY-234 and KY-063) and a TXA_2 antagonist (S-1452).

5 The rank order of potency causing endothelium-independent contraction (EIC) was NKA > NKB > SP. The EIC induced by SP (10^{-7} M) was attenuated by an NK_2 antagonist but not by NK_1 antagonists, cyclo-oxygenase inhibitors, TXA_2 synthetase inhibitors or a TXA_2 antagonist.

6 In conclusion, SP at 10^{-8} M induces EDC via endothelial NK_1 receptors and TXA_2 production, and SP at 10^{-7} M induces EIC via NK_2 receptors in the rabbit intrapulmonary artery.

Keywords: Substance P; intrapulmonary artery; endothelium-dependent contraction (EDC); NK_1 receptor; thromboxane A_2 (TXA_2); neurokinin A; neurokinin B; NK_2 receptor

Introduction

Arterial endothelial cells modulate vascular tone through production of endothelium-derived relaxing or contracting factors (EDRF or EDCF). In most of the peripheral arteries from various species, endothelium-dependent relaxation (EDR) has been predominantly observed and considered to be more important than endothelium-dependent contraction (EDC). In canine cerebral arteries, various vasoactive substances including acetylcholine (ACh), Ca^{2+} ionophore and adenine nucleotides cause EDC while they evoke EDR in peripheral arteries (Usui *et al.*, 1983; Shirahase *et al.*, 1987; 1988a,b). Vasoactive peptides such as angiotensin II, endothelin-1 and somatostatin also induce EDC in cerebral arteries (Manabe *et al.*, 1989; Shirahase *et al.*, 1991; 1993). Thus, we have suggested that EDC plays an important role in the regulation of vascular tone in cerebral arteries (Kurahashi *et al.*, 1994). The rabbit pulmonary artery also responds to acetylcholine with EDC via production of TXA_2 (Altiere *et al.*, 1986). In the pulmonary circulation, neuropeptides such as tachykinins have been considered to play an important role as physiological modulators of vascular tone since the perivascular nerves containing neuropeptides have been identified immunohistochemically (Allen *et al.*, 1989). However, it has not been reported whether the neuropeptides cause EDC in pulmonary arteries. In the present study, we found that substance P (SP), a natural tachykinin causes EDC in isolated intrapulmonary arteries of the rabbit and characterized the SP-induced EDC pharmacologically.

Methods

Male Japanese white rabbits (2–3 kg) (Oriental Bio Service, Kyoto, Japan) were fed regular chow (CR-3, Clea Japan, Osaka, Japan) and allowed access to tap water *ad libitum*. Animals were anaesthetized with sodium pentobarbitone (25 mg kg^{-1}, i.v.) and were exsanguinated from the common carotid arteries. The thoracic cavity was opened and the lungs were excised and placed in aerated Krebs-Henseleit solution. The composition of the solution was as follows (mM): NaCl 120, KCl 4.7, $MgSo_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 10. The peripheral portion of the intrapulmonary arteries (diameter 0.3–1.0 mm) was isolated from the lungs and cleaned of lung parenchymal, fat and connective tissue carefully. The arteries were cut helically and the strips were fixed vertically between hooks in a 10 ml organ bath containing a nutrient solution maintained at 37 ± 0.5°C and bubbled with a mixture of 95% O_2 and 5% CO_2. The pH of the solution was 7.4. The end of each strip was attached to the lever of a force-displacement transducer (NEC San-Ei Instrument Co. Ltd., Tokyo, Japan) connected to an ink-writing oscillograph (NEC San-Ei Instrument Co. Ltd.) and isometric changes in tension were recorded. The applied tension was adjusted to 1.5 g. Each strip was allowed to equilibrate for 1 h, during which the nutrient solution was changed every 10 min and the applied tension was re-adjusted. In several experiments, the intact and endothelium-removed strips were prepared from the same artery. The functional integrity of the endothelium in the intact preparations was checked with ACh, which causes EDR in the presence of active tone (Altiere *et al.*, 1986). The endothelium was removed by intimal rubbing. The rubbed preparations showed no ACh-induced relaxations. The elimination of endothelium was verified morphologically by scanning electron microscopy as described previously (Shirahase *et al.*, 1987). We also verified that the endothelium-independent contraction induced by 9,11-epithio-11,12-methano-thromboxane A_2 (STA_2) was not affected by removal of the endothelium. Various concentrations of SP, neurokinin A (NKA) and neurokinin B (NKB) were added non-cumulatively. Various concentrations of receptor antagonists or en-

[1] Author for correspondence.

zyme inhibitors were applied 20 min before the administration of SP at 10^{-8} or 10^{-7} M.

SP, NKA, NKB and [Sar[1],Ala[8]]-angiotensin II were purchased from Peptide Institute, Osaka, Japan. A-23187, histamine, atropine, aspirin, indomethacin, phentolamine and diphenhydramine were purchased from Sigma, St Louis, MO. STA_2 (Ono Pharmaceutical Co. Ltd., Osaka, Japan), sodium (E)-3[*p*-(1H-imidazol-1-ylmethyl)phenyl]-2-propenoate (OKY-046) (Ono Pharmaceutical Co. Ltd., Osaka, Japan), (+)-(5Z)-7-[3-endo-[(phenyl-sulfonyl)amino]bicyclo[2.2.1]hept-2-exo-yl]heptenoic acid (S-1452) (Shionogi & Co., Osaka, Japan), N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-prolyl]-N-methyl-N-phenylmethyl-3-(2-naphthyl)-L-alaninamide (FK-888) (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan), (2s,3s)-cis-2(diphenylmethyl)-N-[(2-methoxyphenyl)-methyl]-1-azabicyclo [2,2,2]octan-3-amine (CP-96345) (Pfizer Inc., Groton, CT), (+)-(2s, 3s)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99994) (Pfizer Inc.), (S)-1-{2-[3-(3,4,-dichlorophenyl)-1-(3-isopropoxyphenyl-acetyl)piperidin-3-yl]-ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]octane chloride (SR-140333) (Sanofi Recherche, Cedex, France), (S)-N-methyl-N-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl]benzamide (SR-48968) (Sanofi Recherche), 1-[3-(4-benzhydryl-1-piperazinyl) propyl]-3-(1H-imidazol-1-ylmethyl)-1H-indole-6-carboxylic acid trihydrochloride (KY-234) (Kyoto Pharmaceutical Industries, Kyoto, Japan), 5-hexyloxy-3-(1-imidazolylethyl)indoline-1-propanoic acid (KY-063) (Kyoto Pharmaceutical Industries) and 1-[{5′-(3″-methoxy-4″-hydroxyphenyl)-2′,4′-pentadienoyl} amino-ethyl]-4-diphenylmethoxypiperidine (TMK-777) (Terumo, Tokyo, Japan) were kindly provided by the indicated manufacturer.

Data were expressed as the mean ± s.e.mean. The response to SP in the presence of various concentrations of receptor antagonists or enzyme inhibitors was expressed as relative values compared to the corresponding response in the absence of drugs. The statistical significance was analysed by Student's *t* test for paired data. A *P* value less than 0.05 was considered significant.

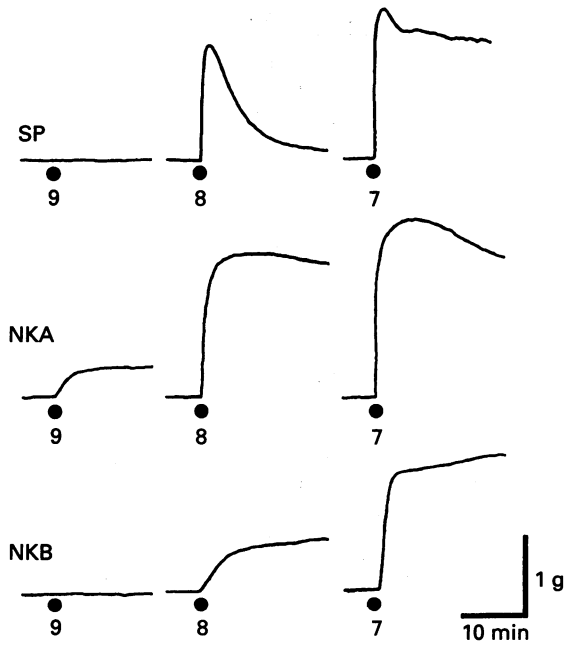

Figure 1 Representative tracings of contractions induced by substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) in the rabbit intact intrapulmonary artery. The numeral with a dot shows the concentration of the peptide (–log M).

Results

Response to SP, NKA and NKB in endothelium-intact intrapulmonary artery

Peptides at 10^{-9}–10^{-7} M were added to the organ bath non-cumulatively. SP (10^{-8} and 10^{-7} M), NKA (10^{-9}–10^{-7} M) and NKB (10^{-8} and 10^{-7} M) caused concentration-dependent contractions in rabbit intrapulmonary arteries (Figure 1). The contraction induced by SP (10^{-8} M) was transient. SP (10^{-7} M), NKA (10^{-9}–10^{-7} M) and NKB (10^{-8} and 10^{-7} M) caused sustained contraction.

Effect of removal of endothelium on responses to SP, NKA and NKB

The contraction induced by SP at 10^{-8} M was nearly abolished by removal of endothelium (Figure 2). However, removal of the endothelium did not affect the contractile response to SP at 10^{-7} M. The contraction induced by NKA at 10^{-9} and 10^{-8} M and NKB at 10^{-8} and 10^{-7} M was slightly enhanced by removal of the endothelium (Figure 2). The rank order of potency causing the EIC was NKA > NKB > SP (Figures 1 and 2).

Effects of tachykinin, NK_1 and NK_2 receptor antagonists on the EDC induced by SP

The endothelium-intact arteries were treated with various concentrations of tachykinin NK_1 and NK_2 receptor antagonists prior to application of SP (10^{-8} M). FK-888 (Fujii *et al.*, 1992) (10^{-10}–10^{-8} M), CP-96345 (Snider *et al.*, 1991) (10^{-10} – 10^{-8} M), CP-99994 (Desai *et al.*, 1992) (10^{-10}–10^{-8} M) and SR-140333 (Emonds-Alt *et al.*, 1993) (10^{-11}–10^{-8} M), all tachykinin

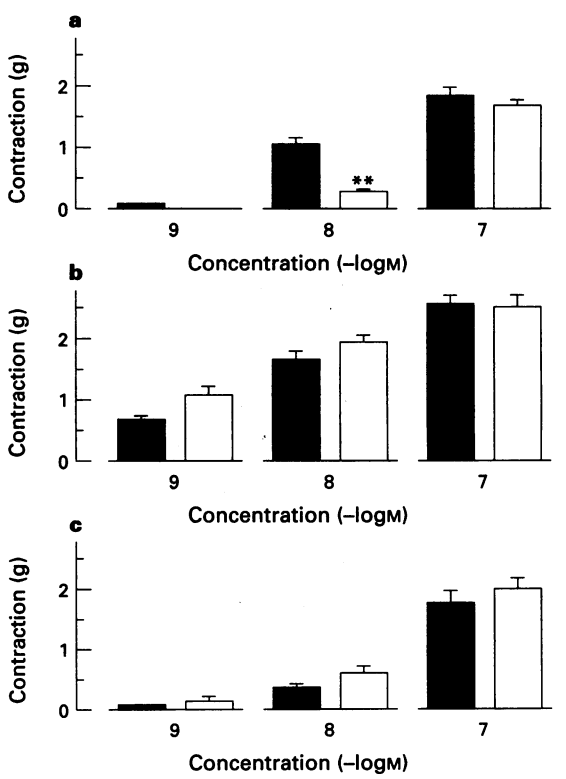

Figure 2 Contractions induced by substance P (SP) (a), neurokinin A (NKA) (b) and neurokinin B (NKB) (c) in the intact (■) and endothelium-free (□) rabbit intrapulmonary artery. Data are mean ± s.e.mean $n = 12$. $^{**}P < 0.01$, Student's test for paired data.

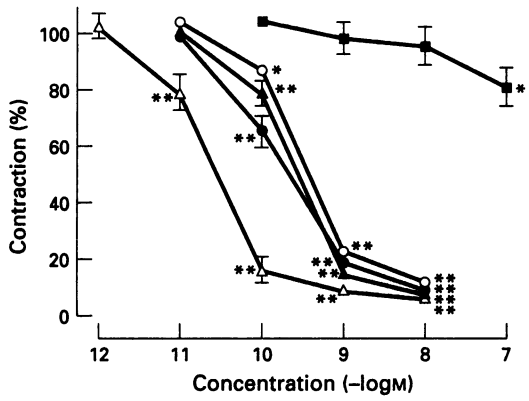

Figure 3 Effects of tachykinin NK_1 and NK_2 receptor antagonists on the endothelium-dependent contraction induced by substance P (10^{-8} M) in the rabbit intact intrapulmonary artery. NK_1 antagonists: (●) FK-888 ($n=9$); (○) CP-96345 ($n=10$); (▲) CP-99994 ($n=10$); (△) SR-140333 ($n=10$). NK_2 antagonist: (■) SR-48968 ($n=9$). The contractions in the absence of NK_1 or NK_2 receptor antagonists were taken as 100%. Data are mean±s.e.mean. $^*P<0.05$, $^{**}P<0.01$, Student's test for paired data.

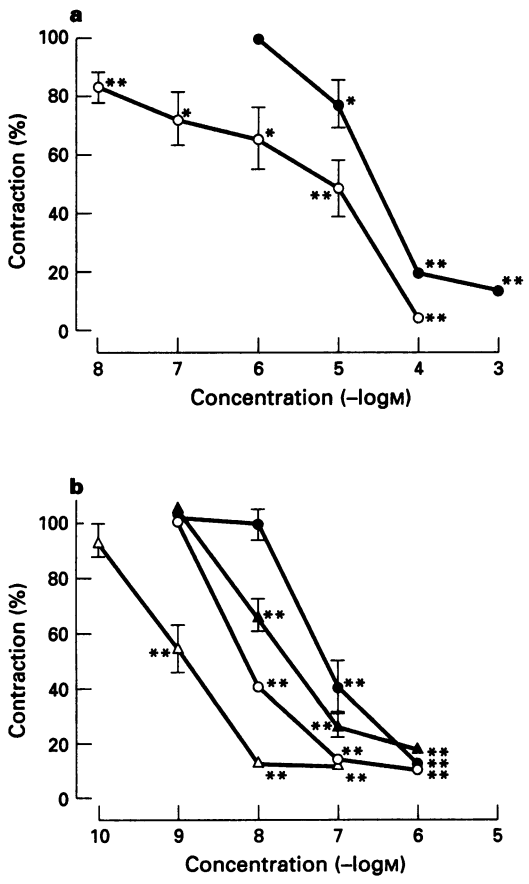

Figure 4 Effects of cyclo-oxygenase inhibitors, TXA_2 synthetase inhibitors and a TXA_2 antagonist on the endothelium-dependent contraction induced by substance P (10^{-8} M) in the rabbit intact intrapulmonary artery. Cyclo-oxygenase inhibitors (a): (●) aspirin; (○) indomethacin. TXA_2 synthetase inhibitors and a TXA_2 antagonist (b): (●) OKY-046; (○) KY-234; (▲) KY-063; (△) S-1452. The contractions in the absence of cyclo-oxygenase inhibitors, TXA_2 synthetase inhibitors or a TXA_2 antagonist were taken as 100%. Data are mean±s.e.mean, $n=9$. $^*P<0.05$, $^{**}P<0.01$, Student's test for paired data.

NK_1 receptor antagonists attenuated the EDC induced by SP in a concentration-dependent manner (Figure 3). SR-140333 attenuated the EDC more strongly than FK-888, CP-96345 and CP-99994. SR-48968 (Emonds-Alt *et al.*, 1992), an NK_2 antagonist had little effect on the EDC at 10^{-10}–10^{-8} M and attenuated it by 22% at 10^{-7} M. NK_1 receptor antagonists and an NK_2 antagonist at concentrations used here had little effect on the STA_2 (10^{-8} M)-induced EIC (data not shown). Other receptor antagonists such as atropine (10^{-6} M), phentolamine (10^{-6} M), diphenhydramine (10^{-6} M) and [Sar^1, Ala^8]-angiotensin II (10^{-6} M) had no effect on the EDC (data not shown).

Effects of cyclo-oxygenase, TXA_2 synthetase inhibitors and a TXA_2 antagonist on the EDC induced by SP

The endothelium-intact arteries were treated with various concentrations of cyclo-oxygenase, TXA_2 synthetase inhibitors and a TXA_2 antagonist prior to application of SP (10^{-8} M). The SP-induced EDC was attenuated by aspirin (10^{-5}–10^{-3} M) and indomethacin (10^{-8}–10^{-4} M), both cyclo-oxygenase inhibitors (Vane 1971), OKY-046 (Iizuka *et al.*, 1981) (10^{-7} and 10^{-6} M), KY-234 (Kanda *et al.*, 1993) (10^{-8}–10^{-6} M) and KY-063 (Shirahase *et al.*, 1994) (10^{-8}–10^{-6} M), all TXA_2 synthetase inhibitors and S-1452 (Narisada *et al.*, 1988) (10^{-9}–10^{-7} M), a TXA_2 antagonist (Figure 4). The STA_2 (10^{-8} M)-induced contraction was attenuated by the TXA_2 antagonist at the concentrations used here but was affected by neither cyclo-oxygenase inhibitors nor TXA_2 synthetase inhibitors (data not shown). TMK-777 (10^{-7} M), a specific 5-lipoxygenase inhibitor (Wakabayashi *et al.*, 1987) did not affect the EDC (data not shown).

Effects of tachykinin NK_1 and NK_2 receptor antagonists on the EIC induced by SP

The arteries without endothelium were treated with various concentrations of tachykinin NK_1 and NK_2 receptor antagonists prior to application of SP (10^{-7} M). The NK_2 antagonist (SR-48968) at 10^{-10}–10^{-8} M attenuated the EIC induced by SP in a concentration-dependent manner (Figure 5). Among the NK_1 antagonists, CP-96345 and CP-99994 at 10^{-8}–10^{-6} M did not affect the EIC (Figure 5). FK-888 and SR-140333 attenuated it slightly and significantly only at 10^{-6} M: 13 and 35% inhibition respectively (Figure 5). Other receptor antagonists such as atropine (10^{-6} M), phentolamine (10^{-6} M), diphenhy-

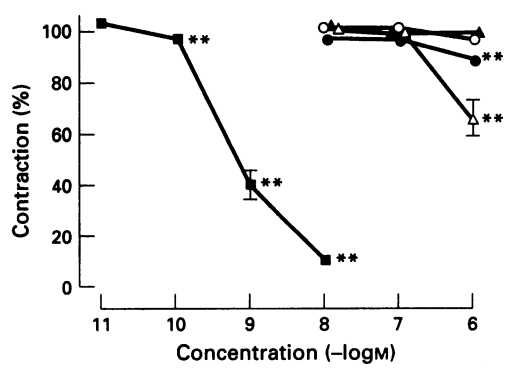

Figure 5 Effects of tachykinin NK_1 and NK_2 receptor antagonists on the endothelium-independent contraction induced by substance P (10^{-7} M) in the endothelium-free intrapulmonary artery of the rabbit. NK_1 antagonists: (●) FK-888 ($n=9$); (○) CP-96345 ($n=9$); (▲) CP-99994 ($n=8$); (△) SR-140333 ($n=9$). NK_2 antagonist: (■) SR-48968 ($n=10$). The contractions in the absence of NK_1 or NK_2 receptor antagonists were taken as 100%. Data are mean±s.e.mean. $^*P<0.05$, $^{**}P<0.01$, Student's test for paired data.

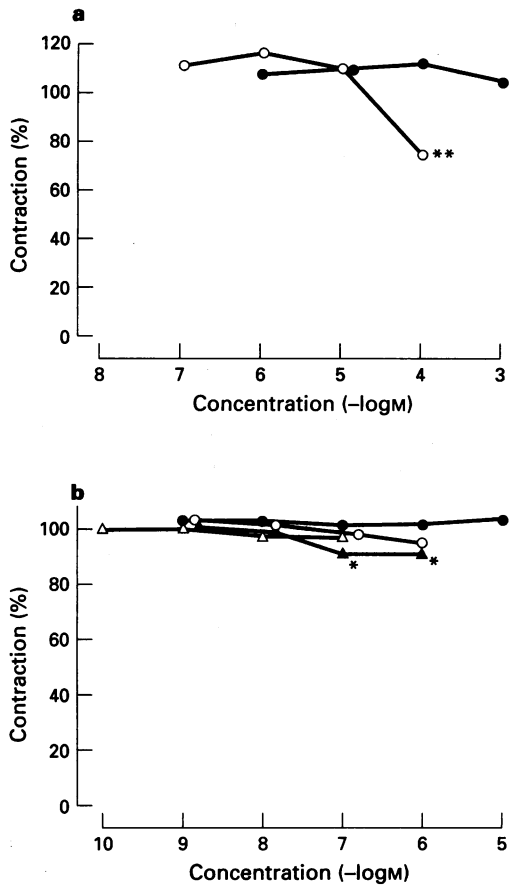

Figure 6 Effects of cyclo-oxygenase inhibitors, TXA_2 synthetase inhibitors and a TXA_2 antagonist on the endothelium-independent contraction induced by substance P (10^{-7} M) in the endothelium-free intrapulmonary artery of the rabbit. Cyclo-oxygenase inhibitors (a): (●) aspirin; (○) indomethacin. TXA_2 synthetase inhibitors and a TXA_2 antagonist (b): (●) OKY-046; (○) KY-234; (▲) KY-063; (△) S-1452. The contractions in the absence of cyclo-oxygenase inhibitors, TXA_2 synthetase inhibitors or a TXA_2 antagonist were taken as 100%. Data are mean ± s.e.mean. $n=8$. $^*P<0.05$, $^{**}P<0.01$, Student's test for paired data.

dramine (10^{-6} M) and [Sar^1,Ala^8]-angiotensin II (10^{-6} M) had no effect on the EIC (data not shown).

Effects of cyclo-oxygenase, TXA_2 synthetase inhibitors and a TXA_2 antagonist on the EIC induced by SP

The endothelium-free arteries were treated with various concentrations of cyclo-oxygenase, TXA_2 synthetase inhibitors and a TXA_2 antagonist prior to application of SP (10^{-7} M). Cyclo-oxygenase inhibitors (aspirin and indomethacin), TXA_2 synthetase inhibitors (OKY-046, KY-234 and KY-063) and a TXA_2 antagonist (S-1452) had little effect on the SP-induced EIC (Figure 6) and 5-lipoxygenase inhibitor (TMK-777) did not affect it (data not shown).

Discussion

The present study demonstrated that SP caused EDC at 10^{-8} M and EIC at 10^{-7} M in rabbit isolated intrapulmonary arteries. SP has been reported to induce EDR in various peripheral arteries including rabbit pulmonary arteries in the presence of active tone (Regoli *et al.*, 1990; Emonds-Alt *et al.*, 1993). ACh also causes EDC in the non-contracted rabbit pulmonary artery while it evokes EDR in the precontracted preparations (Altiere *et al.*, 1986). Either EDR or EDC seems to play a predominant role, depending on the degree of the tone, in the regulation of pulmonary circulation.

SP has been previously reported to cause EDR via release of acetylcholine (Tanaka *et al.*, 1985). However, the SP-induced EDC was not affected by muscarinic, histamine, adrenaline or angiotensin II receptor antagonists. Thus, ACh, catecholamines, histamine or angiotensins are unlikely to be involved in the EDC. SP seems to activate endothelial tachykinin receptors and directly release the EDCF. For tachykinins, three types of receptors are currently recognized, namely NK_1, NK_2 and NK_3 (IUPHAR Committee on Drug Classification and Receptor Nomenclature, 1994). The rank order of potency is SP > NKA > NKB for activating NK_1 receptors, NKA > NKB > > SP for activating NK_2 receptors and NKB > NKA > SP for activating NK_3 receptors. In the present study, the EDC was induced by SP but not NKA or NKB, suggesting the involvement of NK_1 receptors rather than NK_2 or NK_3. Indeed, specific NK_1 receptor antagonists such as FK-888, CP-96345, CP-99994 and SR-140333 but not an NK_2 antagonist, SR-48968 potently attenuated the EDC induced by SP. These findings indicate that SP causes EDC by activating endothelial NK_1 receptors. The presence of functional NK_1 receptors on the endothelium has been demonstrated in human umbilical arteries (Greeno *et al.*, 1993). The tachykinin receptor mediating EDR is also the NK_1 subtype in guinea-pig and rabbit pulmonary arteries (Emonds-Alt *et al.*, 1993; Floch *et al.*, 1994). Whether EDC and EDR are mediated by the same NK_1 receptor remains to be clarified. NK_1 receptors in the guinea-pig bronchopulmonary system and rat vascular system are different pharmacologically (Floch *et al.*, 1994). We have found that unlike EDC, naturally occurring tachykinins all cause EDR according to the rank order of potency SP > NKA > NKB (unpublished data). Further study is needed to determine the possible heterogeneity of the pulmonary endothelial NK_1 receptor causing EDC and EDR.

We have reported that EDCF released by various substances is probably TXA_2 in canine cerebral arteries (Usui *et al.*, 1983; Shirahase *et al.*, 1987; 1988a,b; 1991; 1993; Manabe *et al.*, 1989). In rabbit pulmonary arteries, ACh causes an EDC via production of TXA_2 (Altiere *et al.*, 1986). In the present study, the EDC was abolished by cyclo-oxygenase inhibitors (aspirin and indomethacin), TXA_2 synthetase inhibitors (OKY-046, KY-063 and KY-234) and a TXA_2 antagonist (S-1452). The effective concentration of cyclo-oxygenase inhibitors and TXA_2 synthetase inhibitors was similar in the present experiments and previous reports on the respective enzyme activities (Vane 1971; Iizuka *et al.*, 1981; Kanda *et al.*, 1993; Shirahase *et al.*, 1994). The effective concentration of S-1452 against the EDC was compatible with that reported for the TXA_2 agonist-induced contraction of rat aorta (Narisada *et al.*, 1988). A 5-lipoxygenase inhibitor, TMK-777, did not affect the EDC. From these findings, the SP-induced EDC was concluded to be mediated by TXA_2 production activated via endothelial NK_1 receptors. These findings are also compatible with previous reports showing that SP administered intravenously causes pulmonary vasoconstriction via PG or TXA_2 in anesthetized rabbits (Worthen *et al.*, 1985).

SP at 10^{-7} M, NKA and NKB at concentrations used here caused EIC in the rabbit pulmonary arteries. SP has already been reported to induce EIC via NK_2 receptors (Regoli *et al.*, 1989). We also demonstrated that for EIC, the rank order of potency was NKA > NKB > SP and that the EIC was sensitive to NK_2 but not NK_1 antagonists. The EIC was not affected by cyclo-oxygenase inhibitors, TXA_2 synthetase inhibitors, a TXA_2 antagonist or a 5-lipoxygenase inhibitor. These findings demonstrate that tachykinins cause EIC via direct activation of NK_2 receptors on smooth muscle cells.

The physiological or pathological significance of the EDC and EIC induced by tachykinins remains to be clarified. Pulmonary arteries are innervated by SP immunoreactive fibres (Allen *et al.*, 1989). The C-fibre activator, capsaicin and elec-

trical stimulation of vagal nerves release SP and NKA in the guinea-pig perfused lung (Saria *et al.*, 1988). Therefore, neuronal tachykinins may cause EDC and/or EIC in the pulmonary artery. However, it is not clear whether SP released from the nerve endings diffuse to the endothelium and cause EDC. On the other hand, the concentration of SP in blood is not high enough to cause EDC (Mori *et al.*, 1992). SP is localized in endothelial cells and released by hypoxia and change in flow (Milner *et al.*, 1989; Ralevic *et al.*, 1990). SP may play a role in the regulation of vascular tone as a paracrine autacoid releasing EDCF.

In conclusion, three natural tachykinins cause EIC and only SP induces EDC. The SP-induced EDC is mediated by TXA_2 produced via activation of endothelial NK_1 receptors and the EIC is associated with NK_2 receptors on smooth muscle cells.

References

ALLEN, K.M., WHARTON, J., POLAK, J.M. & HAWORTH, S.G. (1989). A study of nerves containing peptides in the pulmonary vasculature of healthy infants and children and of those with pulmonary hypertension. *Br. Heart J.*, **62**, 353–360.

ALTIERE, R.J., KIRITSY-ROY, J.A. & CATRAVAS, J.D. (1986). Acetylcholine-induced contractions in isolated rabbit pulmonary arteries: Role of thromboxane A_2. *J. Pharmacol. Exp. Ther.*, **236**, 535–541.

DESAI, M.C., LEFKOWITZ, S.L., THADEIO, P.F., LONGO, K.P. & SNIDER, R.M. (1992). Discovery of a potent substance P antagonist: Recognition of the key molecular determinant. *J. Med. Chem.*, **35**, 4911–4913.

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-101–106.

EMONDS-ALT, X., DOUTREMEPUICH, J.D., HEAULME, M., NELIAT, G., SANTUCCI, V., STEINBERG, R., VILAIN, P., BICHON, D., DUCOUX, J.-P., PROIETTO, V., VAN BROECK, D., SOUBRIE, P., LE FUR, G. & BRELIERE, J.C. (1993). In vitro and in vivo biological activities of SR140333, a novel potent non-peptide tachykinin NK1 receptor antagonist. *Eur. J. Pharmacol.*, **250**, 403–413.

FLOCH, A., FARDIN, V. & CAVERO, I. (1994). Characterization of NK_1 and NK_2 tachykinin receptors in guinea-pig and rat bronchopulmonary and vacular systems. *Br. J. Pharmacol.*, **111**, 759–768.

FUJII, T., MURAI, M., MORIMOTO, H., MAEDA, Y., YAMAOKA, M., HAGIWARA, D., MIYAKE, H., IKARI, N. & MATSUO, M. (1992). Pharmacological profile of a high affinity dipeptide NK_1 receptor antagonist, FK 888. *Br. J. Pharmacol.*, **107**, 785–789.

GREENO, E.W., MANTYH, P., VERCELLOTTI, G.M. & MOLDOW, C.F. (1993). Functional neurokinin 1 receptors for substance P are expressed by human vascular endothelium. *J. Exp. Med.*, **177**, 1269–1276.

IIZUKA, K., AKAHANE, K., MOMOSE, D. & NAKAZAWA, M. (1981). Highly selective inhibitors of thromboxane synthetase. I. Imidazole derivatives. *J. Med. Chem.*, **24**, 1139–1148.

IUPHAR COMMITTEE ON DRUG CLASSIFICATION AND RECEPTOR NOMENCLATURE. (1994). 1994 Receptor and ion channel nomenclature supplement. 5th edition. *Trends Pharmacol. Sci.*

KANDA, M., NAKAMURA, S., WADA, K. & SHIRAHASE, H. (1993). Effect of KY-234, a novel anti-asthmatic TXA_2 synthetase inhibitor on rabbit pulmonary arteries and platelets. *Jpn. J. Pharmacol.*, **61**, Suppl. 227p.

KURAHASHI, K., USUI, H., SHIRAHASE, H. & JINO, H. (1994). Endothelium-dependent contraction of cerebral arteries. In *The Human Brain Circulation*. ed. Bevane, R.D. & Bevane, J.A. pp. 167–178. Totowa: Humana Press.

MANABE, K., SHIRAHASE, H., USUI, H., KURAHASHI, K. & FUJIWARA, M. (1989). Endothelium-dependent contractions induced by angiotensin I and angiotensin II in canine cerebral artery. *J. Pharmacol. Exp. Ther.*, **251**, 317–320.

MILNER, P., RALEVIC, V., HOPWOOD, A.M., FEHER, E., LINCOLN, J., KIRKPATRICK, K.A. & BURNSTOCK, G. (1989). Ultrastructural localisation of substance P and choline acetyltransferase in endothelial cells of rat coronary artery and release of substance P and acetylcholine during hypoxia. *Experientia*, **45**, 121–125.

MORI, K., ASAKURA, S., MORIKAWA, N. & TAKEYAMA, M. (1992). Effect of terfenadine on the plasma concentrations of substance P and vasoactive intestinal polypeptide in volunteers. *J. Pharm. Pharmacol.*, **44**, 856–858.

NARISADA, M., OHTANI, M., WATANABE, F., UCHIDA, K., ARITA, H., DOTEUCHI, M., HANASAKI, K., KAKUSHI, H., OTANI, K. & HARA, S. (1988). Synthesis and in vitro activity of various derivatives of a novel thromboxane receptor antagonist, (±)-(5Z)-7-[3-endo-[(phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-exo-yl]heptenoic acid. *J. Med. Chem.*, **31**, 1847–1854.

RALEVIC, V., MILNER, P., HUDLICKA, O., KRISTEK, F. & BURNSTOCK, G. (1990). Substance P is released from the endothelium of normal and capsaicin-treated rat hind-limb vasculature, in vivo, by increased flow. *Circ. Res.*, **66**, 1178–1183.

REGOLI, D., DION, S., RHALEB, N.-E., ROUISSI, N., TOUSIGNANT, C., JUKIC, D., D'ORLEANS-JUSTE, P. & DRAPEAU, G. (1989). Selective agonists for receptors of substance P and related neurokinins. *Biopolymers*, **28**, 81–90.

REGOLI, D., DION, S., RHALEB, N.-E., DRAPEAU, G. & D'ORLEANS-JUSTE., P. (1990). Vasoactive peptides and their receptors. *Blood Vessels*, **27**, 137–145.

SARIA, A., MARTLING, C.R., YAN, Z., THEODORSSON-NORHEIM, E., GAMSE, R. & LUNDBERG, J.M. (1988). Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenyl piperazinium, and vagal nerve stimulation. *Am. Rev. Respir. Dis.*, **137**, 1330–1335.

SHIRAHASE, H., KAMIYA, S., HASHIZUME, Y., NAKAMURA, K., WADA, K., MATSUI, H. & KANDA, M. (1994). A novel series of TXA_2 synthetase inhibitors, 5-alkyloxy-3-(1-imidazolylethyl)indoline-1-propanoic acids (alkyloxy-IIPAs) with free radical scavenging and antiperoxidative activities. In *Frontiers of Reactive Oxygen Species in Biology and Medicine*. ed. Asada, K. & Yoshikawa, T. pp. 363–364. Amsterdam: Excerpta Medica.

SHIRAHASE, H., KANDA, M., SHIMAJI, H., USUI, H., RORSTAD, O.P. & KURAHASHI, K. (1993). Somatostatin-induced contraction mediated by endothelial TXA_2 production in canine cerebral arteries. *Life Sci.*, **53**, 1539–1544.

SHIRAHASE, H., USUI, H., KURAHASHI, K., FUJIWARA, M. & FUKUI, K. (1987). Possible role of endothelial thromboxane A_2 in the resting tone and contractile responses to acetylcholine and arachidonic acid in canine cerebral arteries. *J. Cardiovasc. Pharmacol.*, **10**, 517–522.

SHIRAHASE, H., USUI, H., MANABE, K., KURAHASHI, K. & FUJIWARA, M. (1988a). An endothelium-dependent contraction induced by A-23187, a Ca^{++} inophore in canine basilar artery. *J. Pharmacol. Exp. Ther.*, **247**, 701–705.

SHIRAHASE, H., USUI, H., MANABE, K., KURAHASHI, K. & FUJIWARA, M. (1988b). Endothelium-dependent contraction and -independent relaxation induced by adenine nucleotides and nucleoside in the canine basilar artery. *J. Pharmacol. Exp. Ther.*, **247**, 1152–1157.

SHIRAHASE, H., USUI, H., SHIMAJI H., KURAHASHI, K. & FUJIWARA, M. (1991). Endothelium-independent and -dependent contractions induced by endothelin-1 in canine basilar arteries. *Life Sci.*, **49**, 273–281.

SNIDER, R.M., CONSTANTINE, J.W., LOWE, J.A., LONGO, K.P., LEBEL, W.S., WOODY, H.A., DROZDA, S.E., DESAI, M.C., VINICK, F.J., SPENCER, R.W. & HESS, H.-J. (1991). A potent non-peptide antagonist of the substance P (NK_1) receptor. *Science*, **25**, 435–437.

TANAKA, D.T. & GRUNSTEIN, M.M. (1985). Vasoactive effects of substance P on isolated rabbit pulmonary artery. *J. Appl. Physiol.*, **58**, 1291–1297.

USUI, H., KURAHASHI, K., ASHIDA, K. & FUJIWARA, M. (1983). Acetylcholine-induced contractile response in canine basilar artery with activation of thromboxane A2 synthesis sequence. *IRCS Med. Sci.*, **11**, 418–419.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol.*, **231**, 232–235.

WAKABAYASHI, T., OZAWA, S., ARAI, J., TAKAI, M., KOSHIHARA, Y. & MUROTA, S. (1987). Antiallergic action of TMK-777, a leukotriene biosynthesis inhibitor. *Adv. Prostaglandin Thromboxane Leukot. Res*, **17**, 186–188.

WORTHEN, G.S., GUMBAY, R.S., TANAKA, D.T. & GRUNSTEIN, M.N. (1985). Opposing hemodynamic effects of substance P on pulmonary vasculature in rabbits. *J. Appl. Physiol.*, **59**, 1098–1103.

(Received October 18, 1994
Revised April 4, 1995
Accepted April 21, 1995)