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Role of the 5-HT receptor in neurogenic inflammation in Fisher 344 rat airways

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Abstract

The increased plasma protein extravasation in the airways of Fisher 344 rats upon stimulation of sensory nerves is in part due to the degranulation of mast cells. In this study, we examined the role of 5-HT and histamine receptors in the capsaicin-induced increase in plasma protein extravasation in Fisher 344 rat airways, using Evans blue as an intravascular marker. We found that only 5-HT_2 receptor agonists increased baseline plasma protein extravasation. Furthermore, the 5-HT_2 receptor antagonist ketanserin reduced the capsaicin-induced increase in plasma protein extravasation. Combining ketanserin with the tachykinin NK_1 receptor antagonist (±)-RP 67,580 ((3*aR*,7*aR*)-(7,7-diphenyl-2(1-imino-2-(2-methoxyphenylethyl)-perhydraisoinositol-4-one))) abolished the neurogenic increase in plasma protein extravasation. Finally, using selective receptor agonists and antagonists, we demonstrated that there was no modulation of the capsaicin-induced rise in plasma protein extravasation by stimulation of either histamine receptors or 5-HT_1, 5-HT_3 and 5-HT_4 receptors. We conclude that, in the airways of Fisher 344 rats, the neurogenic increase in plasma protein extravasation is caused by activation of both tachykinin NK_1 receptors and 5-HT_2 receptors.

Keywords: Plasma protein extravasation; 5-HT receptor; Histamine receptor; Tachykinin; Airways; Capsaicin; (Fisher 344 rat)

1. Introduction

Increased microvascular permeability and plasma extravasation have been implicated in the pathogenesis of asthma. Increased microvascular leakage might contribute to desquamation of the epithelium, mucus plug formation and oedema of mucosa and submucosa with infiltration of inflammatory cells, all of which are pathological features of asthma. The swelling of the airway wall and the ensuing loss of elastic recoil also contribute to airway hyperresponsiveness in asthma (Moreno et al., 1986; Persson, 1988).

In rodent airways, antidromic release of tachykinins from capsaicin-sensitive sensory C-fibres is known to cause an increase in vascular permeability, plasma extravasation and oedema (Lundberg and Saria, 1982; Lundberg et al., 1983). Previously, we described that substance P and neurokinin A activate Fisher 344 rat lung mast cells in vivo, causing the release, into bronchoalveolar lavage fluid, of both serotonin (5-hydroxytryptamine, 5-HT) and histamine (Joos and Pauwels, 1993). More recently, we demonstrated that, in the central airways of the rat, tachykinin NK_1 receptors mediate the plasma protein extravasation caused by capsaicin. In Fisher 344 rats, the 5-HT receptor antagonist methysergide and depletion of mast cells with compound 48/80 reduce the neurogenic increase in plasma protein extravasation. These findings indicated that the neurogenic increase in plasma protein extravasation in the airways of the Fisher 344 rat is partly due to an additional mechanism involving activation of mast cells and release of 5-HT (Germonpré et al., 1995).

Recently a number of studies have examined the role of serotonin and histamine receptors in the neurogenic inflammation in several organs in different species.

It has been shown that activation of 5-$HT_{1B/D}$ receptors inhibits the neurogenic increase in plasma protein extravasation induced by both electrical nerve stimulation and systemic administration of capsaicin, in rat and guinea pig dura mater (Buzzi and Moskowitz, 1990). However, in rat skin, pretreatment with 5-HT potentiates the substance P-induced vasodilatation and increase in plasma protein extravasation (Khalil and Helme, 1990). In rat trachea, the release of the sensory neuropeptide calcitonin gene-related

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peptide (CGRP) from capsaicin-sensitive nerves is increased by activation of 5-HT_3 receptors (Hua and Yaksh, 1993). Buckner et al. (1991) found that in vivo activation of 5-HT_{1like} and 5-HT_3 receptors causes the release of sensory neuropeptides in guinea pig airways. Ward and co-workers, however, described an inhibitory, 'atypical' 5-HT receptor on sensory C-fibres in isolated guinea pig bronchi (Ward et al., 1994). Similarly, in rat skin and in guinea pig airways, substance P release and neurogenic inflammation are inhibited by prejunctional histamine H_3 receptors and potentiated by H_1 receptors (Ichinose et al., 1990; Ohkubo et al., 1995).

These studies showed that serotonin and histamine can have potentiating as well as inhibitory effects on the neurogenic rise in plasma protein extravasation in different organ systems of different animal species. Thus, to determine the role of 5-HT and histamine receptors in the neurogenic increase in plasma protein extravasation in the central airways of the Fisher 344 rat, we examined the effect of selective agonists and antagonists on both the baseline plasma protein extravasation and the capsaicin-induced increase in plasma protein extravasation in trachea and main bronchi.

2. Materials and methods

2.1. Animals

Fisher 344 rats were purchased from Harlan CBP (Zeist, Netherlands). All rats were highly inbred and specific pathogen-free. They were male and weighed 220–270 g. After arrival, the animals were maintained in a conventional animal house for at least 1 week before they were tested.

2.2. General procedure

We measured the changes in plasma protein extravasation in response to various agents using the Evans blue technique, as previously described (Germonpré et al., 1995). Briefly, the rats were anaesthetized by an intraperitoneal injection of pentobarbital (Nembutal, 60 mg/kg body weight) and allowed to breathe spontaneously. The external jugular vein was cannulated with a small polyethylene catheter for administration of drugs. Blood pressure and heart rate were monitored via a femoral artery catheter using polyethylene tubing with an inner diameter of 1.77 mm (Intramedic; Clay Adams, Parsippany, NY, USA) and a pressure transducer (Statham P23; Gould Medical, Bilthoven, Netherlands).

Evans blue (30 mg/ml) was administered intravenously, 30 mg/kg body weight, 5 min after the intravenous pretreatment (or 30 min after intraperitoneal pretreatment with thioperamide). Immediately thereafter 5-HT receptor agonists, histamine receptor agonists or capsaicin was injected intravenously. Vehicle pretreatment or treatment was used as control. After a further 5 min the chest was opened, and the circulation was perfused with 0.9% saline for 2 min at 120 cmH_2O. The trachea and the main bronchi were dissected, blotted and weighed (= wet weight).

Evans blue was extracted from the trachea and main bronchi by incubation for 16 h at 37°C in formamide. The Evans blue concentration of the tissue extracts was determined using a Titertek Multiskan MCC microplate reader (Flow Laboratories, Brussels, Belgium), and the amount of extravasated Evans blue was calculated as nanogram per milligram wet weight tissue.

Table 1
List of the 5-HT receptor agonists and antagonists used

Full name	Abbreviation	Specificity
5-HT receptor agonists		
5-Carboxytryptamine	5-CT	5-HT_1
8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide	8-OH-DPAT	5-HT_{1A}
3-(1,2,5,6-Tetrahydropyrid-4-yl)-pyrrolo[3,2-*b*]pyrid-5-one	CP 93129	5-HT_{1B}
3-[2-Diethylamino]ethyl-*N*-methyl-1*H*-indole-5 methane sulfonamide	sumatriptan	5-$HT_{1B/D}$
α-Methyl-5-hydroxytryptamine	α-Methyl-5-HT	5-HT_2
2-Methyl-5-hydroxytryptamine	2-Methyl-5-HT	5-HT_3
Cisapride		5-HT_4
5-Methoxytryptamine	5-MeOT	5-HT_4
5-HT receptor antagonists		
1-(2-Methoxyphenyl)-4-[4-(2-phtalimmido)butyl]piperazine hydrobromide	NAN-190	5-HT_{1A}
4-Iodo-*N*-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl benzamide	pMPPI	5-HT_{1A}
Ketanserin		5-HT_2
Tropisetron		5-HT_3
1-[2-(Methylsulfonyl)amino]-ethyl]-4-piperidinyl]-methyl-1-methyl-1*H*-indole-3-carboxylate	GR 113808	5-HT_4
(1-Butyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate	SB 204070	5-HT_4

2.3. Experimental protocols

In a first set of experiments we investigated the effect of the specific 5-HT receptor agonists listed in Table 1 and of the specific histamine H_3 receptor agonist *R*-(−)-α-methylhistamine on both the baseline plasma protein extravasation and the capsaicin-induced increase in plasma protein extravasation.

In a second set of experiments we looked at the effect of the 5-HT receptor antagonists listed in Table 1 and of the histamine H_3 receptor antagonist thioperamide on the capsaicin-induced increase in plasma protein extravasation (Buckner et al., 1991; Buzzi et al., 1991; Hoyer et al., 1994; Ohkubo et al., 1995).

Finally, we examined whether the combination of ketanserin and the tachykinin NK_1 receptor antagonist (±)-RP 67,580 ((3*aR*,7*aR*)-(7,7-diphenyl-2-(1-imino-2-(2-methoxyphenylethyl)-perhydraisoinositol-4-one))) completely blocks the increase in plasma protein extravasation induced by capsaicin.

In the experiments involving capsaicin, we used a submaximal dose of 100 μg/kg (Germonpré et al., 1995).

2.4. Chemicals

The following drugs were used: capsaicin (Sigma, St. Louis, MO, USA), cimetidine (Smith-Kline Beecham, Genval, Belgium), Evans blue (Merck, Darmstadt, Germany), heparin (B. Braun Pharma, Melsungen, Germany), pentobarbital (Ceva, Brussels, Belgium), serotonin creatine sulfate (5-hydroxytryptamine; Fluka AG, Buchs, Germany), NAN-190, pMPPI, tropisetron, thioperamide, 8-OH-DPAT, α-methyl-5-HT, 2-methyl-5-HT, *R*-(−)-α-methylhistamine (Research Biochemicals International, Natick, MA, USA). (±)-RP 67,580 was a gift from Rhone-Poulenc (Paris, France). GR 1138080 and sumatriptan were a gift from Glaxo (Stevenage, UK). All other drugs were kindly provided by Janssen Pharmaceutica (Beerse, Belgium).

Capsaicin was dissolved in equal parts ethanol 96% and Tween-80 (100 mg/kg stock solution) and further diluted in 0.9% saline. Evans blue was dissolved in 0.9% saline with heparin 300 IU/ml. CP 93129, GR 113808, NAN-190, pMPPI and SB 204070 were dissolved in 10% dimethylsulfoxide (DMSO). RP 67580 was dissolved in 2.2 mM methanesulfonic acid. All other drugs were dissolved in 0.9% saline.

2.5. Analysis of data

Plasma protein extravasation is expressed as ng Evans blue/mg wet tissue, and statistical differences were calculated from the absolute values. The responses in drug-treated animals are expressed as percentage of the baseline plasma protein extravasation or the plasma protein extravasation following capsaicin administration in control vehicle-treated animals carried out at the same time.

Differences between two groups of rats were assessed by the Mann-Whitney U-test. A P value < 0.05 was considered as significant. Reported values are means ± S.E.M..

3. Results

3.1. Characterisation of the receptors involved in the effect of 5-HT on baseline plasma protein extravasation in rat airways

As shown in Fig. 1, 5-HT (0.5 μmol/kg body weight i.v.), the 5-HT_2 receptor agonist α-methyl-5-HT (0.5 μmol/kg body weight i.v.) and the 5-HT_4 receptor agonist 5-MeOT (0.5 μmol/kg body weight i.v.) significantly increased plasma protein extravasation in both trachea and main bronchi. Cisapride (1 μmol/kg body weight i.v.), a 5-HT_4 receptor agonist with weak 5-HT_2 receptor antagonistic activity, failed to increase baseline plasma pro-

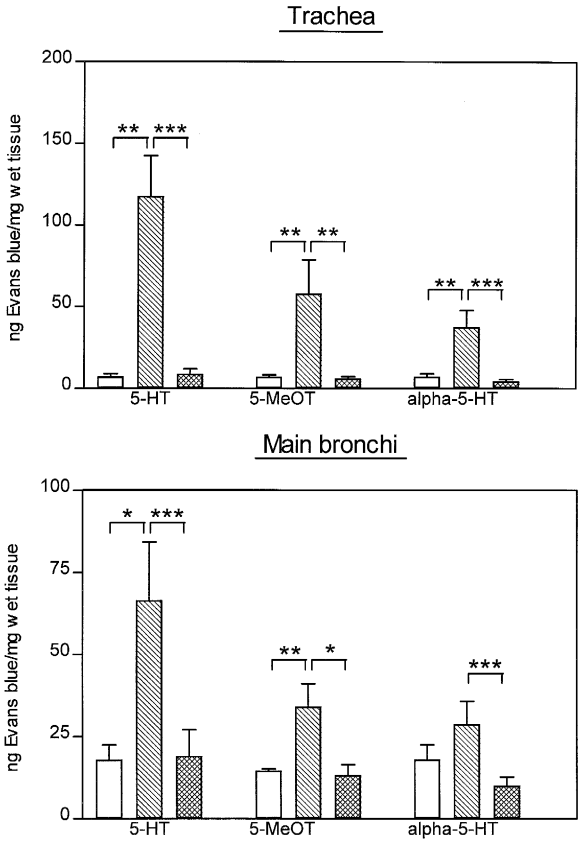

Fig. 1. Effect of intravenous pretreatment with ketanserin (1.8 μmol/kg body weight) on the increase in plasma protein extravasation induced by 5-HT, α-methyl-5-HT or 5-MeOT 0.5 μmol/kg body weight i.v., in the trachea (*top panel*) and main bronchi (*bottom panel*) of Fisher 344 rats ($n = 6$ for each group; cross-hatched bars). Pretreatment with saline was used as control ($n = 6$; hatched bars), and saline/saline treatment was used to assess baseline plasma protein extravasation ($n = 5$; open bars). Data are reported as means ± S.E.M. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$ (all Mann-Whitney U-test).

Table 2
Effect of 5-HT receptor agonists, histamine and a histamine H_3 receptor agonist on baseline plasma protein extravasation in the airways of Fisher 344 rats

Compound	Dose	Percentage of baseline plasma protein extravasation	
		Trachea	Main bronchi
5-CT	0.03 nmol/kg	125.6 ± 10.8	156.9 ± 37.8
	1 μmol/kg	112.2 ± 12.5	99.3 ± 10.4
8-OH-DPAT	0.9 μmol/kg	103.5 ± 9.6	133.2 ± 12.0
Sumatriptan	0.5 μmol/kg	123.1 ± 41.7	106.0 ± 43.9
2-methyl-5-HT	1 μmol/kg	104.5 ± 4.28	90.9 ± 6.6
Cisapride	1 μmol/kg	80.0 ± 9.2	70.5 ± 7.1
Histamine	1 μmol/kg	102.5 ± 8.8	127.5 ± 13.4
	10 μmol/kg	169.2 ± 25.0	169.7 ± 26.0 [a]
R-(−)-α-Methyl-histamine	10 μmol/kg	67.3 ± 12.4	81.4 ± 8.3

Results are expressed as means ± S.E.M. ($n = 8$ for 5-HT receptor agonists and $n = 5$ for histamine receptor agonists).
[a] $P < 0.02$ compared to the control group (Mann-Whitney U-test).

tein extravasation (Table 2). Pretreatment with the selective 5-HT_2 receptor antagonist ketanserin (1.8 μmol/kg body weight i.v.) completely inhibited the 5-HT, α-methyl-5-HT and 5-MeOT-induced increase in plasma protein extravasation (Fig. 1). The selective 5-HT_4 receptor antagonist SB 204070 (1 μmol/kg body weight i.v.) however, had no effect on the increase in plasma protein extravasation induced by 5-HT and 5-MeOT (Fig. 2).

The potent 5-HT_1 receptor agonist 5-CT (up to 1 μmol/kg body weight i.v.), the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.9 μmol/kg body weight i.v.), the 5-$HT_{1B/D}$ receptor agonist sumatriptan (0.5 μmol/kg body weight i.v.) and the 5-HT_3 receptor agonist 2-methyl-5-HT (1 μmol/kg body weight i.v.) all failed to cause significant changes in baseline plasma protein extravasation (Table 2).

3.2. Characterisation of the 5-HT receptors involved in the capsaicin-induced increase in plasma protein extravasation in rat airways

The 5-HT receptor agonists 5-CT (up to 1 μmol/kg body weight i.v.), 8-OH-DPAT (0.9 μmol/kg body weight i.v.), CP 93129 (0.3 μmol/kg body weight i.v.), sumatriptan (0.3 μmol/kg body weight i.v.), α-methyl-5-HT (0.2 μmol/kg body weight i.v.), 2-methyl-5-HT (1 μmol/kg body weight i.v.) and 5-MeOT (0.5 μmol/kg body weight i.v.), had no significant effect on the capsaicin-induced increase in plasma protein extravasation in the airways of the Fisher 344 rat (Table 3). Cisapride (1 μmol/kg body weight i.v.), a weak 5-HT_2 receptor antagonist, caused a small inhibition of the capsaicin-induced increase in plasma protein extravasation in the trachea (Table 3).

Pretreatment with the selective 5-HT_2 receptor antagonist ketanserin (1.8 μmol/kg body weight i.v.) signifi-

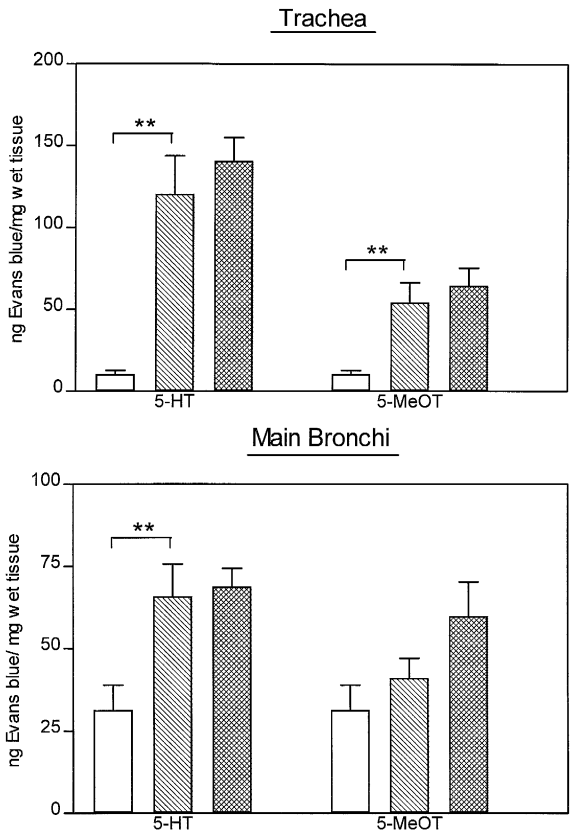

Fig. 2. Effect of intravenous pretreatment with SB 204070 (1 μmol/kg body weight) on the increase in plasma protein extravasation induced by 5-HT or 5-MeOT 0.5 μmol/kg body weight i.v., in the trachea (top panel) and main bronchi (bottom panel) of Fisher 344 rats ($n = 6$ for each group; cross-hatched bars). Pretreatment with saline was used as control ($n = 6$; hatched bars), and saline/saline treatment was used to assess baseline plasma protein extravasation ($n = 5$; open bars). Data are reported as means ± S.E.M. $^{**}P < 0.01$ compared to the saline/agonist group (Mann-Whitney U-test).

Table 3
Effect of 5-HT receptor agonists and a histamine H_3 receptor agonist on the increase in plasma protein extravasation induced by capsaicin (100 μg/kg body weight i.v.) in the airways of Fisher 344 rats

Compound	Dose	Percentage of plasma protein extravasation following capsaicin	
		Trachea	Main bronchi
5-CT	0.03 nmol/kg	98.9 ± 9.9	108.8 ± 8.6
	1 μmol/kg	91.4 ± 5.7	90.5 ± 3.8
8-OH-DPAT	0.9 μmol/kg	98.8 ± 8.8	107.7 ± 5.7
CP 93129	0.3 μmol/kg	113.2 ± 12.3	110.0 ± 14.0
Sumatriptan	0.3 μmol/kg	93.7 ± 11.9	93.0 ± 6.8
α-Methyl-5-HT	0.2 μmol/kg	110.9 ± 9.3	100.3 ± 5.8
2-Methyl-5-HT	1 μmol/kg	102.2 ± 9.0	87.4 ± 3.8
5-MeOT	0.5 μmol/kg	93.3 ± 14.7	99.5 ± 11.5
Cisapride	1 μmol/kg	81.0 ± 4.5 [a]	91.4 ± 2.7
R-(−)-α-Methyl-histamine	10 μmol/kg	96.7 ± 13.1	90.4 ± 16.7

Results are expressed as means ± S.E.M. ($n = 8$ for each group).
[a] $P < 0.02$ compared to the control group (Mann-Whitney U-test).

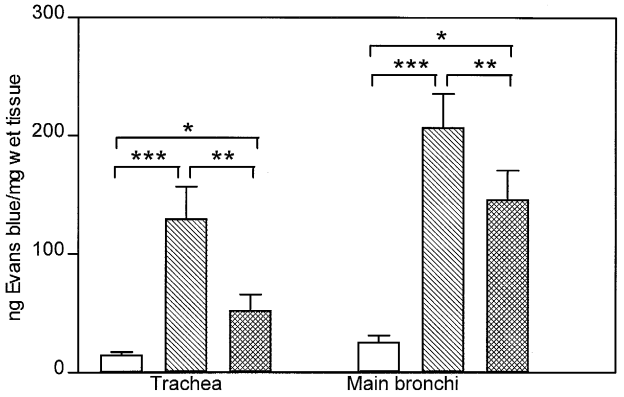

Fig. 3. Effect of intravenous pretreatment with ketanserin (1.8 μmol/kg body weight) on the increase in plasma protein extravasation induced by capsaicin, 100 μg/kg body weight i.v., in the trachea and main bronchi of Fisher 344 rats ($n = 8$ for each group; cross-hatched bars). Pretreatment with saline was used as control ($n = 8$; hatched bars), and saline/solvent treatment was used to assess baseline plasma protein extravasation ($n = 6$; open bars). Data are reported as means ± S.E.M. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$ (all Mann-Whitney U-test).

cantly reduced the capsaicin-induced increase in plasma protein extravasation in trachea and main bronchi by 67% and 34%, respectively (Fig. 3). Combination of the tachykinin NK_1 receptor antagonist RP 67580 (1 mg/kg body weight i.v.) with ketanserin (1.8 μmol/kg body weight i.v.) completely inhibited the increase in plasma protein extravasation caused by capsaicin (Fig. 4).

The selective 5-HT_{1A} receptor antagonists pMPPI (1 μmol/kg body weight i.v.) and NAN-190 (1 μmol/kg body weight i.v.), the 5-HT_3 receptor antagonist tropisetron (1 μmol/kg body weight i.v.), and the 5-HT_4 receptor antagonists GR 113808 (1 μmol/kg body weight i.v.) and

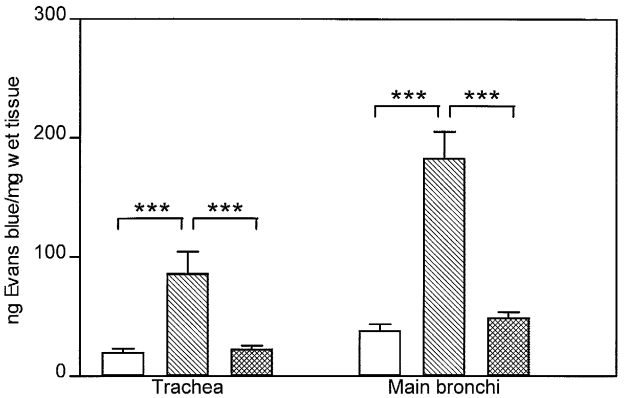

Fig. 4. Effect of intravenous pretreatment with the combination of RP 67,580 (1 mg/kg body weight) and ketanserin (1.8 μmol/kg body weight) on the increase in plasma protein extravasation induced by capsaicin, 100 μg/kg body weight i.v., in the trachea and main bronchi of Fisher 344 rats ($n = 8$ for each group; cross-hatched bars). Pretreatment with saline was used as control ($n = 9$; hatched bars), and saline/solvent treatment was used to assess the baseline plasma protein extravasation ($n = 6$; open bars). Data are reported as means ± S.E.M. $^{***}P < 0.005$ compared to the saline/solvent group (Mann-Whitney U-test).

Table 4
Effect of 5-HT receptor antagonists and a histamine H_3 receptor antagonist on the increase in plasma protein extravasation induced by capsaicin (100 μg/kg body weight i.v.) in the airways of Fisher 344 rats

Compound	Dose	Percentage of plasma protein extravasation following capsaicin	
		Trachea	Main bronchi
GR 113808	1 μmol/kg	115.9 ± 20.9	97.6 ± 7.7
NAN-190	1 μmol/kg	68.3 ± 17.6	69.0 ± 16.1
pMPPI	1 μmol/kg	93.5 ± 29.4	74.9 ± 19.1
SB 204070	1 μmol/kg	109.0 ± 20.9	92.6 ± 13.5
Tropisetron	1 μmol/kg	80.0 ± 19.1	93.3 ± 10.5
Thioperamide	2 mg/kg	137.6 ± 25.6	121.6 ± 19.8

Results are expressed as means ± S.E.M. ($n = 8$ for each group).

SB 204070 (1 μmol/kg body weight i.v.) had no effect on the increase in plasma protein extravasation induced by capsaicin (Table 4).

3.3. Characterisation of the histamine receptors involved in plasma protein extravasation in rat airways

Intravenous administration of a high dose of histamine (10 μmol/kg body weight i.v.) caused a small increase in plasma protein extravasation in Fisher 344 rat airways, which reached statistical significance in the main bronchi only. Lower doses of histamine (1 μmol/kg body weight i.v.) and the histamine H_3 receptor agonist *R*-(−)-α-methyl-histamine (10 μmol/kg body weight i.v.) had no significant effect on baseline plasma protein extravasation (Table 2).

The increase in plasma protein extravasation induced by capsaicin (100 μg/kg body weight i.v.) was not affected by pretreatment with either the histamine H_3 receptor agonist *R*-(−)-α-methyl-histamine (10 μmol/kg body weight i.v.) or the histamine H_3 receptor antagonist thioperamide (0.5 mmol/kg body weight i.p.) (Tables 3 and 4).

4. Discussion

In Fisher 344 rats, 5-HT_2 receptors are involved in the neurogenic increase in plasma protein extravasation in the airways, as demonstrated by the increase in baseline plasma protein extravasation elicited by 5-HT_2 agonists and by the inhibition of the capsaicin-induced increase in plasma protein extravasation by 5-HT_2 receptor antagonists.

We previously found that methysergide (0.1 mg/kg i.v.) and depletion of mast cell mediators with compound 48/80 had no significant effect on baseline plasma protein extravasation (Germonpré et al., 1995). These data suggest that neither serotonin nor histamine is a major contributor to baseline plasma protein extravasation in the trachea of the Fisher 344 rat.

5-Hydroxytryptamine, α-methyl-5-HT and 5-MeOT

caused a significant increase in plasma protein extravasation in the airways of the Fisher 344 rat. Although the 5-HT_2 receptor agonist α-methyl-5-HT can also stimulate 5-HT_4 receptors (Hoyer et al., 1994), this receptor appears not to be involved, as the selective 5-HT_4 receptor antagonist SB 204070 had no effect on the increase in plasma protein extravasation induced by 5-HT or 5-MeOT. The lack of effect on baseline plasma protein extravasation of cisapride, a 5-HT_4 receptor agonist with weak 5-HT_2 antagonism (Moriarty et al., 1987), further indicates that 5-HT_4 receptors are not involved. Pretreatment with the selective 5-HT_2 receptor antagonist ketanserin completely blocked the increase in plasma protein extravasation induced by 5-HT, α-methyl-5-HT and 5-MeOT. The 5-HT_1 agonists 5-CT, 8-OH-DPAT, and sumatriptan, as well as the 5-HT_3 receptor agonist 2-methyl-5-HT had no significant effect on baseline plasma protein extravasation. These findings show that activation of 5-HT_2 receptors increases plasma protein extravasation in the airways of the Fisher 344 rat.

The highly selective 5-HT_2 receptor antagonist ketanserin significantly reduced the capsaicin-induced increase in plasma protein extravasation in the central airways of the Fisher 344 rat. Previously, we have shown that the tachykinin NK_1 receptor antagonist (±)-RP 67,580 only partially inhibits the tachykinin-induced increase in airway plasma protein extravasation in Fisher 344 rats and that this effect is not due to non-specific effects such as calcium-channel blockade (Germonpré et al., 1995). Pretreatment of these rats with both (±)-RP 67,580 and ketanserin completely prevented the increase in plasma protein extravasation caused by capsaicin. These results indicate that the capsaicin-induced increase in baseline plasma protein extravasation is mediated by activation of both NK_1 receptors and 5-HT_2 receptors.

The capsaicin-induced increase in plasma protein extravasation in the airways of Fisher 344 rats is not modulated by either 5-HT_1, 5-HT_3 or 5-HT_4 receptors. Although the increase in plasma protein extravasation caused by stimulation of capsaicin-sensitive nerves in the dura mater of Sprague-Dawley rats can be inhibited by administration of sumatriptan or CP 93,129 (Buzzi and Moskowitz, 1990; Huang et al., 1993), we found no inhibition of the capsaicin-induced increase in plasma protein extravasation in the airways of the Fisher 344 rat when using similar doses of sumatriptan or CP 93,129. Ward and colleagues showed that the eNANC bronchoconstriction in guinea pigs is inhibited by activation of an 'atypical' 5-HT receptor, with a rank order of potency 5-CT ≥ 5-HT > 8-OH-DPAT > α-methyl-5-HT(EC_{50} for 5-CT = 0.13 μM) (Ward et al., 1994). Our results show that in Fisher 344 rat airways neither 5-CT, 8-OH-DPAT nor α-methyl-5-HT inhibited the neurogenic increase in plasma protein extravasation. Activation of 5-HT_3 receptors in the trachea of Sprague-Dawley rats facilitates the release of calcitonin gene-related peptide (Hua and Yaksh, 1993), a sensory neuropeptide which is co-stored and co-released with substance P (Maggi and Melli, 1988; Hua and Yaksh, 1992), and which potentiates the substance P-induced increase in plasma protein extravasation (Brokaw and White, 1992). Similarly, activation of 5-HT_3 receptors in guinea pig airways causes the release of endogenous peptides from capsaicin-sensitive nerve fibres in vivo (Buckner et al., 1991). Using the 5-HT_3 receptor agonist 2-methyl-5-HT and the selective 5-HT_3 receptor antagonist tropisetron, we were unable to detect a modulation of the capsaicin-induced increase in plasma protein extravasation by stimulation of 5-HT_3 receptors.

In this study, none of the agonists which failed to increase baseline plasma protein extravasation caused significant hypotension, and none of the agonists/antagonists which failed to modulate the capsaicin-induced rise in plasma protein extravasation significantly altered the fall in blood pressure induced by capsaicin (results not shown). This indicates that the lack of effect of the 5-HT_1, 5-HT_3 and 5-HT_4 receptor agonists and antagonists is not secondary to effects of these drugs on the systemic circulation. However, as changes in the calibre of airway blood vessels can occur without there being a major effect on the systemic blood pressure, we cannot rule out significant effects of 5-HT_1, 5-HT_3 and 5-HT_4 receptors on the blood flow supplying the central airways.

Although histamine is co-released with serotonin upon stimulation of airway mast cells with tachykinins (Joos and Pauwels, 1993), histamine is not involved in the neurogenic increase in plasma protein extravasation in the Fisher 344 rat airways. Saria et al. described an 8-fold increase in plasma protein extravasation in rat trachea upon intravenous injection of histamine (Saria et al., 1983). When using an even higher dose of histamine (10 μmol/kg body weight), we observed only a 1.7-fold increase of baseline plasma protein extravasation in the airways of Fisher 344 rats (compared to a 3- to 6-fold increase induced by serotonin at a dose of 0.5 μmol/kg body weight). Previously, we demonstrated that histamine H_1 and H_2 receptor antagonists have no effect on the capsaicin-induced increase in plasma protein extravasation in Fisher 344 rat airways (Germonpré et al., 1995). In contrast to the inhibition of the neurogenic increase in plasma protein extravasation in rat skin and guinea pig airways by prejunctional inhibition of neuropeptide release (Ichinose et al., 1990; Ohkubo et al., 1995), we found that neither the histamine H_3 receptor agonist *R*-(−)-α-methylhistamine, nor the histamine H_3 receptor antagonist thioperamide affected the increase in plasma protein extravasation induced by capsaicin in Fisher 344 rat airways.

In conclusion, tachykinins released upon activation of capsaicin-sensitive nerves in the airways of the Fisher 344 rat increase plasma protein extravasation through both a direct effect on NK_1 receptors on the venular endothelium and an indirect mechanism involving the release of serotonin. The released serotonin causes part of the capsaicin-

induced increase in plasma protein extravasation by stimulating 5-HT_2 receptors, whereas histamine plays no role in this neurogenic increase in plasma protein extravasation. In contrast to the observations for rat skin and dura mater, we found no evidence for prejunctional modulation of the neurogenic increase in plasma protein extravasation by either histamine or serotonin.

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