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Modulation by 5-HT_{1A} receptors of the 5-HT_2 receptor-mediated tachykinin-induced contraction of the rat trachea *in vitro*

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1 In the Fisher 344 rat, tachykinins have been shown to cause the release of 5-hydroxytryptamine (5-HT) from airway mast cells, which then causes direct smooth muscle activation as well as the release of acetylcholine from cholinergic nerves. The aim of the present study was to examine the modulatory effects of 5-HT receptors on the neurokinin A (NKA)-induced release of endogenous 5-HT and airway smooth muscle contraction in the isolated Fisher 344 rat trachea.

2 The selective 5-HT_2 receptor antagonist ketanserin (0.1 μM) produced an almost complete inhibition of the contractions caused by NKA ($n=4$, $P<0.0001$, two-way ANOVA), and a significant rightward shift of the concentration-response curve to 5-HT ($n=8$, $P<0.001$, two-way ANOVA).

3 The partial agonist for 5-HT_{1A} receptors, 8-OH-DPAT (1 μM), and the full agonist for 5-HT_1 receptors, 5-CT (0.3 μM), potentiated the submaximal contractions induced by the 5-HT_2 receptor agonist α-methyl-5-HT (0.1 μM) ($n=4$; $P<0.005$ and $P<0.05$, respectively). 8-OH-DPAT (1 μM), as well as the 5-HT_{1A} receptor antagonists pMPPI, SDZ 216525 and NAN-190 (0.1 μM each), caused significant inhibition of the tracheal contractions induced both by NKA (10 nM – 3 μM) and 5-HT (10 nM – 10 μM) ($n=4-10$). This suggests that activation of 5-HT_{1A} receptors potentiates the 5-HT_2 receptor-mediated contractions.

4 SDZ 216525 (0.1 μM) significantly reduced the maximal contraction produced by 1 μM NKA ($n=10$, $P<0.001$), without affecting the release of endogenous 5-HT. These data rule out the involvement of a 5-HT_{1A} receptor-mediated positive feedback mechanism of the 5-HT release from mast cells.

5 Even in the presence of atropine (1 μM), 8-OH-DPAT (1 μM) further reduced the maximal NKA-induced contraction ($n=4$, $P<0.0001$), while the contractions of the rat isolated trachea induced by electrical field stimulation and the concentration-response curve to carbachol were unaffected by pMPPI (0.1 μM), SDZ 216525 (0.1 μM), NAN-190 (0.1 μM) and 8-OH-DPAT (1 μM) ($n=4-6$). These data demonstrate that the 5-HT_{1A} receptor-mediated potentiation of contractile responses is not due to non-specific inhibition of airway smooth muscle contraction or to modulation of postganglionic nerve activation.

6 The selective 5-$HT_{1B/1D}$ receptor antagonist GR 127935, the selective 5-HT_3 receptor antagonist tropisetron and the selective 5-HT_4 receptor antagonists SB 204070 and GR 113808 (0.1 μM each) had no effect on the concentration-response curve for NKA ($n=6-10$), ruling out the involvement of 5-$HT_{1B/1D}$, 5-HT_3 and 5-HT_4 receptors.

7 The α-adrenoreceptor antagonist phentolamine (1 μM) had no effect on the 5-HT-induced contractions ($n=4$), ruling out the involvement of α-adrenoreceptors.

8 In conclusion, the tachykinin-induced contraction of the F334 rat isolated trachea is mediated by the stimulation of 5-HT_2 receptors. Activation of 5-HT_{1A} receptors located on airway smooth muscle potentiates the direct contractile effects of 5-HT_2 receptor activation. The 5-$HT_{1B/1D}$, 5-HT_3 and 5-HT_4 receptors are not involved in the NKA-induced contraction of rat airways.

Keywords: 5-HT receptors; tachykinins; neurokinin A; cholinergic nerve activation; bronchoconstriction; airway smooth muscle

Introduction

The tachykinins substance P and neurokinin A (NKA) are present in airway sensory nerves and are known to cause bronchoconstriction, vasodilatation, increased vascular permeability, increased secretion from submucosal glands and goblet cells, potentiation of cholinergic neurotransmission as well as stimulation of inflammatory cells. In view of these potential effects initiated by activation of sensory airway nerves, the tachykinins have been implicated in the pathogenesis of asthma (Barnes *et al*., 1991a, b; Frossard & Advenier, 1991; Solway & Leff, 1991; Joos *et al*., 1994a).

Previously, we demonstrated that tachykinins cause contraction of the Fisher 344 (F344) rat airways mainly by an NK_1 receptor-mediated, indirect mechanism involving the release of 5-hydroxytryptamine (5-HT) from mast cells (Joos *et al*., 1994b). *In vivo* depletion of sensory nerve fibres with capsaicin significantly inhibited the NKA-induced contraction of rat isolated trachea, which suggests that tachykinins can stimulate C-fibres by a positive feedback mechanism (Joos *et al*., 1997). We and others recently showed that activation of 5-HT_2 receptors causes both direct smooth muscle contraction and release of acetylcholine from cholinergic nerves in rat airways (Aas, 1983; Joos *et al*., 1988; 1994b; Szarek *et al*., 1995; Germonpré *et al*., 1996). Thus the contraction of the rat isolated trachea provides us with a model for activation of sensory and cholinergic nerve fibres, mast cell degranulation and direct smooth muscle activation.

Both in man and in rodents, prejunctional 5-HT receptors have been shown to modulate neurotransmitter release from

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cholinergic and non-cholinergic airway nerves (Fozard & Kilbinger, 1985; Buckner *et al*., 1991; Buzzi *et al*., 1991; Van Oosterhout *et al*., 1991; Hua & Yaksh, 1992; Rizzo *et al*., 1993; Szarek *et al*., 1993; Ward *et al*., 1994; Takahashi *et al*., 1995; Kojima & Shimo, 1996). These studies showed that 5-HT can have potentiating as well as inhibitory effects on both cholinergic and sensory nerves. Therefore, the aim of the present study was to examine the modulatory effects of 5-HT receptors on the tachykinin-induced contraction of the F344 rat trachea *in vitro*.

Methods

Animals

Fisher 344 (F344) rats were obtained from Harlan CBP (Zeist, The 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.

General procedure

The rats were killed by intraperitoneal injection of an overdose of pentobarbitone (Nembutal, 350 mg kg^{-1} body weight). The trachea and lungs were quickly removed. From the distal part of the trachea two rings, each containing three to four cartilaginous rings, were prepared. Each tracheal ring was placed in a 2 ml organ bath (Finney *et al*., 1985) containing Krebs solution (composition in mM: NaCl 118, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, $NaHCO_3$ 24.9, KH_2PO_4 1.15, glucose 5.5), which was maintained at 37°C and bubbled with carbogen (5% CO_2 in oxygen). The two rings displayed a similar response to contractile agents such as carbachol and 5-HT. The optimal resting tension was 0.75 g, as determined by length-tension experiments (Joos *et al*., 1994c). The rings were allowed to stabilize for 15 min at a resting tension of 0.75 g. After this stabilization period, carbachol was added to the organ bath in a non-cumulative way (0.33 μM, 3.3 μM and 33 μM). The contact time for each concentration was 15 min. Between administrations, an interval of 15 min was allowed, with two changings of the bathing medium. The contractions were measured isometrically with Grass FT03 transducers and recorded on a Graphtec Linearecorder type WR3701. The contractions induced by 33 μM carbachol were 1.67 ± 0.12 g mg^{-1} tissue ($n=8$).

Agonist-induced contractions

Concentration-response curves to NKA were made by adding increasing concentrations to the organ bath in a non-cumulative way (10 nM to 3 mM in steps of 0.5 log[concentration]) because it was observed that it was difficult to obtain sustained contractions to NKA. The contact time for each concentration was at least 5 min. Due to the occurrence of tachyphylaxis, only one concentration-response curve for NKA could be obtained. When the effects of selective 5-HT receptor antagonists (0.1 μM), were evaluated, concentration-response curves were made for NKA on two rings obtained from one animal, the antagonist being continuously present in one organ bath and the solvent in the other organ bath.

Increasing concentrations of carbachol (10 nM to 0.1 mM), 5-HT (10 nM to 10 μM) or 5-HT receptor agonists (10 nM to 0.1 mM) were added to the organ bath in a cumulative way (in steps of 0.5 log[concentration]), whereby the contact time for each concentration was at least 5 min. Preliminary experiments showed that two cumulative concentration-response curves for 5-HT could be constructed, with a 30 min interval, without the occurrence of tachyphylaxis (pEC_{50} curve 1, 6.53 ± 0.02 vs curve 2, 6.55 ± 0.02; E_{max} curve 1, $38.3 \pm 3.7\%$ vs curve 2, $39.7 \pm 2.8\%$ of the effect of carbachol 33 μM; $n=8$). The influence of atropine (1 μM), phentolamine (0.1 μM) or selective 5-HT receptor antagonists (0.1 μM) was studied by incubating the antagonists for 30 min before the construction of the second concentration-response curve. Parallel control experiments, whereby the respective solvent was added before the second dose-response curve was constructed, showed that the drug vehicles did not affect the tissue responsiveness.

Preliminary experiments showed that the selective 5-HT_2 receptor agonist α-methyl-5-HT caused a concentration-dependent contraction with pEC_{50} 6.29 ± 0.11 and E_{max} $18.64 \pm 3.08\%$ of the effect of carbachol 33 μM ($n=8$). In a separate set of experiments, we examined the effect of 5-HT_{1A} receptor agonists on the contraction produced by a submaximal concentration of α-methyl-5-HT (0.1 μM).

Only one agonist and one antagonist was tested in each preparation. The concentrations used were based on previously published data (Szarek *et al*., 1993; 1995; Hoyer *et al*., 1994; Joos *et al*., 1994c; Kung *et al*., 1994; 1995).

Transmural nerve stimulation

The tracheal rings were placed between two platinum electrodes. Electrical field current was delivered by a Grass S44 stimulator. Parameters used were: supramaximal voltage of 50 V, 230 mA, pulse duration 0.5 ms, frequency 1 to 50 Hz. Thirty second trains at increasing frequency were applied at 5 min intervals. Preliminary experiments showed that two frequency-response curves could be constructed, with a 30 min interval, without the occurrence of tachyphylaxis (E_{max} curve 1, $57.7 \pm 0.7\%$ vs curve 2, $57.0 \pm 1.2\%$ of the effect of carbachol 33 μM; frequency whereby 50% of the E_{max} was reached: curve 1, 3.8 ± 0.1 Hz vs curve 2, 3.7 ± 0.3 Hz; $n=4$). The effect of selective 5-HT receptor antagonists (0.1 μM) was studied by incubating the antagonists for 30 min before the construction of the second frequency-response curve.

In vitro *release of 5-HT*

At the moment of maximal contraction induced by NKA (1 μM), 100 μl was taken from the organ bath to measure 5-HT. Previous experiments showed that the maximal 5-HT release occurred from one min after application of NKA. 5-HT was measured by use of an enzymo-immunoassay which is based on the competition of acylated 5-HT and the acylated enzyme-conjugate 5-HT-acetylcholinesterase for an antibody against acylserotonin (Immunotech, Marseilles, France). The sensitivity of this assay is 0.5 nM.

Chemicals

The following drugs were used: capsaicin, carbachol (Sigma, St. Louis, MO), pentobarbitone (Ceva, Brussels, Belgium), 5-hydroxytryptamine creatine sulphate (Fluka AG, Buchs, Germany), 1-(2-methoxyphenyl)-4-[4-(2-phtalimmido) butyl] piperazine hydrobromide (NAN-190), 4-iodo-N-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-benzamide (pM PPI), tropisetron, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), 2-methyl-5-HT (Research Biochemicals International, Natick, MA), NKA (Peninsula, St. Helens, U.K.). 1-[2-(methylsulphonyl) amino] ethyl]4-piperidinyl]-

methyl1-methyl-1H-indole-3-carboxylate (GR 1138080) and N-[4-methoxy-3-(4-methyl-1-piprazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,-4-oxadiazol-3-yl) [1,1,-biphenyl]-4-carboxamide (GR 127935) were gifts from Glaxo (Stevenage, U.K.). Methyl-4(4-[4-(1,1,3-trioxo-2H-1,2-benziosothiazol-2-yl) butyl]-1-pi-perazinyl)1H-indole-2-carboxylate (SDZ 216-525) was a gift from Sandoz (Basel, Switzerland). 5-Carboxytryptamine (5-CT), ketanserin and (1-butyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate (SB 204070) were kindly provided by Janssen Pharmaceutica (Beerse, Belgium).

Stock solutions of 1 mM 8-OH-DPAT and 10 μM pMPPI were made in distilled water, which were further diluted in Krebs solution. NAN-190, GR 113808, GR 127935, phentolamine and SDZ 216525 were dissolved in dimethylsulphoxide (DMSO) at a concentration of 10 mM and further diluted in Krebs solution. A stock solution of 10 mM 5-CT was prepared in ethanol 70% and was further diluted with Krebs solution. A stock solution of NKA, 10 μM in distilled water, was kept at -20°C. Each day fresh dilutions of the peptide were made and kept on ice during the experiments. All other drugs and chemicals were dissolved in distilled water at a concentration of 10 mM and further diluted in Krebs solution.

Analysis of data

The contractile responses were expressed as percentages of the maximal contraction induced by carbachol. E_{max} was the maximum contractile effect, expressed as percentages of the contraction induced by 33 μM carbachol. Sigmoidal dose-response curves were fitted by non-linear regression analysis and pEC_{50} were derived with the GraphpadPrism program (Graphpad Inc., San Diego, CA). The results are presented as the mean $\pm$ s.e.mean.

Concentration-response curves for contractile agents in the absence and the presence of antagonists were compared by two-way analysis of variance (ANOVA). When significance was reached, the difference between the results at each concentration of the contractile agent and between the pEC_{50} values were assessed by Student's unpaired t test. Differences were regarded as significant when $P<0.05$. The statistical analyses were accomplished with SPSS for Windows 6.1.2 software (SPSS Inc., Chicago, Illinois).

Results

Modulation by 5-HT receptors of the contraction induced by NKA

The NKA-induced contraction was almost completely inhibited by the 5-HT_2 receptor antagonist ketanserin (0.1 μM) (pEC_{50} control 7.39 ± 0.21 vs ketanserin 6.93 ± 0.05 and E_{max} control $18.6\pm2.2\%$ vs ketanserin $2.0\pm0.2\%$ of the effect of carbachol 33 μM; $P<0.0001$, two-way ANOVA) (Figure 1). The selective 5-$HT_{1B/1D}$ receptor antagonist GR 127935, the selective 5-HT_3 receptor antagonist tropisetron, as well as the selective 5-HT_4 receptor antagonists SB 204070 and GR 113808 (0.1 μM each), had no effect on the concentration-response curve for NKA ($n=6-10$; Table 1).

The 5-HT_{1A} receptor antagonists pMPPI ($n=4$), SDZ 216-525 ($n=6$) and NAN-190 ($n=5$) (0.1 μM each) caused a significant inhibition of the maximal contraction induced by NKA (45%, 40% and 39% inhibition, respectively; $P<0.001$, $P<0.005$ and $P<0.0005$, two-way ANOVA, respectively) (Figure 2 and Table 2). 8-OH-DPAT (1 μM), had a small inhibitory effect on the concentration-response curve to NKA (12% inhibition of the maximal contraction to NKA), which did not reach statistical significance ($n=6$; $P=0.170$, two-way ANOVA) (Figure 2 and Table 2). The potent 5-HT_1 receptor agonist 5-CT, at a concentration which does not cause 5-HT_2 receptor-mediated contraction (3×10^{-7} M), had no effect on the concentration-response curve to NKA ($n=6$; $P=0.153$, two-way ANOVA).

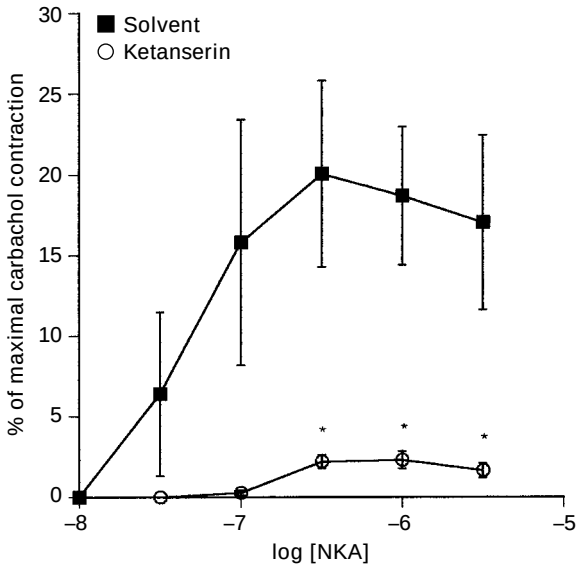

Figure 1 Effect of the 5-HT_2 receptor antagonist ketanserin on the contractile responses evoked by neurokinin A. The tracheal segments were untreated or incubated with 0.1 μM ketanserin for 30 min before the generation of concentration-response curves to neurokinin A. Each point represents the mean, and vertical lines s.e.mean, of 4 preparations and is expressed as a percentage of the contractile response to 33 μM carbachol ($P<0.0001$, two-way ANOVA). *Indicates values that are significantly different from values obtained in the control experiment ($P<0.05$).

Table 1 Effect of selective 5-HT receptor antagonists on the maximum response and pEC_{50} values obtained with NKA

	NKA	
Treatment	*pEC_{50}*	*E_{max} (%)*
GR 127935		
Control	7.32 ± 0.04	36.9 ± 0.6
0.1 μM	7.07 ± 0.12	32.2 ± 2.5
Tropisetron		
Control	7.16 ± 0.02	20.7 ± 2.2
0.1 μM	7.13 ± 0.13	23.8 ± 3.1
SB 204070		
Control	8.06 ± 2.19	20.8 ± 2.0
0.1 μM	7.22 ± 0.12	18.8 ± 1.0
GR 113808		
Control	7.16 ± 0.07	22.3 ± 3.9
0.1 μM	7.03 ± 0.04	28.9 ± 4.3

The pEC_{50} values and E_{max} values (percentage of the effect of carbachol 33 μM) shown represent the mean $\pm$ s.e.mean of 6–10 preparations.

Modulation of the 5-HT_2 receptor-mediated contractions by 5-HT_{1A} receptors

Ketanserin (0.1 μM), a selective competitive 5-HT_2 receptor antagonist, produced a rightward shift of the concentration-

response curve for 5-HT by approximately 2 log-units (pEC_{50}: control 6.47 ± 0.04 vs ketanserin 4.32 ± 0.13; $n = 8$ rings from 8 animals for each group; $P < 0.001$, two-way ANOVA) (Figure 3).

The 5-HT_{1A} receptor antagonists pMPPI ($n = 4$) and SDZ 216-525 ($n = 8$) (0.1 μM each) caused a significant inhibition of the maximal contraction obtained with 5-HT (67% and 58% inhibition, respectively; $P < 0.0001$ and $P < 0.001$, two-way ANOVA) (Figure 4 and Table 2). Another 5-HT_{1A} receptor antagonist, NAN-190 (0.1 μM; Figure 4 and Table 2), produced a rightward shift of the concentration-response curve for 5-HT by 0.4 log-units, which did not reach statistical significance ($n = 10$; $P = 0.055$, two-way ANOVA).

The 5-HT_{1A} receptor agonist 8-OH-DPAT failed to cause a significant contraction of the trachea up to a concentration of 10 μM ($n = 4$). Instead, 8-OH-DPAT (1 μM) casued a 44% inhibition of the concentration-response curve for 5-HT (Figure 4 and Table 2; $n = 6$; $P < 0.05$, two-way ANOVA). The contractions caused by a submaximal concentration (0.1 μM) of the selective 5-HT_2 receptor agonist α-methyl-5-HT, were significantly potentiated by 1 μM 8-OD-DPAT (solvent 0.64 ± 0.20% vs 8-OH-DPAT 5.35 ± 1.25% of the effect of carbachol 33 μM; $n = 8$; $P < 0.005$). The potent 5-HT_1 receptor agonist 5-CT, at a concentration which does not cause contraction of the rat trachea (0.3 μM), caused a similar potentiation of the contractile responses produced by α-

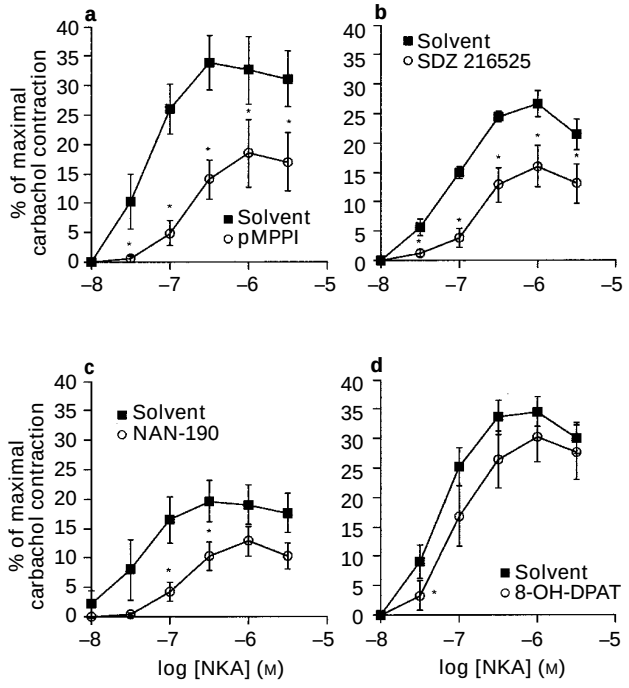

Figure 2 Effect of the 5-HT_{1A} receptor antagonists pMPPI (a), SDZ 216525 (b), NAN-190 (c) and the 5-HT_{1A} receptor agonist 8-OH-DPAT (d) on the contractile responses evoked by neurokinin A. The tracheal segments were untreated or incubated with 0.1 μM pMPPI, 0.1 μM SDZ 216525, 0.1 μM NAN-190 or 1 μM 8-OH-DPAT for 30 min before the generation of concentration-response curves to neurokinin A. Each point represents the mean, and vertical lines s.e.mean, of 4–6 preparations and is expressed as a percentage of the contractile response to 33 μM carbachol. *Indicates values that are significantly different from values obtained in the control experiment ($P < 0.05$).

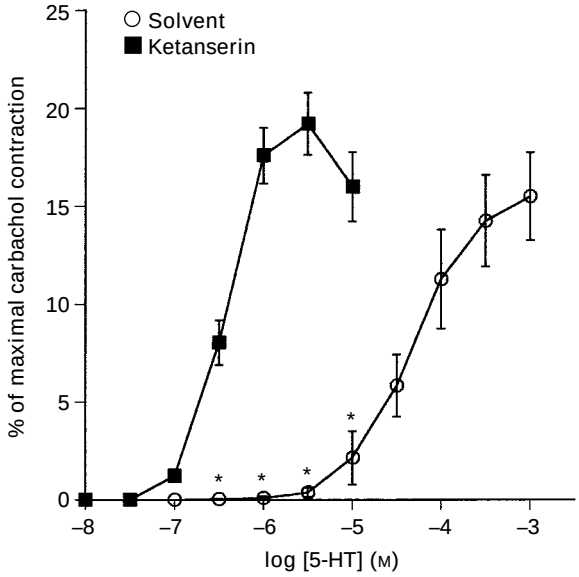

Figure 3 Effect of the 5-HT_2 receptor antagonist ketanserin on the contractile responses evoked by 5-HT. The tracheal segments were untreated or incubated with 0.1 μM ketanserin for 30 min before the generation of concentration-response curves to 5-HT. Each point represents the mean, and vertical lines s.e.mean, of 8 preparations and is expressed as a percentage of the contractile response to 33 μM carbachol ($P < 0.001$, two-way ANOVA). *Indicates values that are significantly different from values obtained in the control experiment ($P < 0.05$).

Table 2 Effect of 5-HT_{1A} receptor agonist and antagonists on the maximum response and pEC_{50} values obtained with NKA and 5-HT

	NKA		*5-HT*	
Treatment	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)
pMPPI				
Control	7.36 ± 0.07	32.7 ± 1.2	6.50 ± 0.04	37.3 ± 1.2
0.1 μM	6.79 ± 0.06*	17.8 ± 0.8*	5.91 ± 0.02*	12.2 ± 0.2*
SDZ 216525				
Control	7.16 ± 0.18	24.5 ± 2.3	6.40 ± 0.02	24.0 ± 0.5
0.1 μM	6.82 ± 0.11	14.5 ± 1.2*	6.23 ± 0.01*	10.0 ± 0.1*
NAN-190				
Control	7.40 ± 0.08	18.8 ± 0.7	6.40 ± 0.07	21.9 ± 1.2
0.1 μM	6.90 ± 0.10*	11.5 ± 1.0	6.00 ± 0.02*	19.7 ± 0.3*
8-OH-DPAT				
Control	7.30 ± 0.11	32.9 ± 1.8	6.54 ± 0.03	39.0 ± 0.9
1 μM	7.08 ± 0.06	28.9 ± 1.1	6.61 ± 0.05	21.8 ± 0.6

The pEC_{50} values and E_{max} values (percentage of the effect of carbachol 33 μM) shown represent the mean $\pm$ s.e.mean of 4–10 preparations. *Indicates values that are significantly different from values obtained in the control experiment ($P < 0.05$).

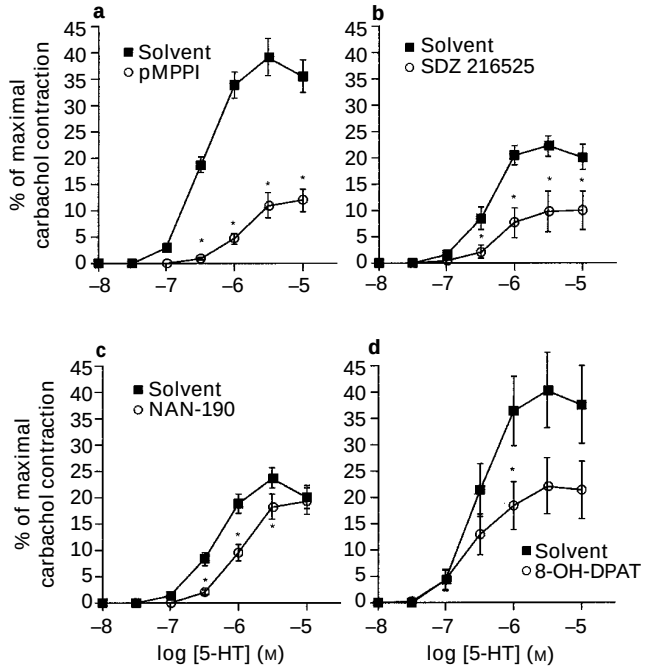

Figure 4 Effect of the 5-HT_{1A} receptor antagonists pMPPI (a), SDZ 216525 (b), NAN-190 (c) and the 5-HT_{1A} receptor agonist 8-OH-DPAT (d) on the contractile responses evoked by 5-HT. The tracheal segments were untreated or incubated with 0.1 μM pMPPI, 0.1 μM SDZ 216525, 0.1 μM NAN-190 or 1 μM 8-OH-DPAT for 30 min before the generation of concentration-response curves to 5-HT. Each point represents the mean, and vertical lines s.e.mean, of 4–10 preparations and is expressed as a percentage of the contractile response to 33 μM carbachol. *Indicates values that are significantly different from values obtained in the control experiment ($P<0.05$).

methyl-5-HT (solvent $1.65\pm0.83\%$ vs 5-CT $6.63\pm1.64\%$ of the effect of carbachol 33 μM; $n=8$; $P<0.05$).

Although 5-HT and a number of 5-HT receptor agonists have been known to interact with α-adrenoceptors (Briejer *et al*., 1995; Takahashi *et al*., 1995), the non-selective α-adrenoceptor antagonist phentolamine (0.1 μM) had no effect on the concentration-response curve for 5-HT (pEC_{50} solvent 6.40 ± 0.01 vs phentolamine 6.32 ± 0.10; E_{max} solvent 18.4 ± 3.3 vs phentolamine 17.2 ± 2.6; $n=4$).

Localization of the 5-HT_{1A} receptors involved in the facilitation of the 5-HT_2 receptor mediated tachykinergic contraction

The 5-HT_{1A} receptor antagonist SDZ 216525 (0.1 μM) significantly inhibited the contraction induced by 1 μM NKA. However, it had no effect on the release of 5-HT induced by NKA (Figure 5). These findings indicate that 5-HT_{1A} receptors are not involved in the release of endogenous 5-HT from the airway mast cells induced by NKA.

To examine whether the inhibitory effect of the 5-HT_{1A} receptor antagonists and the 5-HT_{1A} receptor agonist 8-OH-DPAT was due to inhibition of acetylcholine release from the postganglionic cholinergic nerves or a non-specific inhibition of the airway smooth muscle contraction, we studied the effect of these compounds on the contraction induced by transmural electrical field stimulation or exogenous carbachol. SDZ 216525, pMPPI, NAN-190 (0.1 μM each) and 8-OH-DPAT (1 μM) had no significant effect on either the contraction produced by electrical field stimulation or the concentration-response curve for carbachol (Tables 3 and 4).

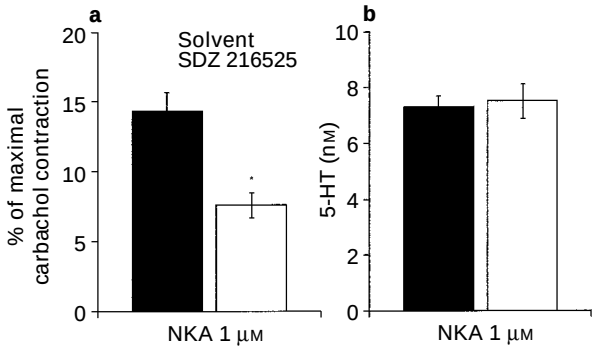

Figure 5 Effect of the 5-HT_{1A} receptor antagonists SDZ 216525 on the contractile responses (a) and the 5-HT release in the organ bath (b), evoked by 1 μM NKA. The tracheal segments were untreated or incubated with 0.1 μM SDZ 216525 for 30 min before the addition of NKA. Each column represents the mean$\pm$s.e.mean of 10 preparations and is expressed as a percentage of the contractile response to 33 μM carbachol (a) or the concentration of 5-HT (b). *Indicates values that are significantly different from values obtained in the control experiment. ($P<0.001$).

Table 3 Effect of 5-HT_{1A} receptor agonist and antagonists on the maximum response and pD_2 values obtained with carbachol

	Carbachol	
Treatment	pD_2	E_{max} (%)
pMPPI		
Control	6.47 ± 0.06	105.9 ± 2.9
0.1 μM	6.47 ± 0.07	105.5 ± 3.1
SDZ 216525		
Control	6.47 ± 0.06	110.0 ± 2.7
0.1 μM	6.44 ± 0.05	105.3 ± 2.4
NAN-190		
Control	6.75 ± 0.05	114.8 ± 2.0
0.1 μM	6.55 ± 0.04	108.7 ± 1.9
8-OH-DPAT		
Control	6.42 ± 0.05	111.3 ± 1.9
1 μM	6.26 ± 0.05	108.9 ± 1.9

The pD_2 values and E_{max} values (percentage of the effect of carbachol 33 μM) shown represent the mean$\pm$s.e.mean of 4–6 preparations.

In previous experiments we observed that atropine (1 μM) inhibits the maximal tension obtained with NKA (Joos *et al*., 1997). In the presence of atropine (1 μM), 8-OH-DPAT (1 μM) further reduced the maximal NKA-induced contraction (E_{max} atropine 11.4 ± 0.7 vs atropine with 8-OH-DPAT $5.9\pm1.0\%$ of the effect of carbachol 33 μM, pEC_{50} atropine 7.55 ± 0.24 vs atropine with 8-OH-DPAT 7.29 ± 0.25; $n=4$; $P<0.0001$, two-way ANOVA), indicating that the contraction induced by direct activation of 5-HT_2 receptors on airway smooth muscle is reduced by the partial 5-HT_{1A} agonist 8-OH-DPAT.

Discussion

In the present study we demonstrated that stimulation of 5-HT_{1A} receptors potentiates the 5-HT_2 receptor-mediated tachykinergic contraction of the Fisher 344 rat trachea *in vitro*.

In this study we observed an almost complete suppression of the NKA-induced contractions and a significant rightward

Table 4 Effect of a 5-HT_{1A} receptor agonist and antagonist on the contractions of the F344 rat trachea induced by electrical field stimulation

	SDZ 216525		*8-OH-DPAT*	
Frequency (Hz)	*Control*	*0.1 μM*	*Control*	*1 μM*
1	24.7±5.8	23.8±5.3	10.0±1.6	13.5±1.9
5	42.2±4.9	41.4±4.0	33.1±1.5	36.3±0.9
10	49.4±3.4	48.4±2.7	42.9±0.9	46.4±0.7
15	54.2±4.0	52.8±2.9	47.2±1.4	50.8±1.4
20	57.9±3.6	56.6±3.3	51.1±1.6	54.0±1.7
25	60.6±2.5	59.1±2.4	52.6±1.4	55.1±1.6
30	58.5±6.3	57.6±5.2	55.1±1.7	57.5±2.3
35	63.4±4.1	61.0±3.2	55.6±1.8	57.8±1.5
40	64.1±3.1	62.8±2.8	56.7±1.9	58.0±1.4
45	65.5±2.8	63.6±2.4	57.0±1.2	58.1±1.6
50	65.7±2.7	64.4±2.2	56.0±1.3	58.3±1.7

The data are expressed as % of the effect of carbachol 33 μM and represent the mean±s.e.mean of 4 preparations.

shift of the concentration-response curve to 5-HT by ketanserin. The competitive 5-HT_{2A} receptor antagonist ketanserin (0.1 μM) caused a 89% reduction of the maximal contractile response to the indirect agonist NKA. This suggests that the pool of endogenous 5-HT which is released by NKA is relatively small (Kenakin, 1993). The present observations as well as recent work from our group (Germonpré *et al*., 1996) confirm the involvement of 5-HT_2 receptors in the tachykinin-induced contractions of the rat trachea. Previously, we demonstrated that tachykinins contract the F344 rat airway mainly by an indirect mechanism involving the release of 5-HT from the airway mast cells. The endogenously released 5-HT causes direct smooth muscle contraction as well as release of acetylcholine from postganglionic cholinergic nerves in rat airways (Aas, 1983; Joos *et al*., 1988; 1994b; Joos & Pauwels, 1993). The 5-HT_2 receptor antagonist ketanserin, at a concentration of 0.1 μM, has been shown to have no effect on the cholinergic contractions in rat airways (Szarek *et al*., 1993). Our results are similar to the findings of Szarek *et al*. (1995), who found that, in the rat isolated intrapulmonary bronchi, exogenously applied 5-HT interacts both with 5-HT_2 receptors on the airway smooth muscle and with 5-HT_2 receptors on the cholinergic nerves, causing the release of acetylcholine.

The selective 5-HT_{1A} ligands pMPPI, SDZ 216525 and NAN-190 inhibited the contractions induced by both NKA and 5-HT. Although these compounds are generally considered as 5-HT_{1A} antagonists (Glennon *et al*., 1988; Schoeffter *et al*., 1993; Kung *et al*., 1994), they may behave as partial agonists depending on the system studied (Hjorth & Sharp, 1990; Lanfumey *et al*., 1993). The potent 5-HT_1 receptor agonist 5-CT potentiated the contractile responses produced by submaximal concentrations of the 5-HT_2 receptor agonist α-methyl-5-HT. 5-CT had no effect on the contractions induced by endogenous 5-HT released following application of NKA. 8-OH-DPAT, which has a 5-HT_{1A} agonist activity, does not produce a significant tracheal contraction up to a concentration of 10 μM. However, 8-OH-DPAT potentiated the contractions caused by submaximal concentrations of α-methyl-5-HT, while it inhibited the contractions evoked by both NKA and 5-HT. These findings confirm the observations of Hadrava *et al*. (1996) that 8-OH-DPAT acts as a partial agonist under conditions were receptors are fully activated. Thus we found that 5-HT_{1A} receptor agonists (5-CT and 8-OH-DPAT) potentiated the contractions evoked by a selective 5-HT_2 receptor agonist, while partial agonists and/or antagonists of the 5-HT_{1A} receptor inhibited the contractions evoked by endogenous or exogenous 5-HT. These results indicate that activation of 5-HT_{1A} receptors potentiates the 5-HT_2 receptor-mediated contractions.

The 5-HT_{1A} receptor antagonist SDZ 216525 significantly reduced the contraction induced by 1 μM NKA, but did not affect the release of endogenous 5-HT. This indicates that the 5-HT_{1A} receptor-mediated inhibition of the tachykinin-induced contraction is not due to inhibition of the endogenous 5-HT from the mast cells. Ward *et al*. (1994) described inhibition of the excitatory non-adrenergic non-cholinergic bronchoconstriction in guinea-pigs by an atypical 5-HT receptor, with rank order of potency 5-CT ≥ 5-HT > > 8-OH-DPAT > α-Me-5-HT. We found no effect of 5-CT on the NKA-induced bronchoconstriction in the rat isolated trachea. Prejunctional 5-HT_{1A} receptors in the guinea-pig proximal colon have been implicated in the inhibition of the acetylcholine release from enteric cholinergic neurones (Fozard & Kilbinger, 1985). However, we observed no effect of these selective 5-HT_{1A} compounds on the contractions induced by electrical field stimulation and, even in the presence of atropine, 8-OH-DPAT still caused inhibition of the 5-HT induced contractions. These observations suggest that 5-HT_{1A} receptors do not modulate acetylcholine release from cholinergic nerves in rat airways. Furthermore, the contractions induced by carbachol were not affected by the 5-HT_{1A} selective compounds, making a non-specific inhibition of smooth muscle contraction unlikely. We therefore conclude that stimulation of 5-HT_{1A} receptors on the airway smooth muscle, potentiates the bronchoconstriction induced by activation of 5-HT_2 receptors.

We observed no effect of the selective 5-$HT_{1B/1D}$ receptor antagonist GR 127935 on the contractions induced by NKA. In rat and guinea-pig dura mater, activation of 5-$HT_{1B/1D}$ receptors has been shown to inhibit the activation of the capsaicin-sensitive sensory nerve fibres (Buzzi *et al*., 1991). The NKA-induced contraction of rat isolated trachea was significantly inhibited by *in vivo* depletion of sensory nerve fibres with capsaicin, suggesting that tachykinins can stimulate the sensory C-fibres by a positive feedback mechanism (Joos & Pauwels, 1993; Joos *et al*., 1994c; 1997).

In our model, the selective 5-HT_3 receptor agonist 2-Me-5-HT had no contractile effect (data not shown), and the selective 5-HT_3 receptor antagonist tropisetron did not modulate the NKA-induced bronchoconstriction. These results suggest that there are no 5-HT_3 receptors present on the sensory nerve fibres, on the mast cells or on the airway smooth muscle cells in the rat trachea. Furthermore, 5-hydroxytryptaminergic activation of postganglionic cholinergic nerve fibres appears not to be modulated by 5-HT_3

receptors. However, in human and guinea-pig isolated airways 5-HT_3 receptors have been shown to facilitate the cholinergic bronchoconstriction induced by electrical field stimulation (Rizzo *et al*., 1993; Takahashi *et al*., 1995). *In vivo* studies in guinea-pigs suggested that 5-HT_3 receptors activate capsaicin-sensitive C-fibres (Buckner *et al*., 1991). Similarly, in rat trachea 5-HT_3 receptors increase the release of the sensory neuropeptide calcitonin gene-related peptide from capsaicin-sensitive nerves (Hua & Yaksh, 1992).

We found that, in the rat, the potent and selective 5-HT_4 receptor antagonists SB 204070 and GR 113808 had no effect on the tracheal contraction caused by NKA. Stimulation of the 5-HT_4 receptors located on the enteric nerves in the guinea-pig intestinal tract facilitates cholinergic neurotransmission (Briejer *et al*., 1995). In the guinea-pig colon, 5-HT_4 receptors appear to release tachykinins from the sensory nerve fibres (Kojima & Shimo, 1996). However, in the rat gastrointestinal tract 5-HT_4 receptors are located predominantly on smooth muscle and mediate relaxation (Briejer *et al*., 1995).

The non-selective α-adrenoceptor antagonist phentolamine, at a concentration which blocked the inhibitory effects of 5-HT on cholinergic contractions in human airways (Takahashi *et al*., 1995), did not affect the 5-HT-induced tracheal contraction, indicating that 5-HT does not interact with α-adrenoceptors in rat isolated trachea. Interaction of the biogenic amine 5-HT with β-adrenoceptors seems very unlikely, because this would cause a non-specific relaxation of the airway smooth muscle.

In conclusion, the 5-HT_2 receptor mediated tachykininergic contraction of rat isolated airways is facilitated by activation of 5-HT_{1A} receptors on airway smooth muscle. The 5-$HT_{1B/1D}$, 5-HT_3 and 5-HT_4 receptors do not appear to be involved in the contractions induced by tachykinins in the F344 rat trachea.

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