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Characterization and localization of thromboxane A_2 receptor in human and guinea-pig nasal mucosa using radiolabelled (+)-S-145

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1 TxA_2 receptor (TP-receptor) antagonists such as S-1452 and Bay u 3405 have been shown to be effective in alleviating nasal blockage in patients with allergic rhinitis as well as guinea-pig allergic rhinitis models. The present study was conducted to examine the existence and localization of the TP-receptor in human and guinea-pig nasal mucosa by *in vitro* receptor binding autoradiography using radiolabelled (+)-S-145, which is a potent and specific TP-receptor antagonist with an extremely slow dissociation rate.

2 We ascertained the binding specificity of [^{3}H]-(+)-S-145 in human and guinea-pig platelet membranes by comparing the ability of four TP-receptor ligands of U-46619, (+)-S-145, I-(+)-S-145 and Bay u 3405 to displace the specific binding of [^{3}H]-(+)-S-145 and [^{3}H]-U-46619. The rank order of potency (K_i) for the displacement was correlated highly with that determined from [^{3}H]-U-46619 binding to the same preparations.

3 Quantitative autoradiography using a radioluminographic imaging plate system, in which the radioactivity of [^{3}H]-(+)-S-145 is expressed as photostimulated luminescence (PSL) per area (mm^2), revealed that specific binding of [^{3}H]-(+)-S-145 to human and guinea-pig nasal mucosa was saturable. Scatchard analysis showed about three fold higher affinity and two fold greater maximal binding to the nasal mucosa of humans than that of guinea-pigs: the K_D and B_{max} values in human mucosa were 2.82 ± 0.35 nM and 6.47 ± 0.33 PSL mm^{-2} and those in guinea-pig mucosa were 8.23 ± 1.93 nM and 3.37 ± 0.66 PSL mm^{-2}, respectively.

4 Specific [^{3}H]-(+)-S-145 binding to cryostat sections of human and guinea-pig nasal mucosa was displaced by another TP-receptor antagonist, Bay u 3405, and a TP-receptor agonist, U-46619. The order of potency (K_i value: nM) was (+)-S-145 (2.5) > Bay u 3405 (15.4) >> U-46619 (359.6) in human nasal mucosa and (+)-S-145 (22.8) > U-46619 (49.8) $\approx$ Bay u 3405 (62.1) in guinea-pig nasal mucosa. These rank orders showed rather good correlation with those obtained for the respective platelet membranes.

5 Autoradiographs of human nasal mucosa demonstrated that specific [^{125}I]-(+)-S-145 binding sites mainly exist on the smooth muscle layers of venous sinusoids and arterioles in the lamina propria, with few or no binding sites in the epithelium and nasal gland.

6 We concluded that radiolabelled (+)-S-145 can be used as a TP-receptor ligand for autoradiographic study, and that the TP-receptor is exclusively located on smooth muscle layers of venous sinusoids and arterioles in the nasal mucosa. The potent vasoconstrictive activity of TxA_2 may cause reduction of local blood flow followed by mucosal oedema probably through mechanisms of vascular injury such as ischaemia-reperfusion.

Keywords: Thromboxane A_2; TP-receptor; S-1452; (+)-S-145; human nasal mucosa; guinea-pig nasal mucosa; receptor autoradiography

Introduction

Thromboxane (TxA_2), an arachidonic acid metabolite, is a potent inducer of platelet aggregation and constrictor of vascular and airway smooth muscles (Hamberg, 1975; Svensson *et al*., 1977), and TxA_2 is considered to be responsible for the pathogenesis of some cardiovascular and thromboembolic disorders and airway obstruction (Halushka & Lefer, 1987; Ogletree, 1987; Coleman *et al*., 1989). The biological effects of TxA_2 are mediated by the cell-surface TxA_2 receptor (TP-receptor) (Coleman *et al*., 1989), the cDNA of which has been cloned for man (Hirata *et al*., 1991), rats (Abe *et al*., 1995) and mice (Namba *et al*., 1992). Therefore, much effort has been directed towards the development of selective TP-receptor antagonists (Lefer & Darius, 1987; Narisada *et al*., 1988; Ohtani *et al*., 1991), and the antagonists have been reported to be effective in a variety of experimental models including coronary thrombosis, cerebral vasospasm, ischaemia and reperfusion arrhythmias, and allergic asthma (Coleman *et al*., 1989; Mihara *et al*., 1989; Nakajima & Ueda, 1989; Arimura *et al*., 1992; 1994a, b; Matsuo *et al*., 1996).

Recently, TP-receptor antagonists, S-1452 (Ca salt of (+)-S-145, Yasui *et al*., 1997), Bay u 3405 (Narita *et al*., 1996) and AA-2414 (Yamasaki *et al*., 1997), have been shown to inhibit antigen-induced nasal plasma exudation and nasal blockage in guinea-pig models, and to alleviate nasal symptoms dramatically, particularly nasal blockage in patients with allergic rhinitis (Terada *et al*., 1998). Thus, TxA_2 seems to play an important role in the pathogenesis of allergic rhinitis. The existence of the TP-receptor has been established in various

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tissues and cells such as vascular smooth muscle cells (Hanasaki *et al*., 1988), platelets (Hanasaki *et al*., 1989; Arita *et al*., 1989), lung membrane (Saussy *et al*., 1991) and kidney (Mannon *et al*., 1996) by binding studies with several radiolabelled TP-receptor ligands such as [^{3}H]-S-145 and [^{125}I]-IBOP, but there has been no account of the existence of a TP-receptor on nasal mucosa.

In the present study, to characterize the TP-receptor in nasal mucosa and establish its location, we performed *in vitro* receptor binding autoradiography on tissue sections from human nasal turbinate, removed at routine surgery, and guinea-pig nasal tissue using a radiolabelled (+)-isomer of S-145 as a TP-receptor ligand. [^{3}H]-(+)-S-145 was expected to be an appropriate ligand for *in vitro* autoradiography because general loss of the bound ligand during washing of the sections can be avoided, due to its slow dissociation rate from TP-receptor as well as high selectivity and affinity for TP-receptor (Kishino *et al*., 1991). Thus, to characterize the TP-receptor and examine the affinity of some TP-receptor agonist/antagonists in nasal mucosa, the well characterized [^{3}H]-(+)-S-145 was used for *in vitro* quantitative autoradiography, and [^{125}I]-(+)-S-145 was also used to make the specific binding sites clearly visible on X-ray films.

Methods

Radiolabelled ligands

(+)-(1**S**, 2**R**, 3**R**, 4**R**)-(5Z)-7-(3-[4-^{3}H]-phenylsulphonylamino-bicyclo[2.2.1.]hept-2-yl)hept-5-enoic acid sodium salt, [^{3}H]-(+)-S-145 sodium salt (18.5 Ci mmol^{-1}, 26.4 Ci mmol^{-1}, radiochemical purity 98.6%), was prepared in our laboratories, as described previously (Nagasaki *et al*., 1992). [^{125}I]-(+)-S-145, (1**S**,2**R**,3**R**,4**R**)-5Z)-7-(3-(4-[^{125}I]-iodo-phenylsulphonylamino)-bicyclo[2.2.1]hept-2-yl)hept-5-enoic acid, was synthesized according to the well-established procedure of D.E. Mais (1991). The synthesis of [^{125}I]-(+)-S-145 consists of N-sulphonation of (+)-(1**S**,2**R**,3**R**,4**R**)-(5Z)-7-(3-amino-bicyclo[2.2.1]hept-2-yl)hept-5-enoic acid methyl ester with 4-iodobenzenesulphonyl chloride to give the methyl ester of I-(+)-S-145 ((1**S**,2**R**,3**R**,4**R**)-(5Z)-7-(3-(4-iodo)-phenylsulphonylamino)-bicyclo[2.2.1]hept-2-yl)hept-5-enoic acid), substitution of iodine with a trimethylstannyl group using hexamethylditin to give key-compound for labelling and radio iodination with [^{125}I]-sodium iodide (Amersham)/chloramine-T followed by hydrolysis with lithium hydroxide on an ultra micro scale. Purification by high performance liquid chromatography (h.p.l.c.) (Nucleosil 5C_{18}, 4.6 mm × 15 cm, CH_3 CN : MeOH : H_2O : AcOH = 3 : 2 : 3 : 0.01, 1.5 ml min^{-1}, UV = 220 nm, Rt = 7.7 min.) gave [^{125}I]-(+)-S-145 (2.11 mCi, 2200 Ci mmol^{-1}, 99.2% radiochemical purity, in 42% radiochemical yield) which was identified by h.p.l.c. and t.l.c. [^{3}H]-U46619 (10–15 Ci mmol^{-1}) was obtained from Dupont/New England Nuclear (Boston, MA, U.S.A.).

Binding and competition studies on human and guinea-pig platelet membranes

Human and guinea-pig platelet membranes were prepared and the binding assays were performed as described previously (Kishino *et al*., 1991). Briefly, freshly drawn human or guinea-pig blood was mixed with EDTA (10 mM). Platelet-rich plasma was obtained by centrifugation at 160 *g* for 10 min, and then the platelets were resuspended in ice-cold lysing buffer (5 mM Tris-HCl, pH 7.4, containing 5 mM EDTA, 10 μM indomethacin and 0.3 mM PMSF) to give a protein concentration of 2 mg ml^{-1} and stored at −80°C until use. Each platelet membrane (40 μg) was incubated with various concentrations of [^{3}H]-(+)-S-145 and [^{3}H]-U46619 in a total volume of 0.2 ml for 90 min at 25°C. After the incubation, the reaction mixture was immediately harvested onto GF/C glass fibre filters (96-well Unifilter plate), and then the filters were dried and counted using TopCount with a MicroScint scintillation cocktail (Packard Instrument Company). Non-specific binding was defined as the binding in the presence of 10 μM unlabelled (+)-S-145 and U-46619. For completion study, the platelet membrane was incubated with 2 nM [^{3}H]-(+)-S-145 or 50 nM [^{3}H]-U-46619 in the presence of various concentrations of (+)-S-145, I-(+)-S-145, Bay u 3405 and U-46619. The half-maximal inhibitory concentrations (IC_{50} value) for the respective ligands in both human and guinea-pig platelet membranes were obtained from the displacement data, and the K_i values of each ligand were calculated with the Cheng-Prusoff equation (Cheng & Prusoff, 1973), using the respective K_D values obtained by Scatchard analysis and the IC_{50} values derived from each displacement curve. The K_D value for [^{3}H]-(+)-S-145 in human platelet membranes was determined in our previous work (Kishino *et al*., 1991) and the other three K_D values, i.e. that for [^{3}H]-(+)-S-145 in guinea-pig platelet membrane and those for [^{3}H]-U-46619 in both tissues, were determined in this study.

Tissue preparation of human and guinea-pig nasal mucosa

Human inferior turbinates were obtained at the time of surgery from three allergy patients with nasal obstructive syndromes. No patient had been taking any TP-receptor antagonists. The tissues were immediately frozen in liquid nitrogen and stored at −80°C. Guinea-pig nasal tissue was obtained from sensitized animals as the human nasal mucosa had been from patients with allergic rhinitis. Male Hartley guinea-pigs weighing 450–550 g, which purchased from Charles River Japan Inc. (Kanagawa, Japan), were sensitized to ovalbumin (OA) twice, 1 week apart, by inhalation of an aerosolized solution of 1% OA as described previously (Yasui *et al*., 1997). One week after the last sensitization, the animals were exsanguinated under anaesthesia with sodium pentobarbital. Nasal septum and nasal turbinate were removed quickly, snap-frozen in isopentane at −40°C and stored at −80°C until use.

Binding studies on cryostat sections of human and guinea-pig nasal mucosa

Frozen sections (15 μm) were cut with a cryostat at −20°C and thaw-mounted on poly-L-lysine coated glass slides. The sections were freeze-dried at −40°C for 24 h. After 15 min pre-incubation in 20 mM Tris-HCl buffer, pH 7.4, containing 135 mM NaCl and 20 mM $MgCl_2$, the sections were incubated for 90 min at room temperture with 0.25 to 30 nM of [^{3}H]-(+)-S-145 in the same buffer. Adjacent sections received [^{3}H]-(+)-S-145 containing an excess (100 μM) of unlabelled (+)-S-145 to define the non-specific binding. After incubation, the sections were transferred through four successive 1-min washings of buffer at 0°C, followed by a dip in ice-cold distilled water. The slides were then rapidly dried under a stream of cold air, and exposed to radioluminographic imaging plates (IP) (type BAS-TR2040; Fuji Photo Film, Tokyo, Japan) (Amemiya & Miyahara, 1988) at room temperature for 10 days. The autoradiographs were recorded and analysed with a radioluminographic IP system (Bio-imaging analyzer

BAS 2000, Fuji Photo Film, Tokyo, Japan) (Motoji *et al*., 1995a, b). For quantification of radioactivity, mucosal areas except cartilage and bone areas were encircled on the radiolumonographic images on the computer display, and the values of photostimulated luminescence (PSL) and the area were directly obtained with a computerized system (BAS 2000). The results were expressed as PSL per area (mm^2). The adjacent sections were stained with haematoxylin and eosin.

Competition studies on cryostat sections of human and guinea-pig nasal mucosa

Under the same assay conditions, consecutive 15 μm cryosections of human and guinea-pig nasal mucosa were incubated with 4 nM [^{3}H]-(+)-S145 in the absence or presence of various concentrations of (+)-S-145, Bay u 3405 and U-46619. The slides were dried and exposed to radioluminographic IPs. Binding radioactivity of [^{3}H]-(+)-S-145 was quantified as mentioned above. The IC_{50} values for the respective ligands were obtained from the displacement data, and the K_i values of each ligand were calculated using the Cheng-Prusoff equation (Cheng & Prusoff, 1973).

Autoradiographic localization studies

Cryostat sections (15 μm) of nasal mucosa obtained from two of three subjects with allergic rhinitis were cut, thawed, and mounted on glass slides. Sections were incubated with 1.5 nM [^{125}I]-(+)-S-145 for 90 min at room temperature in 20 mM Tris HCl buffer containing 135 mM NaCl and 20 mM $MgCl_2$ (pH 7.4). Non-specific binding was determined by parallel incubation of slides in the presence of 100 μM unlabelled (+)-S-145. Following incubation, the slides were washed four successive times, for 1 min, in fresh ice-cold buffer, followed by ice-cold distilled water, and then dried under a stream of cold air. These dried and labelled sections were exposed to X-ray film (Fuji Photo Film, Tokyo, Japan) for three days at room temperature. The adjacent sections were also stained with haematoxylin and eosin.

Drugs

The sodium salt of (+)-S-145 [(+)-(1**S**, 2**R**, 3**R**, 4**R**)-(5Z)-7-(3-phenyl sulphonylamino-bicyclo[2.2.1.]hept-2-yl)hept-5-enoic acid] I-(+)-S-145 ((+)-(1**S**, 2**R**, 3**R**, 4**R**)-(5Z)-7-(3-(4-iodo)-phenyl sulphonylamino)-bicyclo[2.2.1.]hept-2-yl)hept-5-enoic acid) and Bay u 3405 ((+)-(3**R**)-3-p-fluorobenzenesulphonamido)-tetrahydrocarbazole-9-propionic acid), and U-46619 (1, 5, 5-hydroxy-11α, 9α-(epoxymethano) prosta - 5Z, 13E - dienoic acid) were synthesized in our laboratories.

Data analysis

All values are expressed as mean ± s.e.mean. Linear regression analysis of the binding data was performed according to a standard method (Nelder & Mead, 1965).

Results

Competitive inhibition of [^{3}H]-(+)-S-145 and [^{3}H]-U-46619 binding in human and guinea-pig platelet membranes

Before starting autoradiographic studies on the human and guinea-pig nasal mucosa, we first ascertained the binding specificity of [^{3}H]-(+)-S-145 in human and guinea-pig platelet membranes by comparing the ability four TP-receptor agonist/antagonists of U-46619, (+)-S-145, I-(+)-S-145 and Bay u 3405 to displace the specific binding of [^{3}H]-(+)-S-145 and [^{3}H]-U-46619, another specific TP-receptor ligand. All four TP-receptor ligands inhibited the specific binding of both [^{3}H]-(+)-S-145 and [^{3}H]-U-46619 in human and guinea-pig platelet membranes, with the rank orders of binding affinity of four ligands assessed by the K_i value being I-(+)-S-145 > (+)-S-145 > Bay u 3405 > U-46619 for human platelet membranes, and I-(+)-S-145 > (+)-S-145 > Bay u 3405 ≈ U-46619 for guinea-pig platelet membranes (Table 1). The negative logarithms of the K_i values of these four TP-receptor ligands against [^{3}H]-(+)-S-145 binding correlated highly with the corresponding K_i values for inhibition of [^{3}H]-U-46619 binding in both human ($r = 0.99$) and guinea-pig ($r = 0.98$) platelet membranes (Figure 1).

Quantitative autoradiography of [^{3}H]-(+)-S-145 binding in cryostat sections of human and guinea-pig nasal mucosa

The [^{3}H]-(+)-S-145 binding to cryostat sections of human and guinea-pig nasal mucosa were determined using a radioluminographic imaging plate system. The radioluminographs representing total binding of [^{3}H]-(+)-S-145 to human nasal turbinate showed high densities in the lamina propria of nasal mucosa and diffuse staining on the epithelium and nasal glands (Figure 2a and b). The [^{3}H]-(+)-S-145 binding was almost completely abolished by an excess of unlabelled (+)-S-145 (Figure 2c). The radioluminographs of nasal septum and

Table 1 Properties of inhibition of [^{3}H]-(+)-S-145 and [^{3}H]-U-46619 binding to human and guinea-pig platelet membranes by selective TP-receptor ligands

	K_i (nM)			
	Human platelet membrane		*Guinea-pig platelet membrane*	
Compound	*[^{3}H]-(+)-S-145* (2 nM)	*[^{3}H]-U-46619* (50 nM)	*[^{3}H]-(+)-S-145* (2 nM)	*[^{3}H]-U-46619* (50 nM)
(+)-S-145	0.34 ± 0.04	0.69 ± 0.26	0.49 ± 0.08	1.25 ± 0.14
I-(+)-S-145	0.060 ± 0.006	0.124 ± 0.011	0.098 ± 0.004	0.195 ± 0.042
Bay u 3405	0.50 ± 0.02	1.62 ± 0.36	2.38 ± 0.33	7.85 ± 0.73
U-46619	81.8 ± 5.96	76.0 ± 11.30	2.93 ± 0.22	5.63 ± 1.53

K_i values were calculated according to the equation of Cheng & Prusoff (1973), using IC_{50} values derived from inhibition data and dissociation constants (K_D values) obtained by Scatchard analysis. The K_D values for [^{3}H]-(+)-S-145 in human and guinea-pig platelet membranes were 0.20 and 0.54 nM and those for [^{3}H]-U-46619 were 12.3 and 16.9 nM, respectively (see Methods). The values are the means ± s.e.mean of 3 determinants.

turbinate of guinea-pig showed dense binding sites of [^{3}H]-(+)-S-145 in the nasal mucosa and no or few binding sites in the cartilage of the nasal septum or the bone of nasal turbinates (Figure 2d and e). A significant amount of binding was displaced by an excess of unlabelled (+)-S-145 (Figure 2f).

Binding radioactivity was quantified with the mucosal areas except for cartilage and bone areas, if any, encircled on images on the computer display, and expressed as PSL mm^{-2}. Figure 3a and c show the total, non-specific and specific binding of [^{3}H]-(+)-S-145 to human and guinea-pig nasal mucosa. The specific bindings were saturable in human and guinea-pig tissues and represented 90.5±1.0% and 62.5±2.0% of the total binding at 10 nM [^{3}H]-(+)-S-145, respectively (n=3). Scatchard analysis of these data indicated the existence of a single class of binding sites for [^{3}H]-(+)-S-145 in both tissues (Figure 3b and d), and showed almost three fold higher affinity and two fold more maximal binding to human nasal mucosa than that of guinea-pig: the K_D and B_{max} values in human tissues were 2.82±0.35 nM and 6.47±0.33 PSL mm^{-2} and those in guinea-pig tissues were 8.23±1.93 nM and 3.37±0.66 PSL mm^{-2}, respectively.

Competitive inhibition of [^{3}H]-(+)-S-145 binding in cryostat sections of human and guinea-pig nasal mucosa

Competition studies were performed to compare the ability of TP-receptor antagonists, (+)-S-145 and Bay u 3405, and TP-receptor agonist, U-46619, to displace the specific [^{3}H]-(+)-S-145 binding to sections of human and guinea-pig nasal mucosa. As shown in Figure 4, these three TP-receptor ligands inhibited the specific binding of [^{3}H]-(+)-S-145 to cryosections of both tissues in a concentration-dependent manner, and the K_i values are shown in Table 2. The rank order of potency for inhibition of binding to the human nasal mucosa was (+)-S-145 > Bay u 3405 > > U 46619 and that of binding to guinea-pig nasal mucosa was (+)-S-145 > U46619 ≈ Bay u 3405. These rank orders for inhibition of the binding to cryostat sections of human and guinea-pig nasal mucosa by these three compounds display relatively good correlation with those of the binding to their platelet membranes.

Autoradiographic localization of [^{125}I]-(+)-S-145 binding sites in human nasal mucosa

Since the radioluminographs of [^{3}H]-(+)-S-145 were too dim to estimate the detailed distribution of radioactivity, we used ^{125}I-labelled (+)-S-145 for *in vitro* autoradiography of human nasal mucosa instead. The autoradiographs showed dense labelling of [^{125}I]-(+)-S-145 binding sites on the smooth muscle layer of venous sinusoids and arterioles in

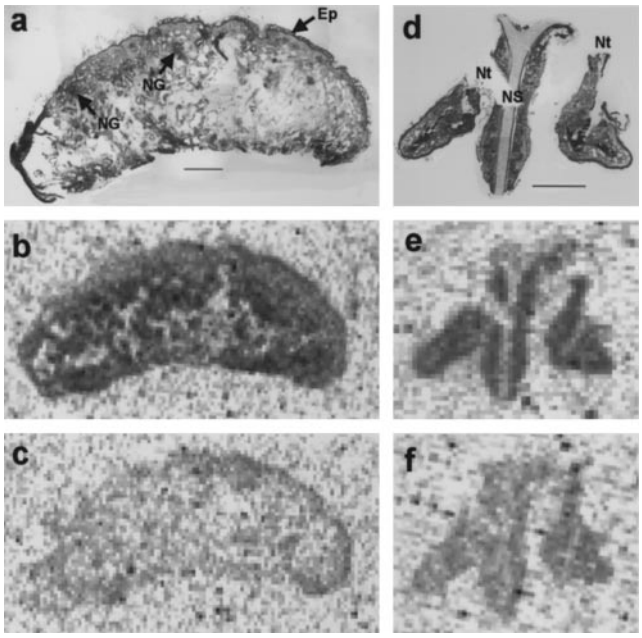

Figure 2 Autoradiographs of [^{3}H]-(+)-S-145 (30 nM) binding in human and guinea-pig nasal tissue printed directly from radioluminographic imaging plates. Photomicrographs show haematoxylin and eosin-stained sections of human (a) and guinea-pig nasal tissue (d), and corresponding radioluminographs showing total binding (b, e) and non-specific binding (c, f) defined by (+)-S-145 (100 μM). Ep = Epithelium, NG = nasal gland, Nt = nasoturbinate, NS = nasal septum. Scale bar = 1 mm.

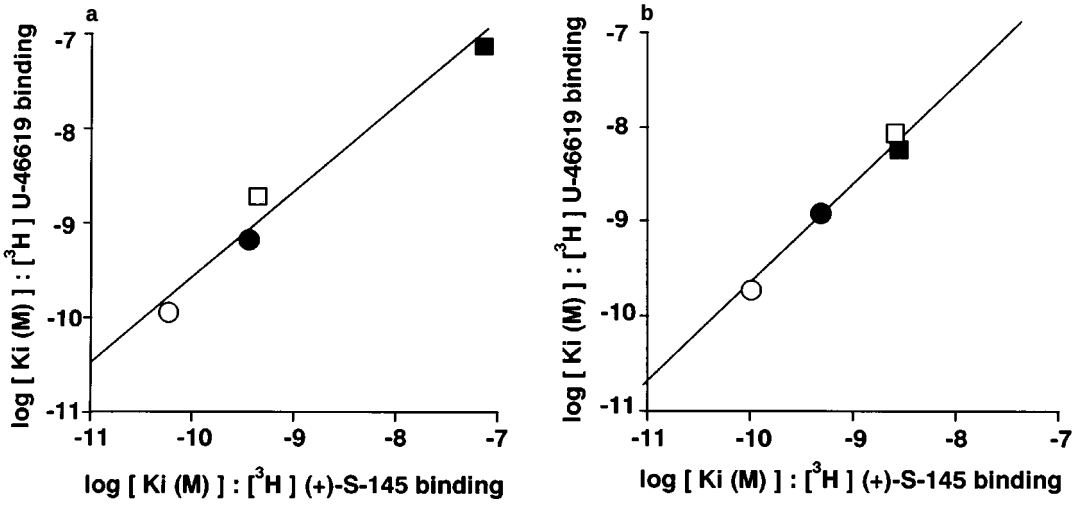

Figure 1 Correlation between negative logarithms of the K_i values of the four TP-ligands against [^{3}H]-(+)-S-145 binding and those against [^{3}H]-U-46619 binding in human (a) and guinea-pig (b) platelet membranes. The correlation coefficients between the negative logarithms of the K_i values for (+)-S-145 (solid circles), I-S-145 (open circles), U-46619 (solid squares) and Bay u 3405 (open squares) against [^{3}H]-(+)-S-145 binding and those against [^{3}H]-U-46619 binding were 0.99 and 0.98 in human and guinea-pig platelet membranes, respectively. The values are the means of 3 determinants.

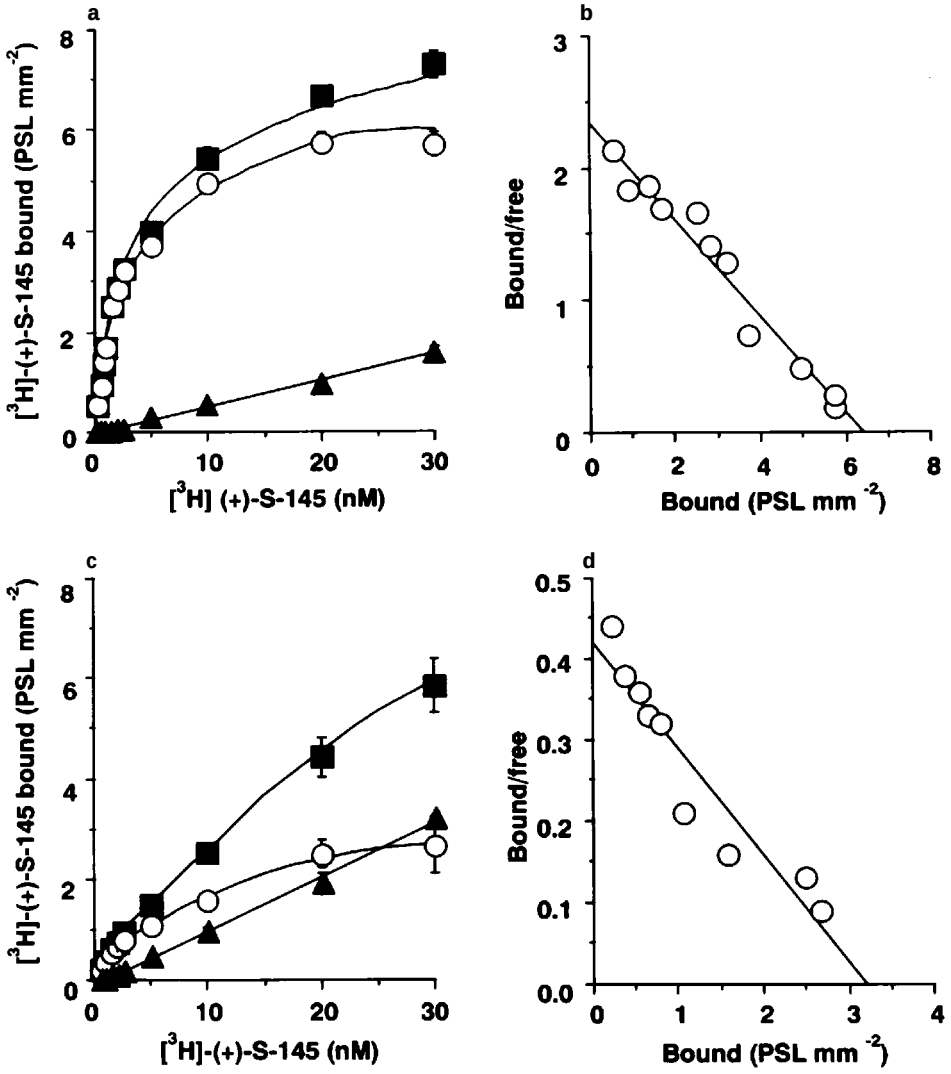

Figure 3 Saturation binding curves for [^{3}H]-(+)-S-145 to cryostat sections of human (a) and guinea-pig nasal mucosa (c). The graph shows total binding (solid squares), specific binding (open circles) and non-specific binding (solid triangles) defined by (+)-S-145 (100 μM). Scatchard transformation (b, d) of specific binding from data in (a) and (c), respectively. Data are means and vertical lines s.e.mean from 3 subjects and 3 animals. K_D and B_{max} values are shown in the text.

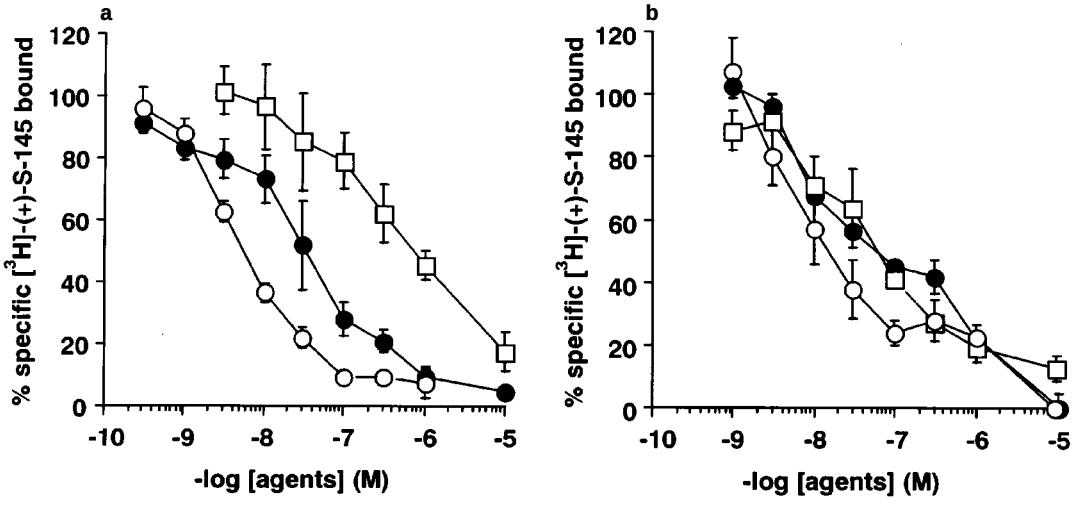

Figure 4 Competition binding curves for [^{3}H]-(+)-S-145 (4 nM) to cryostat sections of human (a) and guinea-pig nasal mucosa (b) by (+)-S-145 (open circles), Bay u 3405 (solid circles) and U-46619 (open squares). K_i values are shown in Table 2. Data are means and vertical lines s.e.mean from 3 subjects and 3 animals.

Table 2 Inhibition constants (K_i) from the displacement of [^{3}H]-(+)-S-145 binding to cryostat sections of human and guinea-pig nasal mucosa by selective TP-receptor ligands

Compound	K_i (nM) *Human nasal mucosa*	*Guinea-pig nasal mucosa*
(+)-S-145	2.5±0.14	22.8±11.94
Bay u 3405	15.4±7.39	62.1±17.77
U-46619	359.6±170.8	49.8±16.97

The values are the means±s.e.mean from 3 subjects and 3 animals.

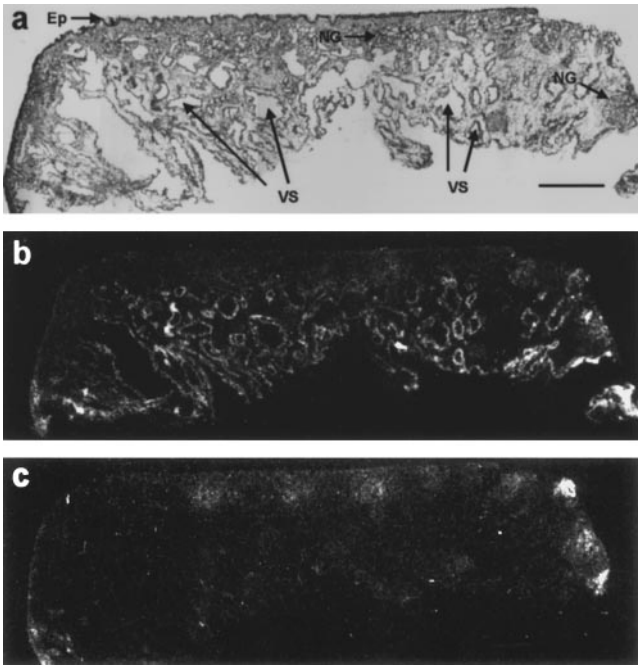

Figure 5 Autoradiograph detection of binding sites for [^{125}I]-(+)-S-145 (1.5 nM) in human nasal tissue. The photomicrograph shows haematoxylin and eosin-stained sections of human nasal turbinate (a), and corresponding autoradiographs showing total binding (b) and non-specific binding (c) defined by (+)-S-145 (100 μM). Ep = epithelium, NG = nasal gland, VS = venous sinusoid. Scale bar = 1 mm.

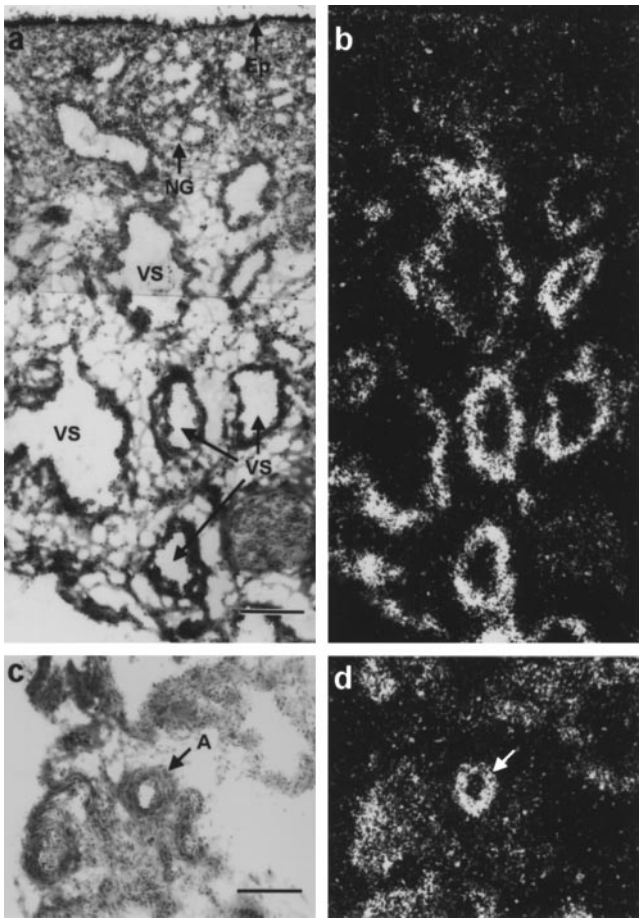

Figure 6 Autoradiographic detection of binding sites for [^{125}I]-(+)-S-145 (1.5 nM) in human nasal tissue. The photomicrographs show higher magnifications of the nasal mucosa shown in Figure 5 (a, b) and deeper area of lamina propria in nasal mucosa obtained from another patient (c, d). Ep = epithelium, NG = nasal gland, VS = venous sinusoid, A = arterioles. Scale bar = 0.2 mm.

the lamina propria, but no or little labelling on the epithelium or nasal glands (Figures 5 and 6). The arterioles were defined by the presence of an internal elastic membrane in the smooth muscle layer by staining with Elastica van Gieson (data not shown). The addition of excess unlabelled (+)-S-145 almost completely displaced the [^{125}I]-(+)-S-145 binding (Figure 5c).

Discussion

This study clearly demonstrated that the TP-receptor exists in human and guinea-pig nasal mucosa by means of receptor autoradiography using radiolabelled (+)-S-145. Among a variety of TP-receptor ligands, we chose (+)-S-145 for the *in vitro* receptor autoradiography, because it has been shown to be a selective TP-receptor antagonist with not only high-affinity binding to the receptor but also an extremely slow dissociation rate from it (Kishino *et al*., 1991). This prevents general loss of the bound ligand during washing of the sections and minimizes background in autoradiographic studies.

We first ascertained the binding specificity of [^{3}H]-(+)-S-145 in human and guinea-pig platelet membranes by comparing the ability of various TP-receptor agonist/antagonists to displace the specific binding of [^{3}H]-(+)-S-145 with that of [^{3}H]-U-46619, which has often been used as a radioligand to characterize TP-receptor on human platelets (Kattelman *et al*., 1986; Liel *et al*., 1987; Morinelli *et al*., 1987; Hanasaki *et al*., 1989). All four TP-receptor ligands used in this study including unlabelled I-(+)-S-145 inhibited the specific binding of [^{3}H]-(+)-S-145 and [^{3}H]-U-46619 to human and guinea-pig platelet membranes. The rank orders of potency for inhibition of the binding in platelet membranes were determined as I-(+)-S-145 > (+)-S-145 > Bay u 3405 > U-46619 for humans, and as I-(+)-145 > (+)-S-145 > Bay u 3405 ≈ U-46619 for guinea-pigs. The negative logarithms of the K_i values of these four TP-receptor ligands against [^{3}H]-(+)-S-145 binding correlated well with the corresponding K_i values for inhibition of [^{3}H]-U-46619 binding in both human and guinea-pig platelet membranes. These findings indicated that [^{3}H]-(+)-S-145 is a useful radioligand for further characterization of TP-receptor in human and guinea-pig tissues and that [^{125}I]-(+)-S-145 can also be useful as a TP-receptor radioligand.

Quantitative autoradiography of [^{3}H]-(+)-S-145 binding to cryostat sections of the human and guinea-pig nasal tissue was performed to establish the existence of the TP-receptor and to characterize it. The radioactivity derived from bound [^{3}H]-(+)-S-145 was determined with a radioluminographic imaging plate system which has been established to be a very sensitive system for soft β particles emitted from either ^{3}H or ^{14}C and to be suitable for quantitative autoradiography because of the good relationship between radioactivity and PSL (Motoji *et al*., 1995b). The radioluminographs demonstrated specific [^{3}H]-(+)-S-145 binding to both crystat sections of human and guinea-pig nasal mucosa. They showed dense labelling of the binding sites in the lamina propria and diffuse staining on the epithelium and nasal glands in human nasal mucosa. In guinea-pig nasal tissue, labelling was not found on the cartilage of nasal septum nor on the bone of nasal turbinates; the radioluminographs did not clearly show any binding sites. Saturation studies revealed a single site for [^{3}H]-(+)-S-145 binding to both human and guinea-pig nasal mucosa. The K_D values obtained from Scatchard analysis indicate that the affinity of (+)-S-145 to human nasal mucosa is almost three times higher than that to guinea-pig tissue.

Several TP-receptor antagonists such as S-1452 (Ca salt of (+)-S-145), Bay u 3405 and AA-2414 have been shown to be effective in the treatment of allergic rhinitis in several guinea-pig models and also in patients (Narita *et al*., 1996; Yasui *et al*., 1997; Yamasaki *et al*., 1997; Terada *et al*., 1998). Therefore, we carried out competitive studies to estimate the affinity of Bay u 3405 and U-46619, a TxA_2 mimetic, as well as (+)-S-145. The rank order of potency for inhibition of specific [^{3}H]-(+)-S-145 binding to the human nasal mucosa was (+)-S-145 > Bay u 3405 >> U 46619 and that obtained in guinea-pig nasal mucosa was (+)-S-145 > U 46619 $\approx$ Bay u 3405. The rank orders of affinity in nasal mucosa of both species correlated well with those obtained in the respective platelet membranes, favouring the possibility that these three ligands recognize a common receptor existing on both nasal mucosa and platelet membrane. Recently, Hirata *et al*. (1996) identified two isoforms of the TP-receptor, TXRα and TXRβ, in human platelets which are produced by alternative splicing of the carboxyl terminus. They also demonstrated that both isoforms activated phospholipase C, but differently regulate adenylyl cyclase activity; TXRα activates adenylyl cyclase, while TXRβ inhibits it. Despite these functional differences, they showed similar binding characteristics, indicating that the subtypes of TP-receptor on nasal mucosa cannot be clarified using a binding technique.

Since the radioluminographs representing the binding sites of [^{3}H]-(+)-S-145 were too dim to estimate the detailed distribution of radioactivity, even though they were sufficient for quantitative autoradiography to characterize the TP-receptor, we used ^{125}I-labelled (+)-S-145 for *in vitro* autoradiography of human nasal mucosa instead of [^{3}H]-(+)-S-145 and exposed the radiolabelled sections to X-ray films. The autoradiographs clearly demonstrated that [^{125}I]-(+)-S-145 binding sites were located on the smooth muscle layer of venous sinusoids and arterioles in the lamina propria, whereas little or no labelling was detected on either the epithelium or nasal glands. Whether the location of TP-receptor differs between human and guinea-pig nasal mucosa is not clear, because we could not clearly show [^{125}I]-(+)-S-145 binding sites in guinea-pig nasal mucosa under the same experimental conditions due to a high background and the tissue being too small for detailed estimation (data not shown).

In guinea-pig models, a TxA_2 mimetic compound, U-46619, has been shown to cause plasma exudation in nasal mucosa and consistently increase intranasal pressure (Yasui *et al*., 1997; Yamasaki *et al*., 1997). Cui *et al*. (1997) have demonstrated that plasma exudation in guinea-pig airway induced by systemic administration of U-46619 is associated with the increased local blood flow which is caused by the increased inflow pressure in the aorta due to its potent vasoconstrictor activity, but not by vasodilatation. Thus, TxA_2-mediated plasma exudation in guinea-pig nasal mucosa may occur via the same mechanism as observed in airway. In contrast, there has been no study on the TxA_2-mediated increase in nasal vascular permeability or nasal airway resistance in man as yet. This suggests that a different mechanism underlies the TxA_2-mediated nasal blockage in subjects with allergic rhinitis.

The venous sinusoids, which express abundantly TP-receptors on the smooth muscle layers, are thought to be capacitance vessels of the nasal mucosa and their progressive distention in response to allergic mediators such as histamine, substance P and prostaglandin D_2 results in corresponding nasal airway obstruction (Atkinson & Kaliner, 1995). TxA_2 is known to be produced by local antigen challenge in nasal mucosa of subjects with allergic rhinitis (Brown *et al*., 1987), but a vasoconstrictor TxA_2 itself is not likely to cause nasal congestion directly via an increase in nasal blood flow, since vasoconstrictors such as α_1-adrenoceptor agonists, used as decongestants, generally improve nasal potency in patients with allergic rhinitis by reduction of nasal blood flow. However, prolonged use of the vasoconstrictor has been demonstrated to cause chronic rhinitis, secondary hyperaemia, and nasal mucosal irritability, the so-called 'rhinitis medicamentosa' (Atkinson & Kaliner, 1995), suggesting that repeated stimulation of nasal mucosa by TxA_2 generated by each antigen exposure may likewise cause nasal blockage, although the changes in nasal blood flow following antigen challenge in patients with allergic rhinitis are contradictory (Konno *et al*., 1982; Bende *et al*., 1984; Holmberg *et al*., 1988; Rangi *et al*., 1990), which may depend on the position and/or time of nasal mucosa in which the blood flow was measured. The precise mechanism of the adverse effect of the vasoconstrictor remains uncertain, but it may involve the nasal oedema triggered by vascular injury such as ischaemia-reperfusion injury, which may occur when reduced local blood flow spontaneously recovers to a normal level following disappearance of the vasoconstrictor or inversely increases due to other allergic mediators possessing a vasodilator effect in the nasal mucosa. Further experiments will be necessary to elucidate the above possibility.

In conclusion, we demonstrated here that the TP-receptor is located on the smooth muscle layer of venous sinusoids and arterioles in human nasal mucosa. The efficacy of TP-receptor antagonists against nasal blockage in clinical studies might result from suppression of nasal mucosal oedema, which is probably caused by ischaemia-reperfusion injury triggered by a decrease in blood flow due to TxA_2 generated by antigen exposure.

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