British Journal of Pharmacology (1998) 123, 984–990

Regulation of H_1-receptor coupling and H_1-receptor mRNA by histamine in bovine tracheal smooth muscle

J.L. Pype, L.J. Dupont, [1]J.C.W. Mak, [1]P.J. Barnes & [2]G.M. Verleden

Katholieke Universiteit Leuven, Laboratory of Pneumology, O&N, Herestraat 49, 3000 Leuven, Belgium and [1]National Heart and Lung Institute, Department of Thoracic Medicine, Dovehouse Street, London SW3 6LY

1 Pretreatment of bovine tracheal smooth muscle (BTSM) with histamine (1–100 μM, 1 h) induced a concentration-dependent desensitization of the contractile response to subsequently administered histamine, with a reduction of the maximum response of $72 \pm 8\%$ ($n = 5$) following pre-exposure to 100 μM histamine. In contrast, concentration-response curves to the muscarinic agonist, methacholine were not affected following histamine pretreatment, indicating a homologous desensitization. Furthermore, concentration-response curves to NaF, a G-protein activator, were not altered following histamine pre-incubation.

2 The histamine H_1-receptor (H_1R) desensitization could be antagonized by mepyramine (an H_1-receptor antagonist, 1 μM) but not by cimetidine (an H_2-receptor antagonist, 10 μM), indicating that the desensitization occurred via stimulation of histamine H_1-receptors, without evidence for the involvement of histamine H_2-receptors.

3 Indomethacin (10 μM) did not block the H_1R desensitization, suggesting no involvement of prostaglandins. Furthermore, histamine pre-incubation in calcium free medium still induced a functional uncoupling of H_1R.

4 GF 109203X, a protein kinase C (PKC) inhibitor, and H-7, a non-selective kinase inhibitor, did not antagonize the homologous H_1R desensitization.

5 The steady-state level of H_1R mRNA, assessed by Northern blot analysis, was not affected by prolonged histamine exposure (100 μM, 0.5, 1, 2, 4, 16 and 24 h).

6 These results suggest that histamine induces desensitization of the H_1R at the level of the receptor protein, which involves a mechanism independent of PKC, PKA, PKG and calcium influx, suggesting the involvement of a receptor-specific kinase.

Keywords: Histamine H_1-receptor; desensitization; airway smooth muscle contraction; gene expression; protein kinase C

Introduction

Histamine-induced contractions in airway smooth muscle are mediated via histamine H_1- receptors (H_1R), which belong to the superfamily of G protein-coupled receptors. Stimulation of H_1R leads to the formation of inositol 1,4,5, triphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol 4,5 biphosphate hydrolysis (Leurs *et al*., 1995). IP_3 increases intracellular Ca^{2+} from the endoplasmic reticulum, via an interaction with a specific receptor, resulting in an initial transient contractile response, while DAG activates protein kinase C (PKC), which is believed to underlie the sustained phase of the smooth muscle contraction (Rasmussen *et al*., 1987). Moreover, PKC seems to play a role in the desensitization of many G-protein-coupled receptors (Chuang *et al*., 1996) and has also been shown to mediate transcriptional down-regulation of some G-protein-coupled receptors (Habecker *et al*., 1993; Koman *et al*., 1993; Rousell *et al*., 1995). Phorbol esters, for instance, have been shown to attenuate H_1R-mediated inositol phosphate formation or to mediate an inhibition of rises in intracellular calcium upon H_1R stimulation, in a number of immortalized cell lines (Smit *et al*., 1992; 1996; Dickenson & Hill, 1993; McCreath *et al*., 1994; Zamani *et al*., 1995). These inhibitory effects of phorbol esters could all be antagonized with selective PKC inhibitors, implicating the involvement of PKC. The mechanism underlying the desensitization process is believed to be a result of receptor phosphorylation. The recent cloning of the genes encoding for human, rat, guinea-pig and bovine histamine H_1 receptors (Yamashita *et al*., 1991; Fujimoto *et al*., 1993; Horio *et al*., 1993; Fukui *et al*., 1994) made it possible to perform more detailed studies into the molecular mechanisms underlying desensitization. The primary sequence of the H_1R protein revealed the existence of seven putative transmembrane domains with a characteristic large third cytoplasmatic loop, which is thought to interact with a G-protein. The third cytoplasmic loop has several serine and threonine residues, which may serve as sites for phosphorylation by various protein kinases (Yamashita *et al*., 1991). H_1R-mediated responses were also found to be affected by exposure to histamine, although the exact mechanism still remains to be elucidated. However, some evidence has already been obtained that homologous H_1R desensitization involves a PKC-independent mechanism (Smit *et al*., 1992; 1996; Dickenson & Hill, 1993; Zamani *et al*., 1995). McGrath *et al*. (1994) also ruled out an involvement of protein kinase A, G and calcium calmodulin-dependent protein kinase II (CaMKII). On the contrary, a recent study in GT1-7 neuronal cells suggested that histamine-induced H_1R desensitization is mediated by an influx of extracellular calcium and by CaMKII, since incubation in calcium free medium as well as KN-62, a putative inhibitor of CaMKII, could prevent desensitization in GT1-7 neuronal cells (Zamani & Bristow, 1996).

In this study, we have used bovine tracheal smooth muscle strips to investigate the homologous short-term desensitization process of the H_1R and the potential role of protein kinase A, G, C and calcium influx. Moreover, since we demonstrated that prolonged PKC activation, with phorbol 12,13 dibutyrate (PDBu), could down-regulate H_1R mRNA (Pype *et al*., 1995a),

[2] Author for correspondence.

we have also examined the effect of prolonged histamine exposure on the H_1R mRNA level.

Methods

Tissue preparation and incubation

Bovine tracheae were obtained from a local abattoir and placed at room temperature in oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit (KH) solution of the following composition (mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.5 and glucose 5.05. Calcium-free medium was prepared by substituting the Ca^{2+} normally present in the Krebs solution with an equivalent molar concentration of Mg^{2+}, with the addition of the calcium chelator, ethylene glycol - O,O′ - bis(2 - aminoethyl) - N,N,N′,N′ - tetraacetic acid (EGTA, 1 mM). The tracheal smooth muscle was dissected free of epithelium and connective tissue and smooth muscle pieces (2×2 mm) and strips (3×10 mm) were prepared in KH solution at room temperature. The bovine tracheal smooth muscle (BTSM) was exposed to one or more of the following agents: histamine (1 – 100 μM), SKF-91488 (100 μM), indomethacin (10 μM), mepyramine (1 μM), cimetidine (10 μM), GF 109203X (1 μM) or H-7 (50 μM). Mepyramine and cimetidine were added 10 min before histamine, whereas GF 109203X, H-7 and indomethacin were added 1 h before histamine.

Contractile responses

After a 1 h incubation time, the strips were washed and mounted in 10 ml organ baths containing oxygenated KH at 37°C. A resting tone of 2 g was applied. The tissues were allowed to equilibrate for 30 min, during which time they were washed with fresh KH every 10 min and cumulative concentration-response curves (CRC) were performed to histamine (10 μM – 10 mM), metacholine (10 nM – 1 mM) or NaF (10 – 80 mM). Only one CRC was constructed for each tissue. Isometric contractils responses were measured with a Grass FT 0.3 force-displacement transducer and visualized on a computer screen by use of a commercially available software programme (Codas; Dataq Instruments, Inc., Ohio). The results are expressed as ratio of mg contraction to mg dry weight (24 h, 60°C).

Northern blot analysis

Total RNA was isolated by phenol/chloroform extraction and isopropanol precipitation (Chomczynski & Sacchi, 1987). Poly $(A)^+$ RNA was prepared by use of the PolyTract mRNA system kit (Promega, Southampton, U.K.) according to the manufacturers' instructions. Northern blots to Hybond-N filters (Amersham, U.K.) were prepared subsequent to size fractionation by gel electrophoresis of the denatured mRNA on 1% agarose/formaldehyde gels containing 20 mM morpholinosulphonic acid (MOPS), 5 mM sodium acetate and 1 mM EDTA (pH 7). A 1.2 kb (EcoRI/SacI) fragment specific to the bovine H_1R mRNA and a 1.2 kb (PstI/PstI) fragment specific to rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, were used as probes for Northern blot analysis.

Prehybridizations and hybridizations were carried out at 42°C with the labelled probes ($1 - 2 \times 10^6$ c.p.m. ml^{-1}) in a buffer containing 50% formamide, 4 × standard sodium citrate (SSC), 50 mM Tris-HCl (pH 7.5), 5 × Denhardt's solution, 0.1% sodium dodecyl sulphate (SDS), 5 mM EDTA and 250 μg ml^{-1} denatured salmon sperm DNA. Following hybridization, the blots were washed to a stringency of 0.1 × SSC, 0.1% SDS at 55°C before exposure to X-OMAT-S film. After suitable exposure time, the autoradiographs were analysed by laser densitometry (PDI, NY, U.S.A.).

Drugs

The drugs used in these experiments were obtained from the following sources: histamine diphosphate salt, metacholine chloride, indomethacin, ethylene glycol-O,O′-bis(2-aminoethyl)-N,N,N′,N′-tetraacetic acid (EGTA), mepyramine, cimetadine and sodium fluoride (Sigma Chemical Co, Bornem, Belgium), SKF-91488 2HC1 (4-(N,N-dimethylamino)butylisothiourea, Research Biochemicals Inc., Sanver Tech, Boechout, Belgium), GF 109203X (3-[1-[3-(dimethylamino)propyl]-1H-indol-3-yl]-4 - (1H-indol-3-yl)-1H-pyrrolyl-2,5-dione) and H-7 (1-(5-isoquinolinylsulphonyl)-2-methyl-piperazine; Biomol, Sanver Tech, Boechout, Belgium). All drugs were dissolved in distilled water except for GF 109203X which was dissolved in dimethylsulphoxide (DMSO) and indomethacin which was dissolved in alkaline phosphate buffer (pH 7.8) of the following composition (mM): KH_2PO_4 20, Na_2HPO_4 120. All control tissues were incubated with an equivalent amount of appropriate vehicle.

Data analysis

All data are expressed as mean ± s.e.mean. Tissue preparations of at least three different animals were used for each type of contraction experiment. EC_{50} values were determined in individual experiments and used to calculate mean values. Statistical analysis was carried out by unpaired t tests or analysis of variance as appropriate, probability values of $P < 0.05$ were considered significant.

Results

Histamine (10 μM – 10 mM) induced a concentration-dependent contraction of BTSM with an EC_{50} value of 81.7 ± 1.1 μM. Pretreatment with histamine (1 – 100 μM, 1 h) induced a concentration-dependent desensitization of the contractile response to subsequently administered histamine, with a reduction of the maximum response of 72 ± 8% ($n = 5$) following pre-exposure to 100 μM histamine (Figure 1a). A recovery of the contractile response after desensitization with 100 μM histamine was observed following a 1 h washing period (Figure 1b). On the other hand, concentration-response curves (CRC) to methacholine, a muscarinic agonist, were not affected following histamine pretreatment (results not shown), indicating a homologous desensitization.

Co-incubation of the tissue with mepyramine (1 μM, an H_1R antagonist) blocked the histamine-induced desensitization, suggesting that the desensitization process occurred via H_1Rs (Figure 2). However, it has also been shown that an elevation of tissue cyclicAMP levels can inhibit inositol phosphate responses to histamine in bovine tracheal smooth muscle slices (Hall *et al*., 1989). Since histamine H_2-receptors are positively coupled to adenylate cyclase, experiments were also performed with cimetidine, a selective histamine H_2-receptor antagonist. However, desensitization following histamine pretreatment (100 μM, 1 h) could not be blocked by cimetidine (Figure 2), suggesting no role for histamine H_2-receptor-induced adenosine 3′ : 5′-cyclic monophosphate (cyclicAMP) in the desensitization process.

To investigate whether the desensitization was mediated by prostaglandins, which has been shown in primate tracheal

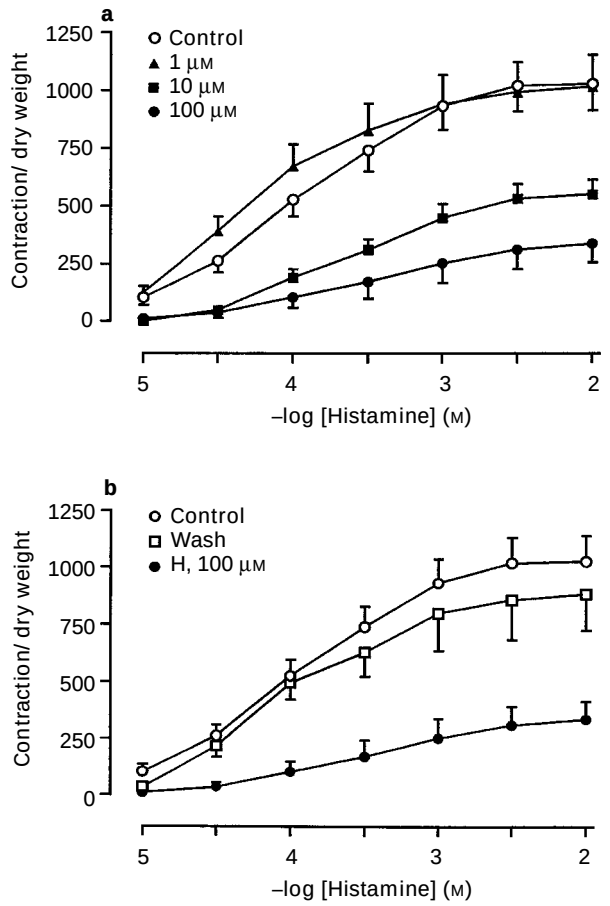

Figure 1 (a) Histamine-induced concentration-response curves in bovine tracheal smooth muscle (BTSM) after 1 h pretreatment with histamine 100 μM ($P<0.001$ versus control), 10 μM ($P<0.01$ versus control) and 1 μM ($P=$ not significant). Data shown are the mean and vertical lines s.e.mean of $\geqslant 4$ different experiments. Values are expressed as ratio of mg contraction to mg dry weight (24 h, 60°C). (b) Histamine-induced concentration-response curves in bovine tracheal smooth muscle (BTSM) after 1 h pretreatment with 100 μM histamine (H). A 1 h washing period, following desensitization with 100 μM histamine for 1 h (wash-out), resulted in a recovery of histamine-induced contractions of bovine tracheal smooth muscle strips. Data shown are the mean and vertical lines s.e. mean of 3 different experiments. Values are expressed as ratio of mg contraction to mg dry weight (24 h, 60°C).

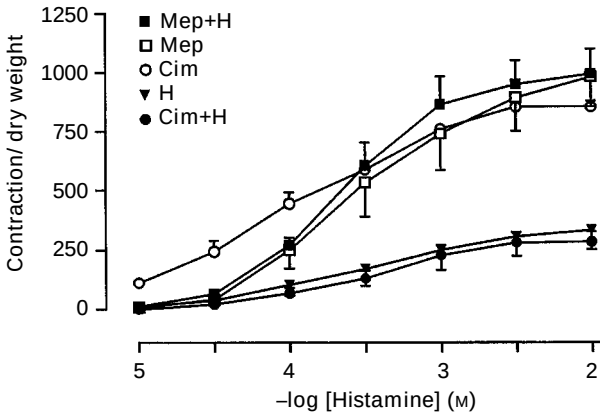

Figure 2 Desensitization of the H_1R following a 1 h pre-exposure to histamine (H, 100 μM) in the presence of mepyramine (mep + H, 1 μM added 10 min, before histamine) or cimetidine (cim + H, 10 μM added 10 min before histamine). Mepyramine (mep) and cimetadine (cim) on their own, had no significant effect on the histamine-induced concentration-response curve (CRC). Data shown are the mean and vertical lines s.e.mean of $\geqslant 4$ different experiments. Values are expressed as ratio of mg contraction to mg dry weight (24 h, 60°C).

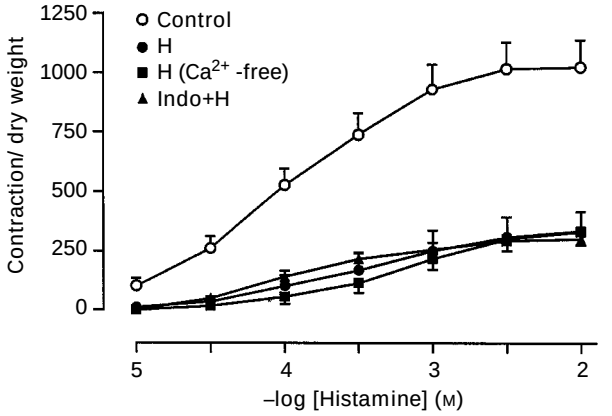

Figure 3 Histamine-induced concentration-response curves (CRC) following pretreatment with histamine (H, Ca^{2+}-free, 100 μM for 1 h) in Ca^{2+}-free KHS or in the presence of indomethacin (indo + H, 10 μM added 1 h before histamine) versus histamine-induced CRC in control and histamine (H)-pretreated tissue. Incubation in Ca^{2+}-free KHS or in the presence of indomethacin had no effect on the histamine-induced concentration-response curve in control tissue. Data shown are the mean and vertical lines s.e.mean of $\geqslant 4$ different experiments. Values are expressed as ratio of mg contraction to mg dry weight (24 h, 60°C).

smooth muscle (Krzanowski *et al*., 1980), experiments were performed with indomethacin (10 μM), an inhibitor of prostaglandin synthesis. As demonstrated in Figure 3, desensitization following histamine (100 μM, 1 h) pre-exposure still occurred in the presence of indomethacin, indicating no involvement of prostaglandins in the observed desensitization process. Histamine pretreatment of BTSM in calcium-free medium did not prevent the desensitization of the H_1R, suggesting no role for extracellular calcium in the regulation of the H_1R desensitization (Figure 3).

To investigate the possible site of the H_1R desensitization (at the level of the receptor or at a site distal in the signalling pathway), CRC to NaF, a G-protein activator, were performed. NaF induced a concentration-dependent contraction of BTSM with an EC_{50} of 15.9 ± 1.2 mM. Pre-exposure to histamine (100 μM, 1 h) had no effect on the NaF-induced contraction of BTSM, suggesting a histamine-induced desensitization at the level of the H_1R or at the interaction with its G-protein (Figure 4).

Pre-incubation with GF 109203X (1 μM, 1 h) failed to antagonize the histamine-induced desensitization (Figure 5), suggesting no role for PKC in the histamine-induced desensitization of the H_1R. Moreover, the non-selective protein kinase inhibitor H-7 (50 μM) was also without significant effect on the desensitization of the histamine-induced contractile reponse (Figure 5). Neither GF 109203X nor H-7, on its own, had any effect on the concentration-response curve to histamine (results not shown).

Furthermore, we investigated the possibility that prolonged histamine exposure could affect H_1R gene expression in BTSM. We measured the steady-state level of H_1R mRNA by Northern blot analysis, following a 0.5, 1, 2, 4, 16, 24 h histamine (100 μM) pretreatment. Northern blot analysis on mRNA extracted from BTSM revealed a band of 3.0 kb in size, corresponding to an H_1R mRNA, in agreement with findings of Yamashita *et al*. (1991) (Figure 6). Figure 6 also demonstrates the lack of effect of histamine (100 μM), in the presence of a selective histamine N-methyltransferase inhibitor

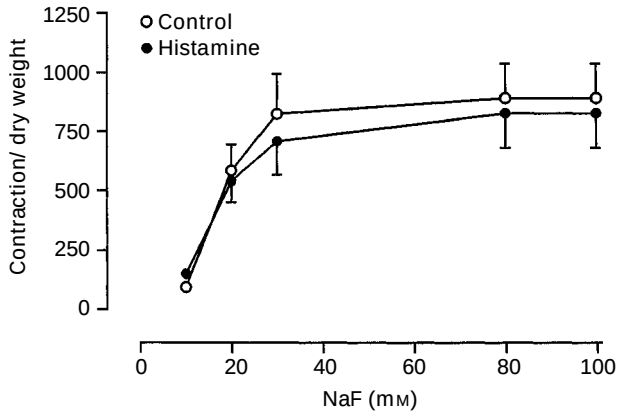

Figure 4 Concentration-response curves to NaF in BTSM following 1 h pretreatment with histamine 100 μM or vehicle. Data shown are the mean and vertical lines s.e.mean of 4 different experiments. Values are expressed as ratio of mg contraction to mg dry weight (24 h, 60°C).

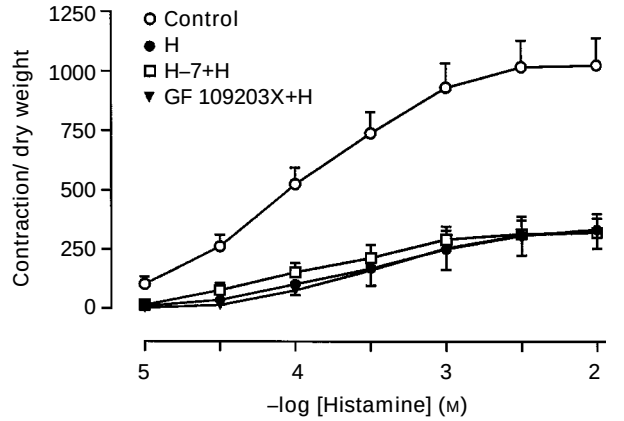

Figure 5 The effect of GF 109203X (1 μM) and H-7 (50 μM), upon the H_1R desensitization induced by a 1 h exposure to 100 μM histamine. GF 109203X and H-7 were added 1 h before histamine. The effect of GF 109203X + histamine (GF 109203X + H) and H-7 + histamine (H-7 + H) were compared with histamine (H) alone. Vehicle-treated tissues served as controls. GF 109203X and H-7 on its own had no effect on the histamine-induced concentration-response curve (data not shown). Data shown are the mean and vertical lines s.e.mean of 4 different experiments. $^*P<0.05$. Values are expressed as ratio of mg contraction to mg dry weight (24 h, 60°C).

SKF-91488 (100 μM), on the H_1R mRNA steady-state level over the time-course investigated.

Discussion

In the present study we have shown that histamine treatment of bovine tracheal smooth muscle induces functional desensitization of the H_1R, without changes in H_1R mRNA steady-state levels. In recent years, H_1R regulation has been described in several cell lines. However, the exact mechanism underlying H_1R desensitization still remains to be elucidated (Smit *et al*., 1992; 1996; Dickenson & Hill, 1993; McCreath *et al*., 1994; Zamani *et al*., 1995). Histamine pretreatment of BTSM induced a concentration-dependent loss of subsequent histamine-induced contractile responses, which completely recovered following a 1 h wash-out period. In contrast, contractions to methacholine or NaF, which directly stimulates G-protein (Hardy *et al*., 1996), were unaltered following histamine pre-exposure, indicating a homologous desensitization of the H_1R, which is mediated at the level of the receptor or at the coupling with its Gq-protein. The desensitization was blocked by mepyramine, an H_1R antagonist, indicating that the desensitization occurred via stimulation of H_1-receptors.

There is evidence to suggest that desensitization of G-protein coupled receptors often involves phosphorylation by protein kinases (Premont *et al*., 1995). Recent cloning of H_1Rs has revealed several phosphorylation sites in the primary sequence of the receptor protein (Yamashita *et al*., 1991). Besides potential protein kinase C phosphorylation sites and a protein kinase A phosphorylation site, many threonine and serine residues, that could be phosphorylated by other kinases, are found in the amino acid sequence of the H_1R (Yamashita *et al*., 1991; Traiffort *et al*., 1994). All these sites can represent possible targets for regulatory actions at the level of the receptor protein. Indeed, in BTSM (Pype *et al*., 1995a) and in several cell lines (Smit *et al*., 1992; 1996; Dickenson & Hill, 1993; McCreath *et al*., 1994; Zamani *et al*., 1995), activation of PKC, by phorbol ester, has already been demonstrated to be involved in H_1R desensitization. On the other hand, there is increasing evidence from recent studies that homologous H_1R desensitization is mediated by a PKC-independent mechanism (Zamani *et al*., 1995; Smit *et al*., 1996), which was also shown in this study, in which GF 109203X, a selective PKC inhibitor, did not antagonize the histamine-induced desensitization in BTSM. To investigate further the mechanisms underlying the desensitization process, we have examined whether activation of cyclicAMP-dependent protein kinase A, following increases in cyclicAMP levels, via histamine H_2-receptor stimulation and/or via histamine-induced prostaglandin release, is involved. Inhibitors of prostaglandin synthesis have been shown to prevent the development of histamine tachyphylaxis in primate tracheal smooth muscle, moreover, exogenous administration of prostaglandin E_2 reduced the histamine-induced response (Krzanowski *et al*., 1980). Furthermore, increases in cyclicAMP levels in BTSM slices and in C6 glioma cells, have been shown to inhibit histamine-stimulated inositol phospholipid hydrolysis, presumably via a protein kinase A-mediated mechanism (Hall *et al*., 1989; Peakman & Hill, 1994). Stimulation of histamine H_2-receptors, which are positively coupled to adenylyl cyclase (Leurs *et al*., 1995), can lead to increases in cyclicAMP levels. However, it seems unlikely that increases in cyclicAMP levels are involved in the H_1R desensitization, since histamine did not increase cyclicAMP above basal levels in BTSM (unpublished information). Furthermore, histamine only potentiated forskolin-induced cyclicAMP accumulation, which was partially mediated by histamine H_2-receptors (Pype *et al*., 1995b). In our study, neither indomethacin (a prostaglandin synthesis inhibitor) nor cimetidine (a histamine H_2-receptor antagonist) had any effect on the desensitization induced by histamine, indicating no involvement of prostaglandins and histamine H_2-receptors. Moreover, in the presence of H-7, a non-selective kinase (PKA, PKC and PKG) inhibitor, no attenuation of the desensitization was observed, ruling out a role for PKA and PKG. Recently, Zamani and Bristow (1996) suggested that the H_1R in GT1-7 neuronal cells is regulated by calcium influx and calcium/calmodulin protein kinase II (CaMKII), since incubation in calcium-free medium or pretreatment with KN-62, a CaMKII inhibitor, could prevent the homologous H_1R desensitization. However, calcium influx did not seem to play any role in the

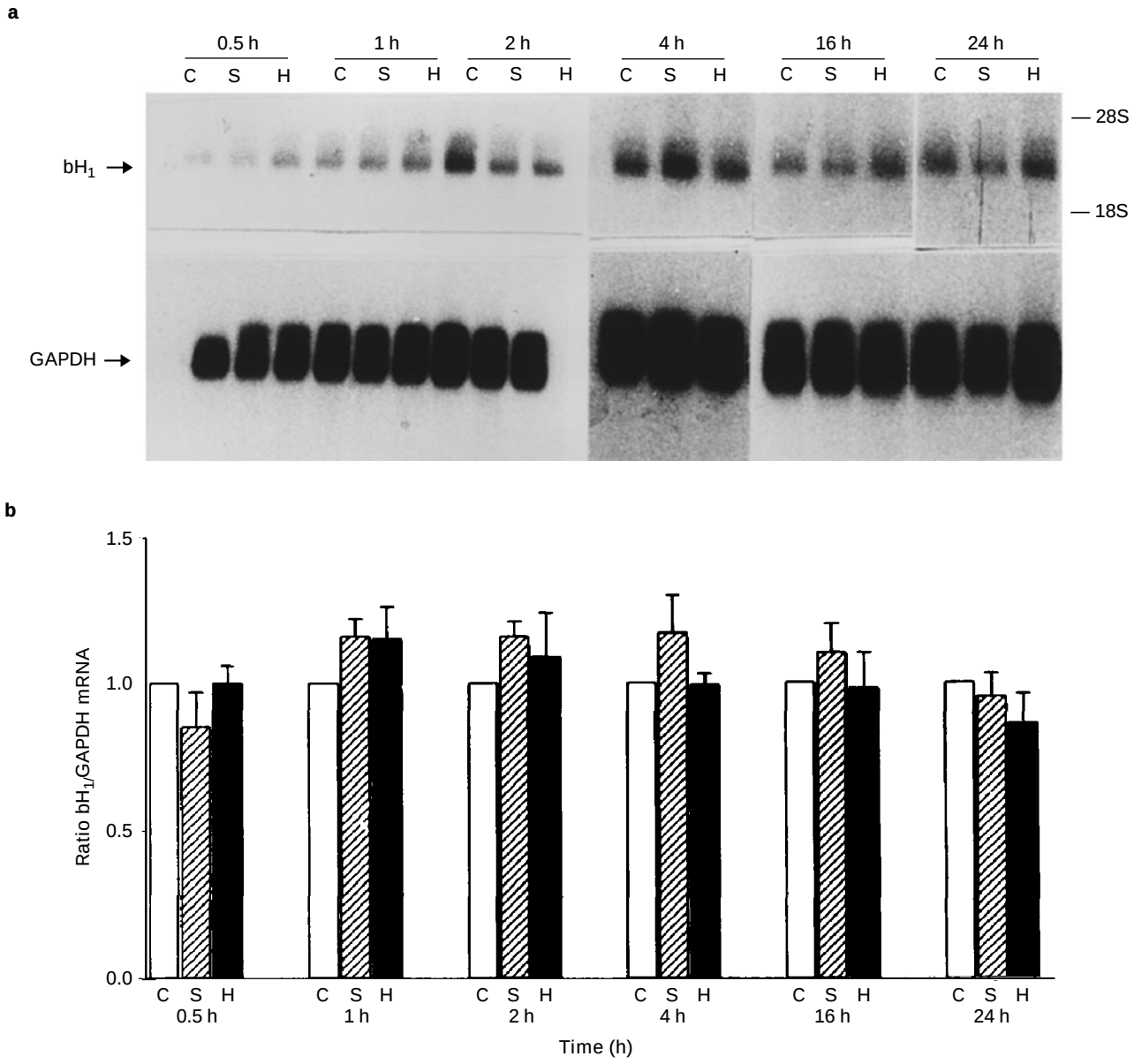

Figure 6 Northern blot analysis was performed with labelled cDNA probes for H_1R and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with poly (A^+) RNA. (a) Representative Northern blot following SKF 91488 (100 μM) and SKF 91488 + histamine (100 μM) pretreatment for the times indicated (C = control, S = SKF 91488 and H = SKF 91488 + histamine). (b) H_1R mRNA levels relative to GAPDH in the presence of SKF 91488 and SKF 91488 + histamine after 0.5, 1, 2, 4, 16 and 24 h preincubation after assessment by laser densitometry. Data shown are the mean of 3 different experiments.

homologous desensitization cascade in our study, since incubation in calcium free medium (+1 mM EGTA) did not appear to prevent the H_1R desensitization. Moreover, in the study of McGreath *et al.* (1994), a CaMKII inhibitor KN-62, had no effect on the histamine-induced desensitization in HUVEC cells, indicating that there might be differences in the mechanisms underlying homologous H_1R desensitization between different cell lines and cell systems. Homologous desensitization seems to involve a family of serine/threonine protein kinases (G protein-coupled receptor kinases, GRK), which exhibit a marked preference for activated (agonist-bound) receptors over inactive or antagonist-bound receptors. Until now, six distinct mammalian GRKs are known and they are grouped in three subfamilies, based on sequence and functional similarities. However, although a larger number of G protein-coupled receptors appear to be regulated by GRKs, so far only rhodospin kinase (GRK1) and βARK (GRK2) have extensively been studied (Premont *et al.*, 1995). Our results support the suggestion that a receptor-specific kinase is involved in the homologous H_1R desensitization (Dickenson & Hill, 1993; McCreath *et al.*, 1994). The development of specific inhibitors of GRKs, therefore, could give more insight into the mechanisms underlying histamine-induced H_1R desensitization (Chuang *et al.*, 1996).

Finally, we have determined the H_1R mRNA steady-state level in BTSM, following histamine pre-exposure. Until now, few studies have focused on the regulation of the H_1R mRNA. In a study of Choi *et al.* (1995), dexamethasone has been demonstrated to increase H_1R mRNA level in bovine adrenaline and noradrenaline chromaffin cells. H_1R mRNA has also been shown to be up-regulated by platelet-derived growth factor in human cultured aortic intimal smooth muscle cells (Takagishi *et al.*, 1995). Recently, it has been found that

the expression of H_1R mRNA is increased in nasal scrapings from patients with allergic rhinitis by use of reverse transcription-polymerase chain reaction (Iriyoshi *et al*., 1996). However, these results should be interpreted with caution, since Simons and Grimm (1996) had some criticism of the methodology used in this study.

Recently, we have demonstrated that phorbol ester pretreatment results in a transcriptional down-regulation of the H_1R gene expression in BTSM, which involves a PKC-dependent mechanism (Pype *et al*., 1995a). In the present study, we looked at the effect of long-term histamine pretreatment on the H_1R mRNA. However, to prevent enzymatic breakdown of histamine during the incubation period, we co-incubated the tissues with SKF 91488, a selective histamine-N-methyltransferase inhibitor, despite the fact that histamine, at a concentration of 100 μM and higher, inhibits the enzymes responsible for histamine metabolism (Beaven & Shaff, 1979). In contrast to PDBu pretreatment, prolonged histamine exposure was unable to affect the steady-state level of H_1R mRNA in BTSM, demonstrating differences in cellular events following PKC activation by PDBu and by histamine, with regard to the regulation of H_1R gene expression.

The discrepancy between PKC activation by phorbol esters and by histamine on the H_1R desensitization and on the H_1R mRNA down-regulation might reflect the presence of multiple PKC isoenzymes within the same tissue and the fact that individual isoenzymes can play a role in distinct cellular functions (Puceat *et al*., 1994). To date, more than 10 distinct isoenzymes have been identified (Nishizuka, 1992) and these are differentially responsive to the phorbol ester phorbol 12-myrisate 13-acetate and to other activating stimuli, which suggests that they play distinct and specific roles (Puceat *et al*., 1994).

In various cell systems, PKC activation by PDBu has been found to attenuate H_1R-mediated responses (Leurs *et al*., 1995) and PDBu has also been shown to induce transcriptional down-regulation of the H_1R gene, involving a PKC-dependent mechanism (Pype *et al*., 1995a). In contrast, since histamine-induced H_1R desensitization seemed to involve a PKC-independent mechanism and since histamine did not have any effect on the H_1R mRNA, one could speculate that PDBu and histamine activate distinct PKC isoforms and that individual isoforms may be involved in specific responses. However, further experiments with selective PKC isoform inhibitors or activators are needed to investigate the role of distinct PKC isoforms on the regulation of H_1R gene expression and function.

In conclusion, we have shown that histamine pretreatment of BTSM induces functional desensitization of H_1Rs which involved a mechanism independent of PKC, PKA, PKG and calcium influx. We also demonstrated that prolonged histamine pretreatment did not affect H_1R mRNA steady state levels.

We wish to thank Prof. H. Fukui (Osaka University, Japan) who kindly donated the bovine H_1R cDNA. This work was supported by Glaxo-Wellcome, Belgium.

References

BEAVEN, M.A. & SHAFF, R.E. (1979). New inhibitors of histamine-N-methyltransferase. *Biochem. Pharmacol.,* **28,** 183–188.

CHOI, A.Y., FUKUI, H. & PERLMAN, R.L. (1995). Glucocorticoids enhance histamine-evoked catecholamine secretion from bovine chromaffin cells. *J. Neurochem.,* **64,** 206–212.

CHOMCZYNSKI, P. & SACCHI, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.,* **162,** 156–159.

CHUANG, T.T., LACOVELLI, L., SALLESE, M. & DE BLASI, A. (1996). G protein-coupled receptors: heterologous regulation of homologous desensitization and its implications. *Trends Pharmacol. Sci.,* **17,** 416–421.

DICKENSON, J.M. & HILL, S.J. (1993). Homologous and heterologous desensitization of histamine H_1- and ATP-receptors in the smooth muscle cell line, DDT1MF-2: the role of protein kinase C. *Br. J. Pharmacol.,* **10,** 1449–1456.

FUJIMOTO, K., HORIO, Y., SUGAMA, K., ITO, S., LIU, Y.Q. & FUKUI, H. (1993). Genomic cloning of the rat histamine H_1 receptor. *Biochem. Biophys. Res. Commun.,* **190,** 294–301.

FUKUI, H., FUJIMOTO, K., MIZUGUCHI, H., SAKAMOTO, K., HORIO, Y., TAKAI, S., YAMADA, K. & ITO, S. (1994). Molecular cloning of the human histamine H_1 receptor gene. *Biochem. Biophys. Res. Commun.,* **201,** 894–901.

HABECKER, B.A., WANG, H. & NATHANSON, N.M. (1993). Multiple second-messenger pathways mediate agonist regulation of muscarinic receptor mRNA expression. *Biochemistry,* **32,** 4986–4990.

HALL, I.P., DONALDSON, J. & HILL, S.J. (1989). Inhibition of histamine-stimulated inositol phospholipid hydrolysis by agents which increase cyclic AMP levels in bovine tracheal smooth muscle. *Br. J. Pharmacol.,* **97,** 603–613.

HARDY, E., FARAHANI, M. & HALL, I.P. (1996). Regulation of histamine H_1 receptor coupling by dexamethasone in human cultured airway smooth muscle. *Br. J. Pharmacol.,* **118,** 11079–11084.

HORIO, Y., MORI, Y., HIGUCHI, I., FUJIMOTO, K., ITO, S. & FUKUI, H. (1993). Molecular cloning of the guinea-pig histamine H_1 receptor gene. *J. Biochem.,* **114,** 408–414.

IRIYOSHI, N., TAKEUCHI, K., YUTA, A. & UKAI, K. (1996). Increased expression of histamine H_1 receptor mRNA in allergic rhinitis. *Clin. Exp. Allergy,* **26,** 3379–3385.

KOMAN, A., CAZAUBON, S., ADEM, A., COURAUD, P.O. & STROSBERG, A.D. (1993). Different regulatory patterns of M1 and M2 muscarinic receptor subtype RNA in SH-SY5Y human neuroblastoma induced by phorbol ester or DMSO. *Neurosci. Lett.,* **149,** 79–82.

KRZANOWSKI, J.J., ANDERSON, W.H., POLSON, J.B. & SZENTIVANYI, A. (1980). Prostaglandin mediated histamine tachyphylaxis in subhuman primate tracheal smooth muscle. *Arch. Int. Pharmacodyn. Ther.,* **247,** 155–162.

LEURS, R., SMIT, M.J. & TIMMERMAN, H. (1995). Molecular pharmacological aspects of histamine receptors. *Pharmacol. Ther.,* **66,** 413–463.

MCCREATH, G., HALL, I.P. & HILL, S.J. (1994). Agonist-induced desensitization of histamine H_1 receptor-mediated inositol phospholipid hydrolysis in human umbilical vein endothelial cells. *Br. J. Pharmacol.,* **113,** 823–830.

NISHIZUKA, Y. (1992). Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science,* **258,** 607–614.

PEAKMAN, M.C. & HILL, S.J. (1994). Endogenous expression of histamine H_1 receptors functionally coupled to phosphoinositide hydrolysis in C6 glioma cells: regulation by cyclicAMP. *Br. J. Pharmacol.,* **113,** 1554–1560.

PREMONT, R.T., INGLESE, J. & LEFKOWITZ, R.J. (1995). Protein kinases that phosphorylate activated G protein-coupled receptors. *FASEB. J.,* **9,** 175–182.

PUCEAT, M., HILAL-DANDAN, R., STRULOVICI, B., BRUNTON, L.L. & BROWN, J.H. (1994). Differential regulation of protein kinase C isoforms in isolated neonatal and adult rat cardiomyocytes. *J. Biol. Chem.,* **269,** 16938–16944.

PYPE, J.L., MAK, J.C. & BARNES, P.J. (1995a). The regulation of H_1-receptor mRNA in bovine airway smooth muscle: the involvement of protein kinase C. *Am. J. Respir. Crit. Care Med.,* **151,** A165.

PYPE, J.L., MAK, J.C., GIEMBYCZ, M.A. & BARNES, P.J. (1995b). Histamine potentiates forskolin-induced cAMP accumulation in bovine tracheal smooth muscle via H_1- and H_2-receptors. *Eur. Respir. J.,* **8,** 412s.

RASMUSSEN, H., TAKUWA, Y. & PARK, S. (1987). Protein kinase C in the regulation of smooth muscle contraction. *FASEB. J.,* **1,** 177–185.

ROUSELL, J., HADDAD, E.B., MAK, J.C. & BARNES, P.J. (1995). Transcriptional down-regulation of m2 muscarinic receptor gene expression in human embryonic lung (HEL 299) cells by protein kinase C. *J. Biol. Chem.,* **270,** 7213–7218.

SIMONS, F.E.R. & GRIMM, P.C. (1996). Histamine H_1-receptors in the nasal mucosa: a mystery solved? *Clin. Exp. Allergy,* **26,** 368–370.

SMIT, M.J., BLOEMERS, S.M., LEURS, R., TERTOOLEN, L.G., BAST, A., DE LAAT, S.W. & TIMMERMAN, H. (1992). Short-term desensitization of the histamine H_1 receptor in human HeLa cells: involvement of protein kinase C dependent and independent pathways. *Br. J. Pharmacol.,* **107,** 448–455.

SMIT, M.J., TIMMERMAN, H., HIJZELENDOORN, J.C., FUKUI, H. & LEURS, R. (1996). Regulation of the human histamine H_1 receptor stably expressed in Chinese hamster ovary cells. *Br. J. Pharmacol.,* **117,** 1071–1080.

TAKAGISHI, T., SASAGURI, Y., NAKANO, R., ARIMA, N., TANIMOTO, A., FUKUI, H. & MORIMATSU, M. (1995). Expression of the histamine H_1 receptor gene in relation to atherosclerosis. *Am. J. Pathol.,* **146,** 981–988.

TRAIFFORT, E., LEURS, R., ARRANG, J.M., TARDIVEL-LACOMBE, J., DIAZ, J., SCHWARTZ, J.C. & RUAT, M. (1994). Guinea pig histamine H_1 receptor. I. Gene cloning, characterization, and tissue expression revealed by in situ hybridization. *J. Neurochem.,* **62,** 507–518.

YAMASHITA, M., FUKUI, H., SUGAMA, K., HORIO, Y., ITO, S., MIZUGUCHI, H. & WADA, H. (1991). Expression cloning of a cDNA encoding the bovine histamine H_1 receptor. *Proc. Natl. Acad. Sci. U.S.A.,* **88,** 11515–11519.

ZAMANI, M.R., DUPERE, J.R. & BRISTOW, D.R. (1995). Receptor-mediated desensitisation of histamine H_1 receptor-stimulated inositol phosphate production and calcium mobilisation in GT1-7 neuronal cells is independent of protein kinase C. *J. Neurochem.,* **65,** 160–169.

ZAMANI, M.R. & BRISTOW, D.R. (1996). The histamine H_1 receptor in GT1-7 neuronal cells is regulated by calcium influx and KN-62, a putative inhibitor of calcium/calmodulin protein kinase II. *Br. J. Pharmacol.,* **118,** 1119–1126.

(Received September 18, 1997
Revised November 11, 1997
Accepted November 27, 1997)