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Regulation of histamine H_1 receptor coupling by dexamethasone in human cultured airway smooth muscle

Elaine Hardy, Mosavar Farahani & [1]Ian P. Hall

Department of Therapeutics, University Hospital of Nottingham, Nottingham NG7 2UH

1 The regulation of histamine-induced [^{3}H]-inositol phosphate and intracellular calcium responses in human cultured airway smooth muscle cells was studied.

2 Histamine induced concentration-dependent [^{3}H]-inositol phosphate formation (EC_{50} 4 μM). This response was inhibited by a range of selective H_1 receptor antagonists but not by the H_2-selective antagonist, tiotidone or the H_3 receptor-selective antagonist, thioperamide, indicating that an H_1 receptor is involved in this response in human cultured airway smooth muscle cells.

3 Preincubation of human cultured airway smooth muscle cells with concentrations of dexamethasone >10 nM for 22 h produced concentration-dependent inhibition of histamine-induced inositol phosphate formation. The maximum inhibition observed was 45% of the response in control cells. The inhibitory effect of dexamethasone was itself reversed by prior exposure to the glucocorticoid receptor antagonist, RU38486 (10 μM). Preincubation for 22 h with 1 μM dexamethasone produced inhibition of the inositol phosphate response to histamine at all concentrations of histamine inducing significant inositol phosphate formation in these cells. In contrast, the response to the G protein activator, NaF (0.1-20 mM) was unaltered by preincubation with dexamethasone.

4 Preincubation of human airway smooth muscle cells with 1 μM dexamethasone for time periods of <6 h failed to inhibit histamine-induced inositol phosphate formation in human airway smooth muscle cells.

5 Histamine also induced concentration-dependent elevation of intracellular calcium levels in Fura 2-loaded human airway smooth muscle cells. This response was inhibited by preincubation with 1 μM dexamethasone.

6 We conclude that signal transduction through the H_1 receptor in human airway smooth muscle is subject to regulation by dexamethasone and that this may in part account for the protective effect of dexamethasone against spasmogen-induced contractile responses in the airways.

Keywords: Airway smooth muscle; histamine H_1 receptors; intracellular calcium; inositol phosphates; dexamethasone; proliferation; asthma

Introduction

Corticosteroids are the major anti-inflammatory medication used in the treatment of asthma. The main effects of corticosteroids in the airways of asthmatic patients are believed to be inhibition of recruitment of inflammatory cells into the airways and inhibition of mediator release from inflammatory cells in the lungs. However, corticosteroids have a number of other actions which may be relevant to their effects in the airways. High local concentrations of corticosteroids may be present in the airways of patients receiving nebulised steroid therapy for asthma. We have been interested in the observation that preincubation of airway smooth muscle preparations with corticosteroids reduces the contractile response of the tissue when subsequently stimulated with contractile agonist (Nabishah *et al.*, 1990). The mechanisms underlying this response are poorly understood. We hypothesized that one mechanism through which corticosteroids may alter contractility of airway smooth muscle preparations is by altering the coupling of contractile agonist receptors to their intracellular signal transduction pathways in this tissue. Contractile agonists such as histamine and acetylcholine are believed to initiate a contractile response in airway smooth muscle via H_1 and M_3 receptors respectively: these receptors are coupled in airway smooth muscle to phospholipase C (Hall & Hill, 1988; Meurs *et al.*, 1989; Coburn & Baron, 1990; Chilvers & Hall, 1993). Stimulation of phospholipase C results in the formation of inositol 1,4,5 trisphosphate, which is able to release calcium from intracellular stores, thus inducing contraction (Hashimoto *et al.*, 1985).

In other smooth muscle preparations, the coupling of a number of G protein-linked receptors to their signal transduction pathways has been shown to be altered by prior exposure to dexamethasone in tissue-specific ways (Liu *et al.*, 1992; Sato *et al.*, 1992; Yasunari *et al.*, 1994). Because these effects require medium to long term exposure to dexamethasone to become apparent, we used primary cultures of human airway smooth muscle. We have previously shown that human cultured airway smooth muscle cells express a histamine receptor coupled to phospholipase C and that the characteristics of the second messenger responses to stimulation of this receptor are similar to those observed in noncultured airway smooth muscle (Daykin *et al.*, 1993; Hall & Kotlikoff, 1995). We therefore investigated the effects of dexamethasone pretreatment upon the inositol phosphate and intracellular calcium responses induced by histamine in human cultured airway smooth muscle cells.

Methods

Culture of human airway smooth muscle cells

Primary cultures of human airway smooth muscle cells were prepared from explants of trachealis muscle obtained from individuals without respiratory disease within 12 h of death as

[1] Author for correspondence.

previously described (Daykin *et al.*, 1993). Tissue was taken from the trachea immediately above the level of the carina. A strip of trachealis about 2 × 1 cm was dissected clear of surrounding tissue and transported to the laboratory in DMEM containing penicillin G (200 u ml^{-1}), streptomycin (200 $\mu g\ l^{-1}$) and amphotericin B (0.5 $\mu g\ l^{-1}$). The tissue was washed several times in 10 ml DMEM containing antibiotics and antifungal agents at double the above concentrations. Overlying mucosa was dissected free from the airway smooth muscle under sterile conditions. Small (0.2 × 0.2 cm) explants of airway muscle were then excised and about 15 explants placed in each 60 mm Petri dish. After allowing explants to adhere, DMEM containing antibiotics, amphotericin B, 10% foetal calf serum (FCS) and glutamine (2 mM) were added to just cover explants. The medium was changed twice each day for the first 3 days to reduce the incidence of fungal infection. Smooth muscle cell growth usually occurred about 7–10 days after placing explants in culture. When growth started, cultures were supplemented with fresh DMEM containing 10% FCS and 2 mM glutamine about every three days. When cells were approaching confluence in some parts of the vessel, explants were removed and 24 h later cells were harvested by trypsinization. Cells from an individual dish or flask were then plated in one 75 cm^2 flask and grown to confluence. When confluent, the contents of each flask was divided between 4 new flasks. Antibiotics and amphotericin were not added to the medium used for all subsequent passages after this stage (passage 2). Cells for experiments were either plated in 24 (× 1 cm) well plates or on glass coverslips in 6 well plates and studied within 2 days of reaching confluence. All primary cell cultures from each donor were examined using anti-smooth muscle alpha actin antibody (1:100 dilution) (Sigma) to confirm the presence of smooth muscle type cells using standard immunocytochemical techniques. Primary cell cultures used for the experiments described in this paper showed >95% of cells staining for smooth muscle actin. Cells from preparations from 4 individuals were used.

Accumulation of total [^{3}H]-inositol phosphates

[^{3}H]-inositol phosphate formation in primary cultures of human tracheal smooth muscle was quantified by an assay described by Daykin *et al.* (1993) with minor modifications. The medium was aspirated from confluent monolayers of cells in 24 well plates and replaced with 300 μl of inositol-free DMEM containing [^{3}H]-myoinositol at a concentration of 2 μ Ci ml^{-1}. Cells were then incubated for a further 24 h. Where dexamethasone was present during the preincubation, this was added with the [^{3}H]-inositol. Preliminary experiments revealed no alteration in the labelling of the inositol phospholipid pool in the presence of dexamethasone during the loading period. The medium was removed and cells washed twice with 1 ml of Hanks/HEPES buffer: 300 μl of Hanks/HEPES containing LiCl (10 mM) was then added to each well and the cells incubated for 15 min at 37°C. Agonists were finally added in a volume of 10 μl. Reactions were stopped by removing the medium from each well and adding 1 ml of a mixture of methanol/0.12 M HCl (1:1 v/v) which had been previously kept at −20°C. Samples were then stored at −20°C for at least 30 min. An 800 μl aliquot of each sample was then neutralised to pH7 with an appropriate volume (typically 4.8 ml) of buffer (composition: 25 mM Tris/0.5 M NaOH/H_2O: 0.238/0.025 0.737 v/v/v) and total [^{3}H]-inositol phosphates were finally separated from free [^{3}H]-myo-inositol by anion-exchange chromatography on Dowex-Cl columns (Daykin *et al.*, 1993).

Calcium measurements

For experiments where the effect of pretreatment with dexamethasone (1 μM) on the peak calcium response to a range of concentrations of histamine (1 μM–1 mM) was investigated, human airway smooth muscle cells were exposed to dexamethasone for 22 h prior to measurement of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). $[Ca^{2+}]_i$ was measured with the calcium-sensitive fluorophore, Fura-2 in cells grown to confluence on glass cover slips. The cells were incubated with 5 μM Fura-2/AM in a physiological buffer (composition, mM: (NaCl 145, glucose 10, KCl 5, $MgSO_4$ 1, HEPES 10, $CaCl_2$ 2, pH 7.45) for 30 min at 37°C. Following this loading period, cells were bathed in fresh buffer (i.e. without Fura-2) for a further 15 min. Coverslips were then mounted in a holder and transferred to a cuvette containing 2.9 ml buffer. Fluorescence measurements were made with a Perkin-Elmer LS-50 spectrometer. The excitation wavelengths were 340 and 380 nm and the emission wavelength was 500 nm. In order to calibrate the fluorescence readings in terms of $[Ca^{2+}]_i$, the cells were first made permeable to Ca^{2+} by adding 10 μM ionomycin in the presence of 2 mM Ca^{2+}. An initial series of calibration experiments demonstrated that 10 μM ionomycin provided a maximal signal in the presence of 2 mM Ca^{2+}. Hence a fluorescence ratio reading for calcium-saturated Fura-2 (R_{max}) was obtained. Sequential aliquots of 10 mM EGTA were then added, to chelate all Ca^{2+} and hence obtain a reading for calcium-free Fura-2 (R_{min}). The autofluorescence of the cells was estimated in each experiment by measuring the fluorescence at 340 nm and 380 nm of cells which had not been loaded with Fura-2. All fluorescence readings were corrected for autofluorescence before calculating $[Ca^{2+}]_i$ using the equation of Grynkiewicz *et al.* (1985).

Materials

FCS was obtained from Advanced Protein Products (England). [^{3}H]-myo-inositol (10–20 Ci $mmol^{-1}$) was purchased from New England Nuclear (Stevenage, England). Fura-2 (acetoxymethyl ester) and ionomycin were purchased from Calbiochem (Nottingham, England). Chlorcyclizine, (+)-chlopheniramine, tiotidine and thioperamide were a gift from Prof. S. J. Hill (Nottingham), and RU38486 was a gift from Dr D. Kendall (Nottingham). All other chemicals were obtained from the Sigma Chemical Co (Poole, England). The antibodies used for immunocytochemistry were anti smooth muscle actin (Sigma A2547) and mouse IgG whole molecule (host goat) (Sigma F0257). Plasticware was obtained from Costar (UK) Ltd. (High Wycombe, England).

Data analysis and statistics

EC_{50} values for histamine were defined in each individual experiment and used to calculate mean values. Each data point in individual experiments was calculated from the mean of triplicate determinations. K_d values for antagonists were calculated in individual experiments using a range of concentrations of antagonist to inhibit the response to a near maximal concentration of agonist. Statistical analysis of data was performed by paired or unpaired *t* tests or analysis of variance as appropriate. All values in the text represent mean ± s.e.mean of *n* separate experiments.

Results

Histamine induced concentration-related [^{3}H]-inositol phosphate formation in human cultured airway smooth muscle cells as previously described (Figure 1). The EC_{50} for this response was 4.3 ± 0.3 μM (n = 12). In order to confirm that this response was mediated through the H_1 receptor subtype, we studied the ability of a number of histamine receptor antagonists to inhibit the inositol phosphate response to a near maximal concentration of histamine (100 μM) in these cells. The mean K_d values obtained from these experiments for H_1 selective antagonists were as follows: mepyramine 1.0 ± 0.3 nM; (+)-chlopheniramine 5.1 ± 1.3 nM; and promethazine 0.18 ± 0.04 nM (all n = 4–6). In contrast, the H_2-selective antagonist, tiotidine (10 μM, n = 4) and the H_3 selective antagonist, thioperamide (10 μM, n = 4) were without

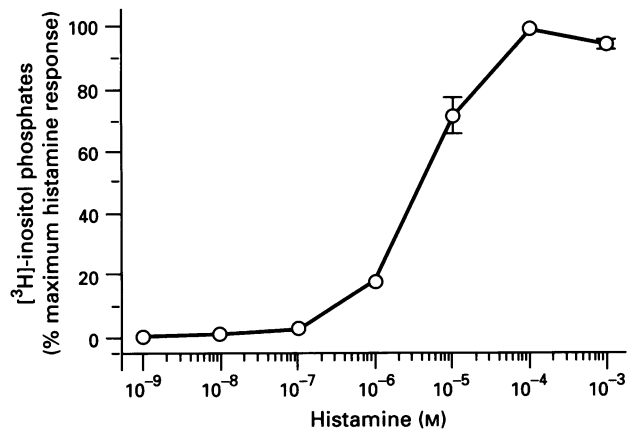

Figure 1 The effects of a range of concentrations of histamine upon [^{3}H]-inositol phosphate formation in human cultured airway smooth muscle cells. Results are shown as the mean %(±s.e.mean) of the response to 100 μM histamine ($n=12$). Where error bars are not shown they lie within data points.

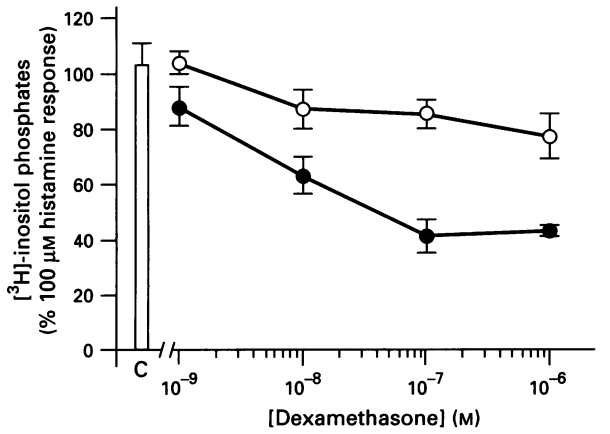

Figure 2 The effect of pre-incubation with a range of concentrations of dexamethasone for 22 h upon the inositol phosphate response to 100 μM histamine. Data shown are the mean±s.e.mean of data obtained in 4 separate experiments performed in the absence (●) or presence (○) of the glucocorticoid receptor antagonist, RU38486 (10 μM). Cells were exposed to dexamethasone for 22 h before stimulation with histamine. Responses are shown as a % of the response observed in naive cells stimulated with 100 μM histamine. The effect of RU38486 was significant ($P<0.001$, ANOVA) at all concentrations of dexamethasone >1 nM. Preincubation with RU38486 alone did not alter the response to histamine (column C).

signficant effect upon the inositol phosphate response to histamine (0.1 μM – 1 mM) in these cells.

To investigate the potential for preincubation with dexamethasone to modulate the inositol phosphate response to histamine in human cultured airway smooth muscle cells, we preincubated the cells with a range of concentrations of dexamethasone for 22 h before stimulation with histamine. As can be seen from Figure 2, dexamethasone caused concentration-dependent inhibition of the inositol phosphate response to histamine challenge ($P<0.05$ for all concentrations >10 nM). When cells were preincubated with 1 μM dexamethasone and then stimulated with a range of concentrations of histamine, the modulatory effect of dexamethasone preincubation was evident at all concentrations of histamine inducing significant inositol phosphate formation (Figure 3). The effect of dexamethasone was evident only after cells had been exposed to the drug for at least 6 h ($P<0.05$ at 6 h, $P<0.001$ at 22 h) (Figure 4). The mean reduction seen after prior exposure to 1 μM dexamethasone for 22 h was 48±5% ($n=12$, $P<0.001$

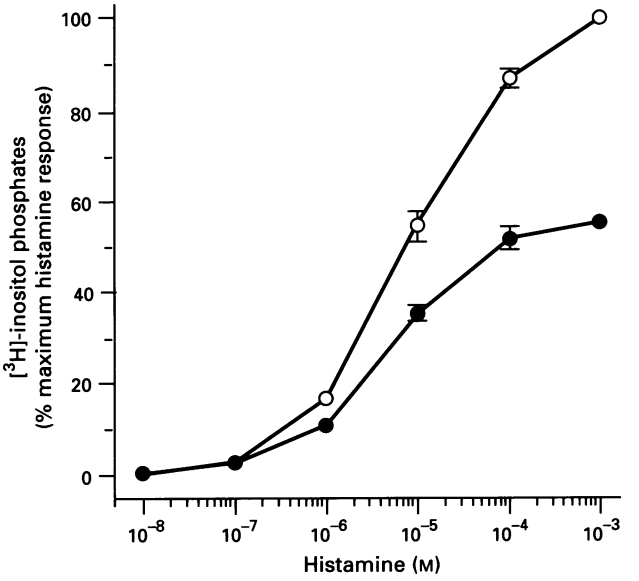

Figure 3 The effect of pre-incubation with 1 μM dexamethasone upon the inositol phosphate response to a range of concentrations of histamine. Data are shown as the % of the response to naive cells stimulated with 100 μM histamine alone. Cells were stimulated with histamine following previous exposure to dexamethasone (●) or vehicle alone (○). Mean data for four experiments are shown. The effect of dexamethasone was significant ($P<0.05$) for all concentrations of histamine >1 μM (ANOVA).

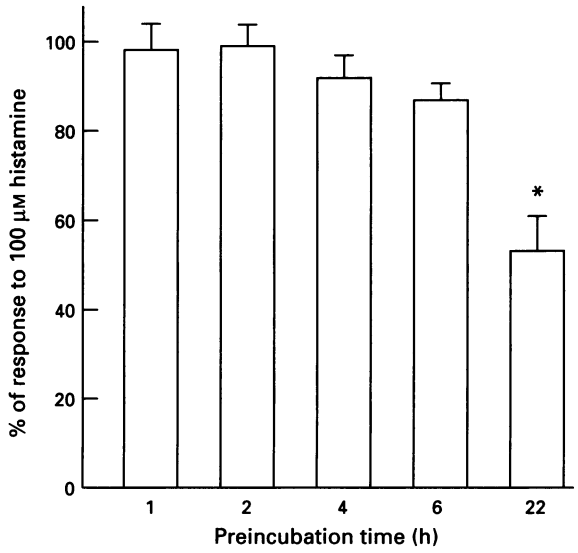

Figure 4 The effect of prior exposure to 1 μM dexamethasone for various times upon the subsequent response of human airway smooth muscle cells to 100 μM histamine. Data are shown as the % of the response in cells exposed to vehicle alone and are the mean data (±s.e.mean) obtained from 6 experiments. Significant inhibition of the inositol phosphate response to histamine was seen at 6 h ($P<0.05$) and 22 h ($P<0.001$) only.

cf. vehicle alone), with the maximum effect being observed with 1 μM dexamethasone in some experiments and with 100 nM dexamethasone in other experiments. The inhibitory effect of dexamethasone was itself reversed by prior incubation for 20 min with the glucocorticoid receptor antagonist, RU 38486 (10 μM), (Veldhuis *et al.*, 1985), indicating that the effect of dexamethasone was mediated through binding of dexamethasone with the glucocorticoid receptor (Figure 2). At higher concentrations of dexamethasone (10 μM and above),

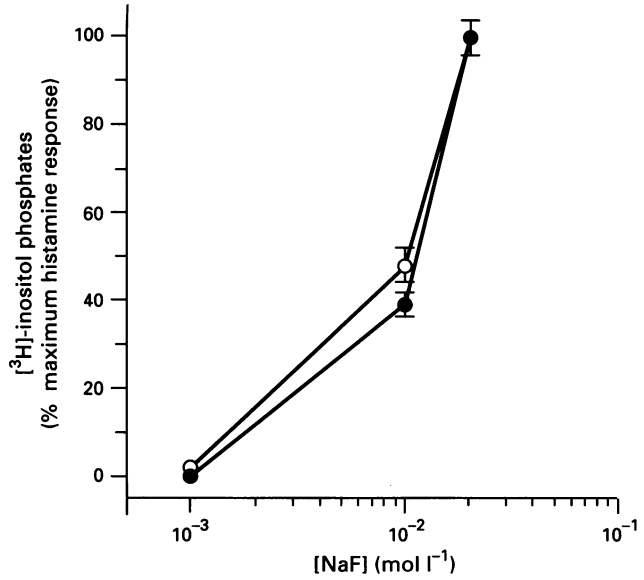

Figure 5 Lack of effect of dexamethasone upon the inositol phosphate response to a range of concentrations of sodium fluoride (NaF). Cells were stimulated with NaF following 22 h incubation in the presence (●) for absence (○) of 1 μM dexamethasone. Data shown are the mean data (±s.e.mean) from three experiments.

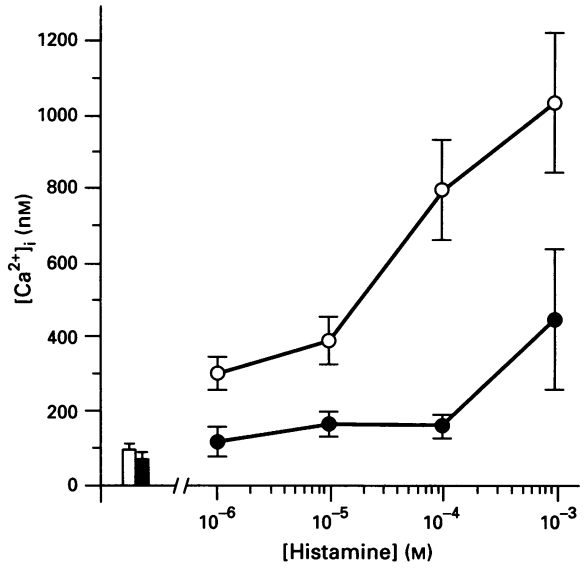

Figure 6 Peak intracellular calcium responses to a range of concentrations of histamine in cells previously exposed for 22 h to vehicle alone (○) or dexamethasone (●) (all $n=4-6$). The basal levels of intracellular calcium are shown by the open column (vehicle-exposed cells) and the solid column (dexamethasone-exposed cells). Each data point is the mean±s.e.mean of values obtained in four to six separate experiments. The effect of dexamethasone was significant at all concentrations of histamine >1 μM ($P<0.05$).

nonspecific inhibition of the inositol phosphate response was observed which was not reversed by RU38486. Therefore, for further experiments, 1 μM dexamethasone was used as the maximally effective concentration.

In order to investigate whether the effect of dexamethasone was being mediated at the level of the receptor/G protein interaction or at a site more distal in the signalling pathway, we investigated the potential for dexamethasone to inhibit the inositol phosphate response to NaF, which is believed to induce an inositol phosphate response in these cells by direct activation of the G protein (Hall *et al.*, 1990). As can be seen from Figure 5, the inositol phosphate response to NaF was unaltered by prior incubation of the cells with a concentration of dexamethasone (1 μM) which in the earlier experiments had been shown to modulate the response to histamine.

We next examined the effect of dexamethasone preincubation upon the intracellular calcium response of human airway smooth muscle cells to histamine. In these experiments, confluent monolayers of cultured human airway smooth muscle cells grown on glass coverslips were exposed to either dexamethasone or vehicle alone for 22 h before being incubated with Fura 2AM. The peak rise in intracellular free calcium seen following histamine addition was reduced for all concentrations of histamine which induced a significant rise in intracellular calcium (Figure 6). Concentrations of histamine >10 μM produced a sustained elevation of intracellular calcium after the initial transient as previously reported (Murray & Kotlikoff, 1990). Preincubation with dexamethasone had less effect upon the magnitude of this sustained rise in calcium. There was also a small reduction in basal (i.e. unstimulated) levels of calcium in these cells after exposure to dexamethasone.

One potential mechanism whereby histamine could be modulating the response to histamine H_1 receptor stimulation in these cells is that dexamethasone could be downregulating (as opposed to uncoupling) the histamine H_1 receptor. We attempted to study this possibility by performing binding studies in membranes prepared from human cultured airway smooth muscle cells using [^{3}H]-mepyramine (Hill, 1990; Bristow *et al.*, 1993). Despite using the maximum practical numbers of cells in each experiment (16 × 162 cc flasks) these studies were consistently hampered by low levels of specific binding in the cell membrane preparations and by the existence of a low affinity binding site for mepyramine which became evident when concentrations of [^{3}H]-mepyramine in excess of 8 nM were used. We performed binding studies on membranes from cells preincubated in the absence or presence of 1 μM dexamethasone in 6 experiments using methods previously described (Dickenson & Hill, 1993). However, due to the problems noted above, quantifiable specific binding was observed in only one experiment. In this experiment dexamethasone preincubation reduced the amount of specific binding to a range of concentrations of [^{3}H]-mepyramine (0.4–6.6 nM) by a mean of 85%. Previous studies attempting to quantify histamine H_1 receptor levels using [^{3}H]-mepyramine as a ligand have suffered similar difficulties (McCreath *et al.*, 1994), although specific binding can be more readily identified with identical methods in high expressing cell lines transfected with the H_1 receptor (Dickenson & Hill, 1993).

Discussion

Histamine is an important contractile agonist for airway smooth muscle cells and is believed to initiate a contractile response in this tissue by stimulation of phospholipase C (Coburn & Baron, 1990; Chilvers & Hall, 1993). This results in an increase in the intracellular concentration of inositol 1,4,5 trisphosphate which in turn causes a rise in intracellular free calcium concentration by inducing calcium release from intracellular stores (Hashimoto *et al.*, 1985). Both this response and the contractile response to histamine are mediated through stimulation of the histamine H_1 receptor subtype (Hall & Hill, 1988, Hill, 1990).

In order to study the longer term regulation of histamine H_1 receptor coupling in human airway tissues, we used human cultured airway smooth muscle cells. These cells provide a useful model for studying the long term regulation of G protein coupled receptors in a non transformed airway cell system in that they constitutively express stable physiologically relevant numbers of important airway receptors over a number of cell passages (Panettieri *et al.*, 1989; Hall & Kotlikoff, 1995). Our initial studies demonstrate that these cells express a clas-

sical histamine H_1 receptor based upon the profile of a range of histamine receptor antagonists. Neither H_2 nor H_3-selective antagonists had significant effects upon histamine-induced inositol phosphate formation in these cells, whereas a range of H_1-selective antagonists was able to induce concentration-dependent inhibition of this response in these cells with K_d values similar to those previously described for the H_1 receptor subtype (Hill, 1990). These results extend our previous work on the histamine H_1 receptor in these cells (Daykin *et al.*, 1993) and confirm that the receptor has similar characteristics to the receptor seen in airway smooth muscle preparations studied *ex vivo*. Histamine also increased intracellular calcium levels in Fura 2-loaded human airway smooth muscle cells in keeping with previous observations (Panettieri *et al.*, 1989; Murray & Kotlikoff, 1991). This response has also been shown previously to be mediated through the H_1 receptor subtype (Murray & Kotlikoff, 1991). These experiments were performed in the presence of extracellular calcium, and in agreement with these previous studies, we observed a sustained elevation of intracellular calcium with higher concentrations of histamine. This response has previously been shown to be dependent upon entry of calcium into the cell through a non-voltage channel dependent calcium entry mechanism (Murray & Kotlikoff, 1991).

We next demonstrated that preincubation of these cells with dexamethasone inhibited the magnitude of both the inositol phosphate and the intracellular calcium response to histamine H_1 receptor stimulation. The effect of dexamethasone was inhibited by RU38486, indicating that this effect is mediated through the glucocorticoid receptor (Veldhuis *et al.*, 1985). The response appears to be mediated at the level of the histamine H_1 receptor, or perhaps its interaction with G_q, the G protein likely to be coupled to this receptor in airway smooth muscle cells, because the response to NaF was unaltered by preincubation with dexamethasone. NaF is believed to interact directly with Gq to induce inositol phosphate formation in airway and other smooth muscle cell types (Hall *et al.*, 1990). The effect of dexamethasone takes at least 6 h to become apparent, suggesting that longer term changes in protein expression may be involved in this response rather than direct modulation of components of the phospholipase C-coupled signal transduction pathway. We therefore considered a number of potential mechanisms whereby dexamethasone could be inhibiting histamine induced inositol phosphate and intracellular calcium responses.

The most probable explanation for our results is that dexamethasone downregulates the expression of the H_1 receptor in cultured airway smooth muscle cells. However, our attempts to perform binding experiments were hampered by the low levels of specific binding found in membranes from these cells, although we did observe a reduction in [^{3}H]-mepyramine binding following dexamethasone preincubation in the single experiment where adequate binding was observed.

Another possibility we considered to account for our results was that dexamethasone might be inhibiting cell proliferation and hence might artifactually alter the magnitude of the inositol phosphate and calcium responses to histamine merely by altering cell number. Prolonged incubation with dexamethasone inhibits human airway smooth muscle cell proliferation in culture (Hall & Pearl, 1994). However, this mechanism can be discontinued for two reasons. Firstly, the inositol phosphate response to NaF was unaltered by preincubation with dexamethasone. Secondly, the inositol phosphate experiments were performed in confluent cells, with the loading period (during which dexamethasone was present) taking place in serum-free medium. Under these conditions little or no cell division takes place (Panettieri *et al.*, 1989). In our previous experiments we found that whilst dexamethasone can inhibit serum-induced cell division, this effect is apparent only after a minimum of 48 h and when 10% FCS is present as a mitogenic stimulus (Hall & Pearl, 1994).

In liver macrophages, dexamethasone has been reported to inhibit zymosan-stimulated inositol phosphate formation by inhibiting the incorporation of [^{3}H]-myoinositol into the membrane phospholipid pool (Fitzke & Dieter, 1991). However, we found no difference in incorporation in cells loaded in the presence or absence of dexamethasone as assessed by measuring incorporation of label into the membrane phospholipid pool (data not shown). In addition, if this were the mechanism through which dexamethasone were operating, one would also have expected the response to NaF to be inhibited.

The most probable explanation for our results, therefore, is that dexamethasone is downregulating the expression of histamine H_1 receptors in airway smooth muscle cells, although an additional effect upon coupling with G_q cannot be excluded. The human histamine H_1 receptor has recently been cloned and sequenced (De Backer *et al.*, 1993; Fukui *et al.*, 1994), together with some of the flanking sequence upstream of the gene. Analysis of this upstream region reveals the presence of a number of potential regulatory sites for transcription, including several AP1 sites and also a GRE site, although interestingly the majority of these sites are some distance (>300 base pairs) from the start codon (Fukui *et al.*, 1994). Little is known at present regarding the action of *cis* and *trans* acting influences upon histamine H_1 receptor gene expression, and the important sites for the regulation of expression of this gene have not been defined.

The effects of dexamethasone upon G protein coupled receptor expression and coupling have been examined extensively in vascular smooth muscle. Dexamethasone has been shown to increase expression of the α_{1B} receptor in DDT1-FM cells and enhances noradrenaline and endothelin-induced inositol phosphate formation in rat primary cultured vascular smooth muscle cells (Liu *et al.*, 1992; Sato *et al.*, 1992). Similarly, dopamine D_1 receptor expression was upregulated by dexamethasone in primary cultures of rat vascular smooth muscle cells, an effect that was prevented by a protein synthesis inhibitor (Yasunari *et al.*, 1994). In contrast, endothelin-1 receptors have been shown to be downregulated by prior exposure to dexamethasone in A10 cells, a rat vascular smooth muscle cell line. This effect was believed to occur at the transcriptional levels because a decrease in ET-1 receptor mRNA was observed, but was receptor-specific because the reponses to vasopressin in these cells were unaffected (Nambi *et al.*, 1992). The effect of dexamethasone upon responses to histamine have not been studied in any of these cells. The observation that dexamethasone can apparently increase the expression of some receptor subtypes and inhibit the expression of other receptor subtypes in different tissues suggests that cell-specific factors as well as gene-specific regulatory elements must be important in determining the effects of dexamethasone upon expression of G protein-coupled receptor genes in a given system. In the airways, dexamethasone inhibits inflammatory responses and also can reduce the responsiveness of airway smooth muscle *ex vivo* to contractile agonists (Nabishah *et al.*, 1990). Our results suggest a possible mechanism to account for this latter effect.

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