Up-Regulation of Airway Smooth Muscle Histamine H$_1$ Receptor mRNA, Protein, and Function by β_2-Adrenoceptor Activation

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ABSTRACT

Histamine, released from activated mast cells, causes broncho-constriction mediated by H$_1$ receptors, whereas β_2-agonists are widely used for the relief of bronchoconstriction. In this study, we examined the effects of the β_2-adrenoceptor agonist, fenoterol, on the expression of H$_1$ receptors at the mRNA and protein levels, and functional responses. Incubation of bovine tracheal smooth muscle with fenoterol (10^{-7} M) for 2 h increased H$_1$ receptor mRNA (maximum $\sim$190%). The number of H$_1$ receptors was increased after 12 and 18 h without any change in binding affinity. In the contraction experiments, the concentration-response curves for histamine-induced contraction were shifted significantly to the left after 18-h exposure to fenoterol, consistent with the increase in receptor number. The fenoterol-induced increase in H$_1$ receptor mRNA was concentration-dependent and was abolished by propranolol and ICI 118551, but not by CGP 20712A, indicating that fenoterol acts via β_2-adrenoceptors. These effects were mimicked by other cAMP-elevating agents forskolin and prostaglandin E$_2$, and by the stable cAMP analog 8-bromo-cAMP. Cycloheximide alone produced superinduction of H$_1$ receptor mRNA and augmented the fenoterol-induced increase in H$_1$ receptor mRNA. Fenoterol increased both the stability and the transcription rate of H$_1$ receptor mRNA. Pretreatment with dexamethasone did not prevent fenoterol-induced up-regulation of H$_1$ receptor mRNA. Thus, fenoterol increases the expression of airway smooth muscle H$_1$ receptors via activation of the cAMP system through increased gene transcription and mRNA stability. This mechanism may be involved in the adverse responses encountered with the clinical use of short-acting β_2-agonists.

Histamine is an important mediator of airway smooth muscle contraction, which is mediated via H$_1$ receptors (Chand and Sofia, 1995). H$_1$ receptors have now been cloned from several species (Yamashita et al., 1991; Fujimoto et al., 1993; Horio et al., 1993; Fukui et al., 1994; Inoue et al., 1996). Activation of histamine H$_1$ receptors leads to the formation of inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] and diacylglycerol from phosphatidylinositol 4,5-bisphosphate hydrolysis (Leurs et al., 1995). Ins(1,4,5)P$_3$ binds to a specific receptor on endoplasmic/sarcoplasmic reticulum, which leads to the release of Ca^{2+} from intracellular stores, resulting in an initial transient contractile response, whereas diacylglycerol activates protein kinase C (PKC), which is believed to be responsible for the sustained phase of the smooth muscle contraction (Rasmussen et al., 1987). H$_1$ receptors are ex-pressed in airway smooth muscle and may be regulated at the transcriptional level. PKC phosphorylation sites have recently been identified in the third intracellular loop of the histamine H$_1$ receptors. We have shown that phorbol esters, which activate PKC, result in a marked down-regulation of H$_1$ receptors due to uncoupling of the receptor and a reduction in transcription (Pype et al., 1998).

β_2-Adrenoceptor agonists have been widely used as bronchodilators for the relief of the symptoms of asthma, because they act as functional antagonists that counteract multiple bronchoconstrictors. β_2-Adrenoceptors are localized to airway smooth muscle of all airways from trachea to terminal bronchioles (Barnes, 1995). However, the use of high doses of β_2-agonists has been linked to the development of bronchial hyper-responsiveness (Taylor et al., 1993; Wahedna et al., 1993) and an increase in asthma exacerbations and mortality (Suissa et al., 1994; Barrett and Strom, 1995). Sustained use of β_2-adrenoceptor agonists is associated with loss of protection to bronchoconstrictors, such as adenosine, allergen,

ABBREVIATIONS: Ins(1,4,5)P$_3$, inositol 1,4,5-trisphosphate; PKA, protein kinase A; PKC, protein kinase C; PGE$_2$, prostaglandin E$_2$; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DMEM, Dulbecco's modified Eagle's medium; SSC, standard sodium citrate.

methacholine, and histamine (O'Connor et al., 1992; Cockcroft and Swystun, 1996). β_2-Adrenoceptors are coupled via G_s protein to the membrane-bound enzyme adenylyl cyclase to increase production of cAMP. The formation of cAMP leads to the characteristic cellular response via the activation of a specific protein kinase, protein kinase A (PKA), by dissociating a regulatory (inhibitory) subunit. PKA then phosphorylates serine and threonine residues on specific proteins, such as regulatory proteins within the cell, which results in relaxation of airway smooth muscle. Evidence has emerged to support the concept of regulatory "cross talk" between the β_2-adrenoceptor/cAMP signaling pathway and the phospholipase C-coupled/Ins(1,4,5)P$_3$ transmembrane signaling mechanism (Madison and Brown, 1988; Schramm et al. 1995; Zaagsma et al., 1997).

This study was thus undertaken to investigate the effects of the β_2-adrenoceptor agonist fenoterol on the expression of the histamine H$_1$ receptor gene and functional receptors in bovine tracheal smooth muscle. We have previously shown that this tissue expresses H$_1$ receptors with no detection of H$_2$ receptors (Pype et al., 1998). Because glucocorticoids counteract the effect of β_2-adrenoceptor agonists, the effects of steroid in the presence of fenoterol on the expression of the histamine H$_1$ receptor gene were also investigated.

Experimental Procedures

Materials. [α-^{32}P]dCTP (3000 Ci/mmol) was purchased from Amersham International (Amersham, UK). [^{32}P]UTP (800 Ci/mmol) and [pyridimyl-5-^{3}H]mepyramine (pyrilamine; 20 Ci/mmol) were from DuPont/NEN (Boston, MA). Fenoterol hydrobromide, ($-$)-isoproterenol hydrochloride, histamine dihydrochloride, and triprolidine were from Sigma. Methacholine chloride and HEPES-buffered Dulbecco's modified Eagle's medium (DMEM) were either from Sigma or ICN Biomedicals (Costa Mesa, CA). Scintillant Filtron-X was from National Diagnostics (Hull, UK). Tissue culture supplies were from Life Technologies (Paisley, UK). All other chemicals were of reagent grade and from standard sources.

Tissue Preparation and Incubation. Fresh bovine tracheae were obtained from the abattoir and placed in oxygenated Krebs-Henseleit solution [(in millimolar): NaCl 118, KCl 5.9, MgSO$_4$ 1.2, CaCl$_2$ 2.5, NaH$_2$PO$_4$ 1.2, NaHCO$_3$ 25.5, and glucose 5.6] at room temperature. The tracheal smooth muscle layer was dissected after stripping off epithelium; mucosa and connective tissue and smooth muscle pieces were prepared in Krebs-Henseleit solution at room temperature. After washing, incubation was performed in HEPES-buffered DMEM supplemented with 10% fetal calf serum, 2 mM L-glutamate, 100 I.U./ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B at 37°C in a shaker incubator. For molecular and binding studies, pieces of bovine tracheal smooth muscle were placed in T-75 flasks and incubated in the absence or presence of 10^{-9} to 10^{-5} M fenoterol for the indicated periods of time. The tissues were then frozen and kept at -70°C for RNA extraction and membrane preparation. To examine whether the effects of fenoterol were mediated via specific receptor subtypes, propranolol (nonselective β-adrenoceptor antagonist; 10^{-7} M), ICI 118551 (selective β_2-adrenoceptor antagonist; 10^{-7} M), or CGP 20712A (selective β_1-adrenoceptor antagonist; 10^{-7} M) were added for 1 h before 10^{-7} M fenoterol for a further 2 h. Preincubation of bovine tracheal smooth muscle with the protein synthesis inhibitor cycloheximide (10 μg/ml) for 1 h before addition of 10^{-7} M fenoterol for a further 2 h was carried out to assess whether new synthesis of protein(s) was required. Incubation of bovine tracheal smooth muscle with a direct adenylyl cyclase activator, forskolin (10^{-5} M), a nonhydrolyzable analog of cAMP, 8-bromo-cAMP (10^{-3} M), or other cAMP-elevating

agents such as prostaglandin E$_2$ (PGE$_2$; 10^{-6} M) for 2 h was carried out to see whether the effect of fenoterol is mediated by the elevation of cAMP. The half-life of the H$_1$ receptor mRNA in control and fenoterol-treated tissues was measured by incubation in the absence and presence of 10^{-7} M fenoterol for 2 h before the addition of 5 μg/ml actinomycin D for various times (Rodgers et al., 1985). In some experiments, incubation of bovine tracheal smooth muscle with 10^{-7} M fenoterol for 2 h was carried out in the presence of various concentrations of dexamethasone (10^{-9} to 10^{-6} M).

For contraction experiments, smooth muscle strips ($\sim 5 \times 2 \times 1$ mm) were washed in sterile DMEM and then exposed for 18 h to 10^{-7} M or 10^{-5} M fenoterol, to 10^{-6} M dexamethasone alone or in combination with 10^{-7} M fenoterol, or to vehicle (three to four smooth muscle strips in 10 ml of the same medium used for the incubation of muscle pieces in a 25-cm^2 cell culture flask) at 37°C in a shaking incubator.

Radioligand Receptor Binding Assay. Bovine trachealis (control and fenoterol-treated, 10^{-7} M for various times) was homogenized using a Polytron in ice-cold 50 mM Tris-HCl buffer, pH 7.5, containing 0.32 M sucrose, then centrifuged at 1000g for 10 min to remove unhomogenized debris; the supernatant was centrifuged at 40,000g for 20 min and the resulting pellet was washed and centrifuged again. The final pellet was resuspended in incubation buffer (50 mM Tris-HCl, pH 7.5, at 30°C) to give a final protein concentration of 3 to 5 mg/ml and was stored at -70°C until required for use. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin used as a standard.

The density and affinity of H$_1$ receptors were assessed using saturation isotherms. Membranes (0.3–0.4 mg of protein) were incubated in duplicate with various concentrations of [^{3}H]pyrilamine (0.25–10 nM) for 60 min at 30°C in a total volume of 500 μl. Nonspecific binding was determined in the presence of 10 μM triprolidine. Reactions were terminated with rapid filtration through Whatman GF/C glass fiber filters, presoaked in 0.3% (w/v) polyethylenimine for 5 h, using a Brandel cell harvester. The filters were washed three times with ice-cold buffer, transferred to scintillation vials containing 4 ml of Filtron-X, and counted in a liquid scintillation counter (TRI-CARB 2200CA; Packard) at an efficiency of 36%. Data were analyzed using weighted nonlinear regression-computerized, least-squares curve-fitting programs [EBDA and LIGAND; Munson and Rodbard (1980)].

Contraction Measurements. After 18-h exposure to drugs or vehicle, smooth muscle strips were washed twice and then mounted in 20-ml organ baths for isotonic recording under a 0.5-g preload in gassed Krebs-Henseleit buffer at 37°C. After two 30-min equilibration periods with a change of buffer in between, the strips were precontracted twice by cumulative administration of methacholine (10^{-7} to 10^{-5} M and 10^{-7} to 10^{-4} M, respectively), followed by washing periods of 60 min; in between, maximal relaxation was established with 10^{-7} M ($-$)-isoproterenol immediately followed by a 15-min washing period. Subsequently, cumulative concentration-response curves to histamine were constructed in the presence of 10^{-6} M timolol, to ensure elimination of β-adrenoceptor activation; experiments were performed in duplicate on five independent occasions, except for dexamethasone plus fenoterol ($n = 4$). Histamine-induced contractile responses were expressed as a percentage of the response to 10^{-4} M methacholine as assessed in the second precontraction curve in each individual smooth muscle strip.

Northern Blot Analysis. Total RNA was isolated by phenol/chloroform extraction and isopropanol precipitation (Chomczynski and Sacchi, 1987). An mRNA isolation kit system (PolyATtract IV; Promega, Southampton, UK) was used to prepare poly(A)$^+$ RNA according to the manufacturer's instructions. Samples of poly(A)$^+$ RNA were size-fractionated on a 1% agarose/formaldehyde gel and blotted onto nylon membrane (Magna, MA) by capillary action.

The bovine histamine H$_1$ receptor cDNA (1.2-kb *Eco*RI/*Sac*I fragment; kindly provided by Professor H. Fukui, Osaka University, Japan) and the rat glyceraldehyde-3-phosphate dehydrogenase

(GAPDH) cDNA (1.3-kb *Pst*I fragment) were radiolabeled using a random primer-labeling kit (Multiprime; Amersham) in the presence of [α-^{32}P]dCTP. The blot was prehybridized for 4 to 5 h in 50% formamide, 5 × standard sodium citrate (SSC), 5 × Denhardt's solution, 0.1% SDS, 10 mM NaH$_2$PO$_4$, and 100 mg/ml sonicated denatured salmon sperm DNA, and then hybridized with ^{32}P-labeled cDNA probes for 12 to 16 h at 42°C. After hybridization, the blot was washed at high stringency in 0.1 × SSC/0.1% SDS at 55°C for 30 min. The blot was exposed to Kodak OMAT XS film at −70°C with an intensifying screen for 1 to 3 days. The blot was hybridized first to a ^{32}P-labeled histamine H$_1$ receptor cDNA probe and subsequently to a GAPDH cDNA probe after stripping. The autoradiograms were scanned with a laser densitometer (Gel Documentation and Analysis System GDS8000; UVP, Cambridge, UK). The amount of histamine H$_1$ receptor mRNA was quantified relative to the amount of GAPDH mRNA on the same filter.

Nuclear Run-On Transcription Assay. Nuclear run-on transcription assays were performed to determine whether fenoterol changed the transcription rate of histamine H$_1$ receptor gene. Nuclei were isolated from frozen bovine tracheal smooth muscle after incubating with or without fenoterol (10^{-7} M) for 2 h and stored at −70°C in Keller storage buffer [10 mM Tris-HCl, pH 7.5, 5 mM MgCl$_2$, 0.5 M Sorbitol, 2.5% Ficoll (400,000 mol. wt.), 0.008% spermidine, 1 mM dithiothreitol, and 50% glycerol] at 5 × 10^7 nuclei/100 μl. Each reaction (final volume, 0.4 ml) was carried out in the presence of 5 × 10^7 isolated nuclei, 40 mM Tris-HCl (pH 8.3), 150 mM NH$_4$Cl, 7.5 mM MgCl$_2$, 0.625 mM ATP, 0.313 mM GTP, 0.313 mM CTP (Promega), 0.5 mCi of [^{32}P]UTP, and 120 units/ml recombinant ribonuclease (RNase) inhibitor. Transcription reactions were allowed to proceed for 30 min at 27°C before termination by the addition of 40 units of recombinant RNase inhibitor and 75 units of RQ-1 DNase. After DNase and proteinase K treatments, the radiolabeled RNA formed was purified by phenol-chloroform extraction and precipitated with ethanol three times in the presence of 1.33 M ammonium acetate. An equal number of counts from each sample was added to slot blots, three slots on the same blot on which 10 μg of either pGEM-3Z plasmid (as control) or plasmid containing inserts of bovine histamine H$_1$ receptor cDNA or rat GAPDH cDNA have been immobilized to a nylon membrane. After hybridization for 72 h at 42°C, the blots were washed at a final stringency of 0.1 × SSC and 0.1% SDS at 55°C, including a 30-min digestion with 1 mg/ml RNase A and 20 units/ml RNase T1 at 37°C to digest any single-stranded RNA not hybridized to DNA. After autoradiography, the film was scanned by laser densitometry and quantified by calculating the ratio of histamine H$_1$ receptor cDNA signal to GAPDH cDNA signal.

Statistical Analysis. Data were presented as means ± S.E. For multiple comparison of different groups, analysis of variance was used. Data that appeared statistically significant were compared by paired or unpaired Student's *t* test, with Bonferroni correction. A value of $P < .05$ was considered to be significant.

Results

Radioligand Binding Studies. Saturation studies performed with the selective histamine H$_1$ receptor antagonist [^{3}H]pyrilamine revealed a single class of high-affinity binding sites in airway smooth muscle. Treatment of airway smooth muscle with fenoterol causes a time-dependent increase in the number (B_{max}) of histamine H$_1$ receptors (Fig. 1), without any change in binding affinity (K_d; 1.5 ± 0.2 versus 1.7 ± 0.1 nM, 1.7 ± 0.2 versus 1.6 ± 0.1 nM, 1.7 ± 0.2 versus 1.7 ± 0.2 nM, and 1.6 ± 0.1 versus 1.7 ± 0.1 nM for 2, 6, 12, and 18 h, respectively).

Contraction Studies. Histamine produced concentration-dependent contractions in control bovine tracheal smooth muscle strips, with pD$_2$ (−log EC$_{50}$) values of 5.95 ± 0.10 ($n = 5$). Concentration-response curves after 18-h expo-

sure to fenoterol were shifted significantly, albeit only moderately, to the left by 0.19 ± 0.06 (10^{-7} M fenoterol) and 0.24 ± 0.08 (10^{-5} M fenoterol) log units, reaching pD$_2$ values of 6.14 ± 0.12 and 6.19 ± 0.14 (both $P < .05$ compared with control); there were no significant changes in maximum contraction levels as compared with control. Histamine-induced contractions were shifted to the right, albeit not as significantly, by 0.18 ± 0.08 log units after 18-h exposure to dexamethasone (10^{-6} M), reaching pD$_2$ values of 5.77 ± 0.09. Dexamethasone prevented the increase in contractile potency induced by 10^{-7} M fenoterol, resulting in pD$_2$ values of 5.84 ± 0.11 (not significantly different from control; $P < .05$ compared with fenoterol alone) (Fig. 2).

Histamine H$_1$ Receptor Gene Expression. Northern blot analyses of airway smooth muscle revealed the presence of a 3.0-kb transcript corresponding to the histamine H$_1$ receptor mRNA. Short incubations (up to 2 h) of airway smooth muscle with fenoterol resulted in a maximal (92%) increase in the steady-state levels of histamine H$_1$ receptor mRNA, which returned to baseline with longer incubation periods (up to 18 h; Fig. 3). A fenoterol-induced increase in histamine H$_1$ receptor mRNA after 2 h was not detected until concentration reached 10^{-7} M (Fig. 4). Preincubation with the nonselective β-adrenoceptor antagonist, propranolol, and the selective β_2-adrenoceptor antagonist, ICI 118551, completely inhibited the fenoterol-induced increase in steady-state mRNA at 2 h, whereas the selective β_1-adrenoceptor antagonist, CGP 20712A, had no effect (Fig. 5). This indicated that the effect of fenoterol on histamine H$_1$ receptor mRNA was mediated entirely by β_2-adrenoceptors.

To investigate the mechanism of the fenoterol-induced histamine H$_1$ receptor mRNA up-regulation, experiments were performed using cycloheximide, a protein synthesis inhibitor. Cycloheximide (10 μg/ml) alone produced superinduction (180%) of histamine H$_1$ receptor mRNA. There was a further increase in the histamine H$_1$ receptor mRNA in the presence of cycloheximide and fenoterol for 2 h (Fig. 6), indicating that new protein synthesis was not required.

Airway smooth muscle was also incubated with other agents that elevate cellular cAMP levels. Forskolin (a direct adenylyl cyclase activator), 8-bromo-cAMP (a stable cAMP analog), and PGE$_2$ caused a significant increase in histamine H$_1$ receptor
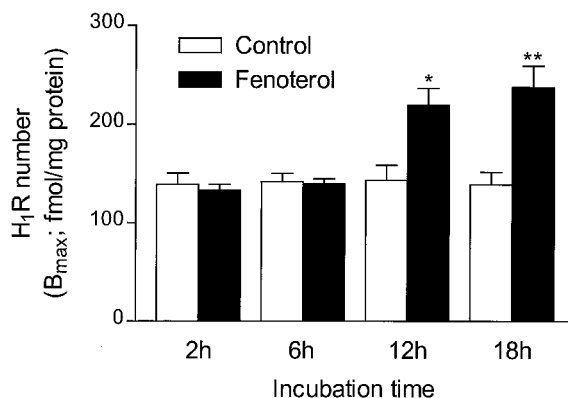
Fig. 1. Time course of the effect of fenoterol (10^{-7} M) on the number of histamine H$_1$ receptors determined by [^{3}H]pyrilamine in bovine tracheal smooth muscle. Means ± S.E. of four to five separate experiments are shown; significance of differences from control values with Student's *t* test: *$P < .05$, **$P < .01$.

mRNA similar to the effect of fenoterol (Fig. 7), suggesting that all these effects are mediated by cellular elevation of cAMP.

To investigate whether fenoterol stimulation increased histamine H_1 receptor mRNA expression by altering mRNA degradation, the RNA polymerase inhibitor actinomycin D (5 μg/ml) was added to untreated and treated airway smooth muscle. The degradation rate of the histamine H_1 receptor mRNA was significantly altered by 2-h fenoterol treatment ($t_{1/2}$ of 3.3 and 4.7 h for control and fenoterol-treated tissues, respectively; $P < .05$)(Fig. 8), suggesting that the fenoterol-induced increase in histamine H_1 receptor mRNA was, at least in part, due to increased stability of the histamine H_1 receptor mRNA.

To investigate whether the rate of histamine H_1 receptor gene transcription was changed following fenoterol incubation, a nuclear run-on assay was performed on nuclei isolated from control and fenoterol-treated airway smooth muscle. As shown in Fig. 9, the rate of transcription of newly synthesized histamine H_1 receptor mRNA was increased by approximately 60% after fenoterol treatment.

The expression of histamine H_1 receptor mRNA after incubation with fenoterol in the presence of various concentrations of dexamethasone was also examined, despite dexamethasone alone having been found to have no significant effect on the steady-state level of histamine H_1 receptor mRNA in this tissue (J. L. Pype, unpublished observation). As shown in Fig. 10, there was no significant inhibitory effect of dexamethasone on fenoterol-induced increase of histamine H_1 receptor mRNA at any concentrations up to 10^{-6} M.

Discussion

These studies demonstrate transregulation of histamine H_1 receptors by β_2-adrenoceptor activation in airway smooth muscle and implicate a cAMP-dependent process in the up-regulation of histamine H_1 receptors.

Specific [^{3}H]pyrilamine binding to histamine H_1 receptors in airway smooth muscle membranes was saturable and best described by interaction of the radioligand with a single class of high affinity binding sites. Stimulation of β_2-adrenoceptors resulted in a time-dependent increase in histamine H_1 receptor density after 12 and 18 h of incubation. However, treatment with fenoterol led to a rapid increase in histamine H_1 receptor mRNA, reaching a maximum at 2 h and returning to control level by 12 h of incubation. The increase in histamine H_1 receptor mRNA occurred well before the increase in histamine H_1 receptor number. Hence, the increased receptor density is likely a result of increased mRNA level, leading to increased receptor translation and expression at the cell surface. This fenoterol-induced increase in the histamine H_1 receptor mRNA level was antagonized by coincubation with the nonselective β-adrenoceptor antagonist propranolol and the selective β_2-adrenoceptor antagonist ICI 118551 but not the selective β_1-adrenoceptor antagonist CGP 20712A, confirming a β_2-adrenoceptor-mediated effect.

To gain insight into the mechanism whereby fenoterol induced histamine H_1 receptor mRNA up-regulation, we investigated the effect of the protein synthesis inhibitor, cycloheximide. Following cycloheximide treatment alone, there was a marked accumulation of the histamine H_1 receptor mRNA, to a similar extent as with fenoterol. This superinduction is thought to result from the loss of labile transcriptional repressors and mRNA-degrading enzymes and is usually regarded as a direct consequence of the inhibition of protein synthesis (Mahadevan and Edwards, 1991). However, in this study, we did not investigate the exact mechanism by which cycloheximide causes superinduction of histamine H_1 receptor gene. Nevertheless, in the presence of cycloheximide, fenoterol caused an additional increase in histamine H_1 receptor mRNA, an additive effect that providing evidence that, subsequent to β_2-adrenoceptor stimulation, de novo
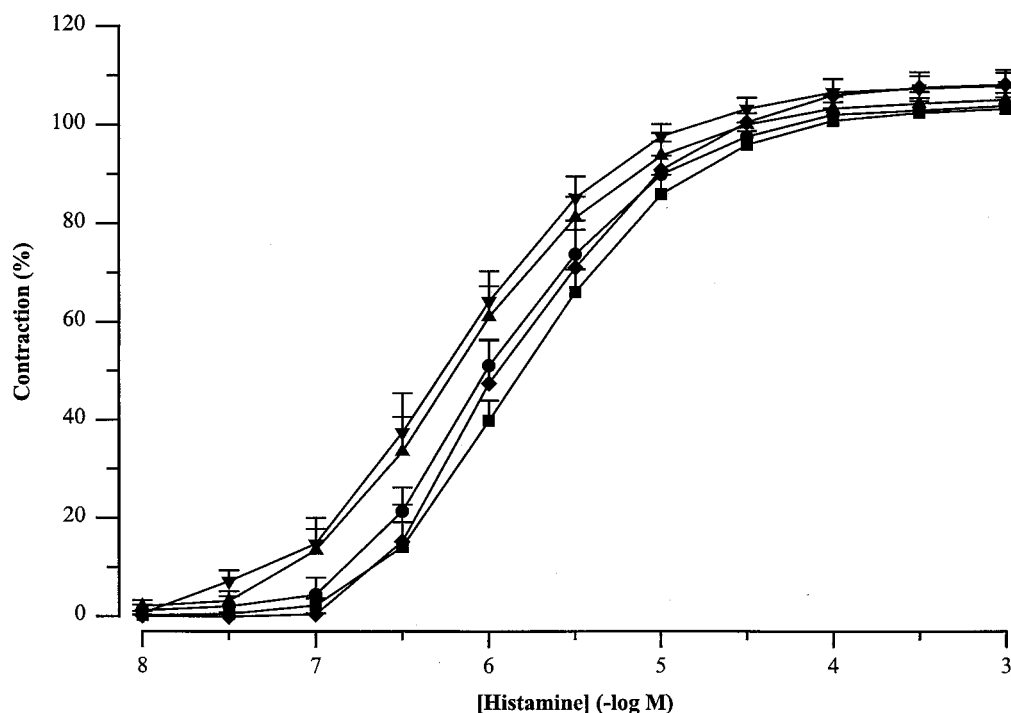
Fig. 2. Concentration-response curves for histamine-induced contraction in bovine tracheal smooth muscle strips incubated for 18 h without (circles) or with the β_2-adrenoceptor agonist fenoterol (upward triangles: 10^{-7} M, downward triangles: 10^{-5} M), 10^{-6} M dexamethasone (squares) or the combination of 10^{-7} M fenoterol and dexamethasone (diamonds). Contraction is presented as percentage of the response to 10^{-4} M methacholine in the second precontraction curve (see *Experimental Procedures*). Means $\pm$ S.E. of five experiments each performed in duplicate, except dexamethasone plus fenoterol ($n = 4$).

protein synthesis was not required for the fenoterol-enhanced expression of histamine H$_1$ receptor mRNA.

The increase in the steady-state level of histamine H$_1$ receptor mRNA after β_2-adrenoceptor activation was mimicked by forskolin (an agent that directly stimulates adenylyl cyclase), 8-bromo-cAMP (a nonhydrolyzable analog of cAMP), and PGE$_2$ (another agent-elevating intracellular cAMP level via receptor activation). These data strongly indicate a cAMP-dependent process in the up-regulation of histamine H$_1$ receptor mRNA. The presence of a putative cAMP-response element in the 5′-flanking region of the bovine histamine H$_1$ receptor gene would be expected, given the regulation of histamine H$_1$ receptor mRNA by cAMP in this study. However, no sequence data are available for the bovine histamine H$_1$ receptor gene promoter except from human studies (De Backer et al., 1998).

Experiments were also performed to determine whether the fenoterol-induced up-regulation of histamine H$_1$ receptor mRNA level was a consequence of alteration in mRNA stability or changes in the rate of gene transcription. Following fenoterol treatment, we found an increase in the histamine H$_1$ receptor mRNA half-life that may partially explain the up-regulation. β-Adrenoceptor agonists have previously been shown to affect mRNA stability, such as m2 muscarinic ace-

tylcholine receptor mRNA levels in chicken heart cells (Jackson and Nathanson, 1995) and tachykinin NK$_2$ receptor mRNA in bovine tracheal smooth muscle (Katsunuma et al., 1999). The rate of transcription of newly synthesized histamine H$_1$ receptor mRNA measured by a nuclear run-on assay was also increased by approximately 60% after fenoterol treatment for 2 h, compared with control, suggesting that both an increase of histamine H$_1$ receptor mRNA stability and an increased rate of transcription of histamine H$_1$ receptor gene are likely to contribute to the transcriptional and post-transcriptional up-regulation of histamine H$_1$ receptors.

The contraction experiments suggest that β_2-adrenoceptor-mediated up-regulation of histamine H$_1$ receptor number may have functional implications. Thus, although the concentration-response curves for histamine-induced contraction were shifted to the left to only a limited extent after prolonged exposure to fenoterol (1.5-fold after 10^{-7} M, 1.7-fold after 10^{-5} M), this increase in contractile potency agrees extremely well with the actual level to which receptor number was increased (i.e., 1.7-fold after 18-h fenoterol 10^{-7} M). Furthermore, the decrease in histamine contractile potency
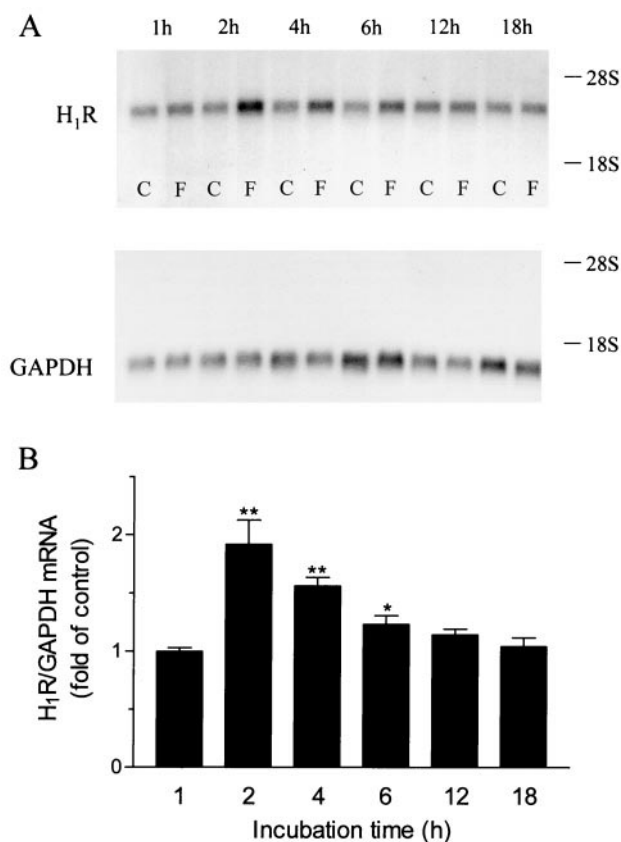
Fig. 3. Time course of fenoterol-induced steady-state level of histamine H$_1$ receptor mRNA in bovine tracheal smooth muscle. A, Northern blot analyses with cDNA for H$_1$ receptor and GAPDH after isolation of poly(A)$^+$ RNA from bovine tracheal smooth muscle in absence (C) and presence of 10^{-7} M fenoterol (F) for indicated times. B, quantification of densitometric measurement as ratio of H$_1$ receptor mRNA relative to GAPDH mRNA. Means ± S.E. of four to six separate experiments are shown; significance of difference from corresponding control value with Student's t test: *P < .05, **P < .01.
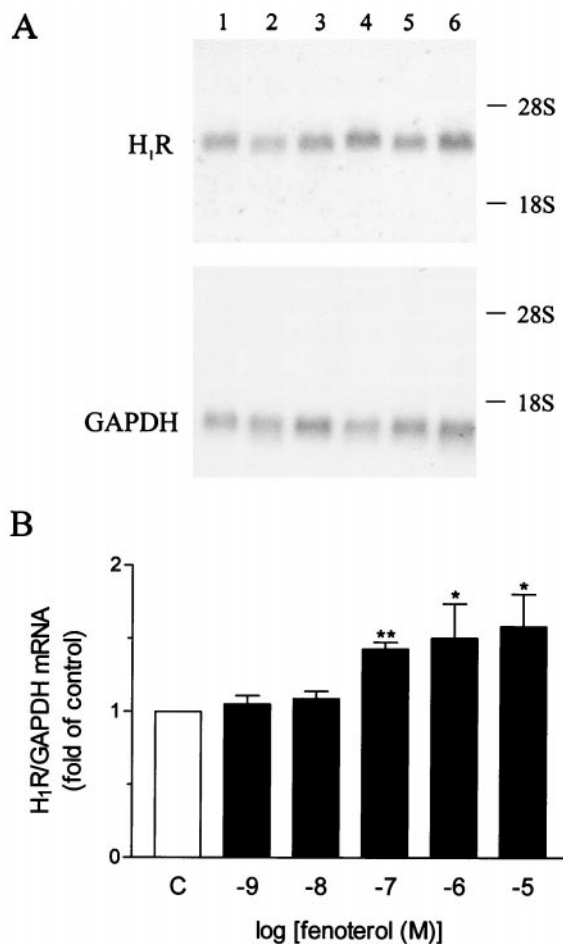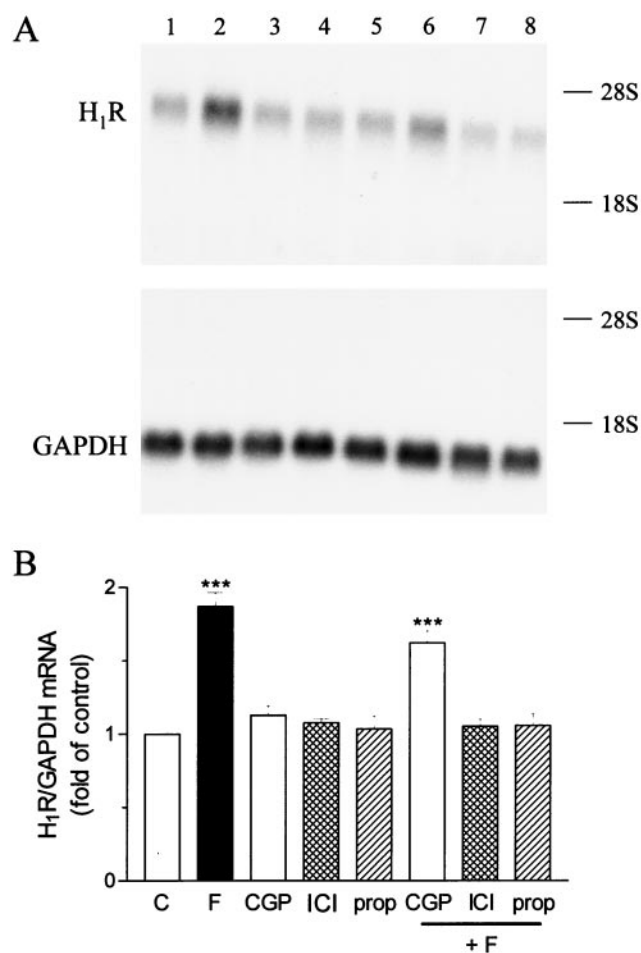

Fig. 4. Concentration-response relationship of fenoterol-induced increases in histamine H$_1$ receptor mRNA in bovine tracheal smooth muscle. A, Northern blot analyses of poly(A)$^+$ RNA isolated after incubation in the absence (C) or presence of increasing concentrations of fenoterol for 2 h. Lane 1, vehicle (control); lane 2, 10^{-9} M fenoterol (F); lane 3, 10^{-8} M; lane 4, 10^{-7} M; lane 5, 10^{-6} M; lane 6, 10^{-5} M. B, densitometric measurements of H$_1$ receptor mRNA, means ± S.E. of five separate experiments are shown; significance of differences from control values with Student's t test: *P < .05, **P < .01.

after 18-h pre-exposure to dexamethasone was also relatively small in terms of shift of the concentration-response curve (1.5-fold), but, similar to the effect observed after fenoterol, this might have functional implications in that the actual contraction levels at low concentrations of the spasmogen were clearly decreased (e.g., from 21 to 14% at 3×10^{-7} M histamine). This regulatory mechanism may well contribute to the improvement of bronchial hyper-responsiveness to histamine in patients suffering from asthma and taking corticosteroids as observed in numerous studies, in addition to the well known anti-inflammatory properties (Barnes et al., 1998). The mechanism by which dexamethasone decreased histamine contractile potency does not appear to reside in direct control of mRNA expression. It has been shown, however, that the exposure of cultured human airway smooth muscle cells to dexamethasone for 22 h resulted in an inhibition of histamine-induced inositol phosphates accumulation as well as calcium mobilization (Hardy et al., 1996). This

mechanism might explain the effect observed in this study. It remains to be established whether reductions in histamine H_1 receptor number and/or alterations in the receptor-G-protein interaction play a role in this functional desensitization.

Similar to our findings on NK_2 receptor expression, dexamethasone completely prevented the increase in histamine contractile potency as induced by prolonged exposure to fenoterol. This protection may be explained by the 37% reduction of fenoterol-induced up-regulation of histamine H_1 receptor mRNA in the presence of the highest dose of glucocorticosteroid, in addition to the putative inhibition of histamine-induced intracellular signaling, which are in contrast to complete protection against fenoterol-induced up-regulation of tachykinin NK_2 receptor mRNA in the same tissue (Katsunuma et al., 1999). Recent observations have shown that salbutamol and salmeterol (partial β_2-agonists) caused direct activation of the glucocorticoid receptors in human lung fibroblasts and vascular smooth muscle cells (Eickelberg et al., 1999). Thus, if this effect on glucocorticoid recep-
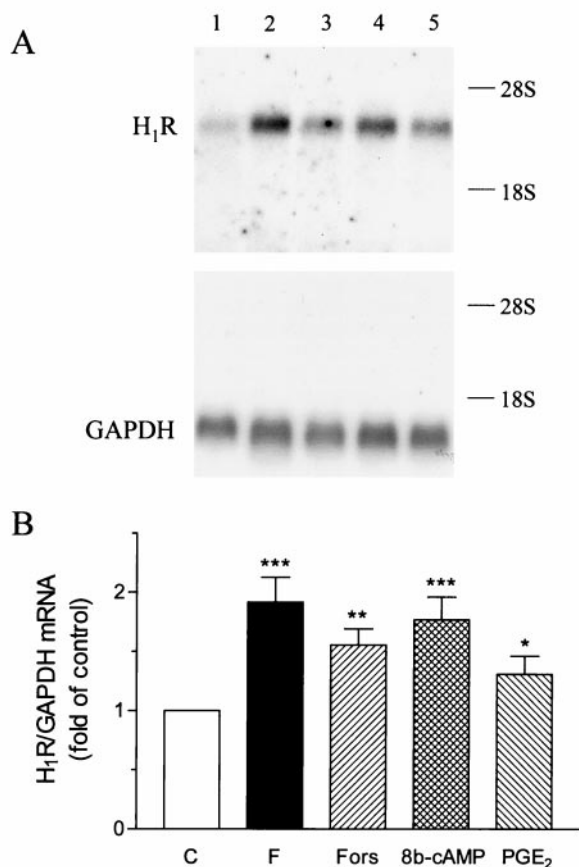

Fig. 5. Effect of the nonselective and selective β-adrenoceptor antagonists propranolol (prop, 10^{-7} M), ICI 118551 (ICI, 10^{-7} M), and CGP 20712A (CGP, 10^{-7} M) on fenoterol-induced increases of histamine H_1 receptor mRNA in bovine tracheal smooth muscle. A, Northern blot analysis of poly(A)$^+$ RNA isolated after incubation in the absence of fenoterol (C, lane 1); in the presence of 10^{-7} M fenoterol (F) alone for 2 h (lane 2); CGP 20712A alone (lane 3); ICI 118551 alone (lane 4); propranolol alone (lane 5); and combinations of CGP 20712A and fenoterol (lane 6), ICI 118551 and fenoterol (lane 7), propranolol and fenoterol (lane 8). B, densitometric measurements of H_1 receptor mRNA, means $\pm$ S.E. of five separate experiments are shown; significance of differences from control values with Student's t test: ***$P < .001$.
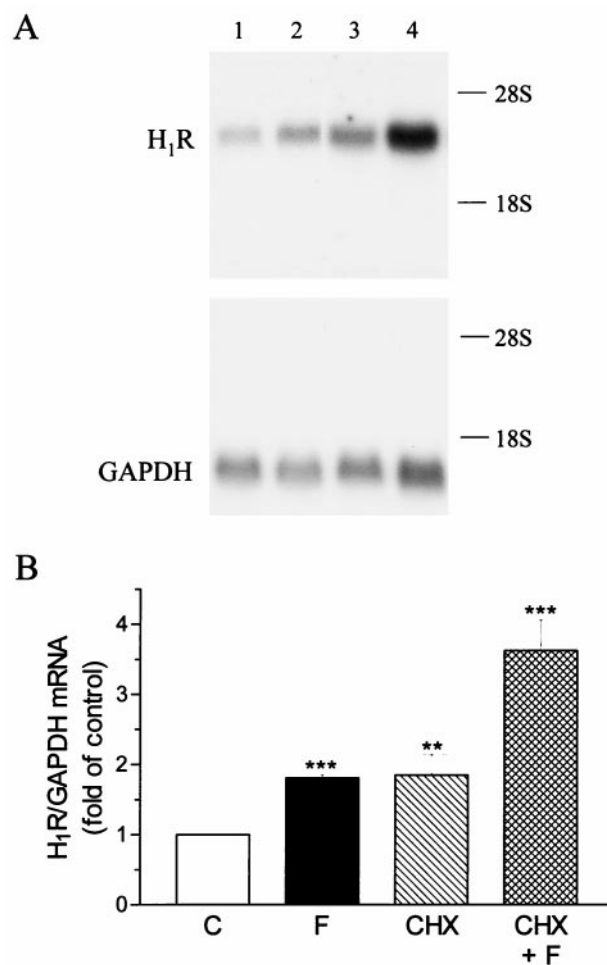

Fig. 6. Effect of the protein synthesis inhibitor cycloheximide (CHX, 10 μg/ml) on histamine H_1 receptor mRNA in bovine tracheal smooth muscle. A, Northern blot analysis of poly(A)$^+$-isolated RNA, isolated in the absence of fenoterol (C, lane 1), in the presence of 10^{-7} M fenoterol (F) alone for 2 h (lane 2), cycloheximide alone (lane 3), and in the combination of fenoterol and cycloheximide (lane 4). B, densitometric measurements of H_1 receptor mRNA, means $\pm$ S.E. of four separate experiments are shown; significance of differences from control values with Student's t test: **$P < .01$, ***$P < .001$.

tors would also occur with fenoterol in bovine trachealis, this would suggest that the effect of β_2-agonist alone is the result of fenoterol-induced up-regulation plus possibly glucocorticoid receptor-mediated down-regulation of histamine H$_1$ re-
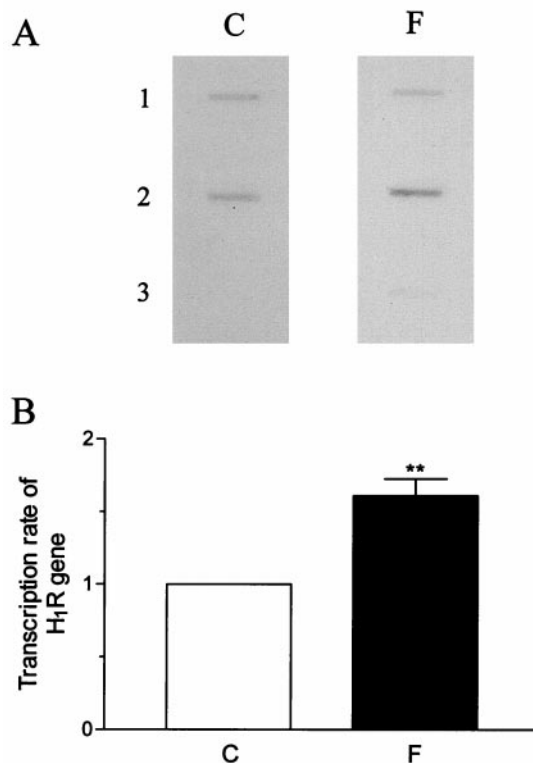
ceptor mRNA. However, we have previously found that dexamethasone alone had no effect on the steady-state level of histamine H$_1$ receptor mRNA (J. L. Pype, unpublished observation), in contrast to the fact that dexamethasone indeed regulates histamine H$_1$ receptor coupling in human cultured airway smooth muscle (Hardy et al., 1996). The observed differences may be due to different mechanisms. From the structure of human histamine H$_1$ receptor gene (De Backer et al., 1998), no glucocorticoid response element is present in its promoter, but multiple potential AP-1 binding sites are present in the region. The mechanism by which fenoterol increases the expression of histamine H$_1$ receptor mRNA appears to be independent of new protein synthesis, in contrast to the requirement for de novo protein synthesis for fenoterol-induced up-regulation of tachykinin NK$_2$ receptor mRNA, suggesting that dexamethasone inhibits new protein synthesis.

β_2-Adrenoceptor agonists are the most effective bronchodilators currently available for the treatment of asthma. However, there are still some concerns that excessive use of β_2-adrenoceptor agonists in some patients may contribute to asthma morbidity or mortality (Chung, 1993). The regular use of inhaled β_2-adrenoceptor agonists such as fenoterol has been associated with an increased risk of death or near death from asthma (Spitzer et al., 1992). Thus, if the increase in histamine H$_1$ receptor expression as observed here would also occur in human airway smooth muscle in vivo, this


Fig. 7. Effect of the direct adenylyl cyclase activator forskolin (Fors, 10^{-5} M), cAMP analog 8-bromo-cAMP (8b-cAMP, 10^{-3} M), and PGE$_2$ (10^{-6} M) on histamine H$_1$ receptor mRNA in bovine tracheal smooth muscle. A, Northern blot analysis of poly(A)$^+$ RNA isolated after incubation in the absence of any drug (C, lane 1), in the presence of 10^{-7} M fenoterol (F, lane 2), forskolin (lane 3), 8-bromo-cAMP (lane 4), and PGE$_2$ (lane 5) for 2 h. B, densitometric measurements of H$_1$ receptor mRNA, means ± S.E. of four separate experiments are shown; significance of differences from control values with Student's *t* test: *$P < .05$, **$P < .01$, ***$P < .001$.
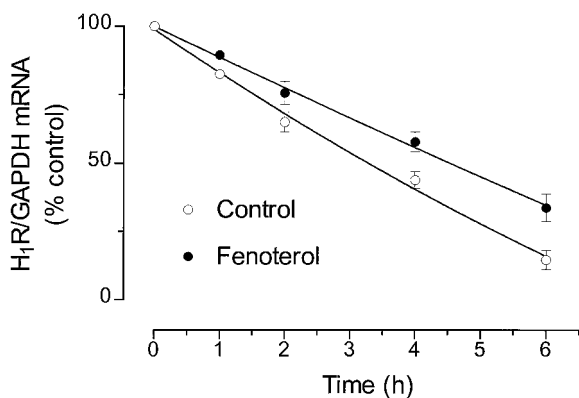
Fig. 8. Effect of fenoterol on stability of histamine H$_1$ receptor mRNA in bovine tracheal smooth muscle. Actinomycin D (5 μg/ml) was added at 0 min to bovine tracheal smooth muscle that had been pretreated for 2 h with vehicle (○) or 10^{-7} M fenoterol (●), and incubation was continued for the times indicated. Means ± S.E. of four separate experiments are shown.

Fig. 9. Effect of fenoterol on transcription of histamine H$_1$ receptor gene in bovine tracheal smooth muscle after incubation of in the absence (control) and presence of 10^{-7} M fenoterol for 2 h by nuclear run-on assay. A, a representative autoradiogram showing quantification by GAPDH (1), histamine H$_1$ receptor (2), and pGEM-3Z (3). B, densitometric measurement showing transcription rate of histamine H$_1$ receptor gene as the ratio to GAPDH in control and fenoterol-treated tissues. Means ± S.E. of three separate experiments are shown; significance of difference from control values with Student's *t* test: **$P < .01$.

might contribute to the adverse effects with the regular or sustained use of β_2-adrenoceptor agonists. Consistent with the contraction experiments, contraction levels were increased markedly especially at low concentrations of the spasmogen (e.g., from 21% to approximately 35% of methacholine maximum at 3×10^{-7} M histamine). Such increased contraction, and thus shortening of the muscle, would result in quite important reductions in airflow, given that this is dependent on airway radius.

In summary, the molecular transregulation of histamine H_1 receptors by β_2-adrenoceptor activation, which results in up-regulation of the functional histamine H_1 receptors, is preceded by an increase in steady-state levels of mRNA and is controlled at the gene level through a dual mechanism involving transcriptional and post-transcriptional mechanisms. The up-regulation of histamine H_1 receptor mRNA could also be mimicked by other agents that elevate cAMP levels, suggesting that cAMP has a role in this process.
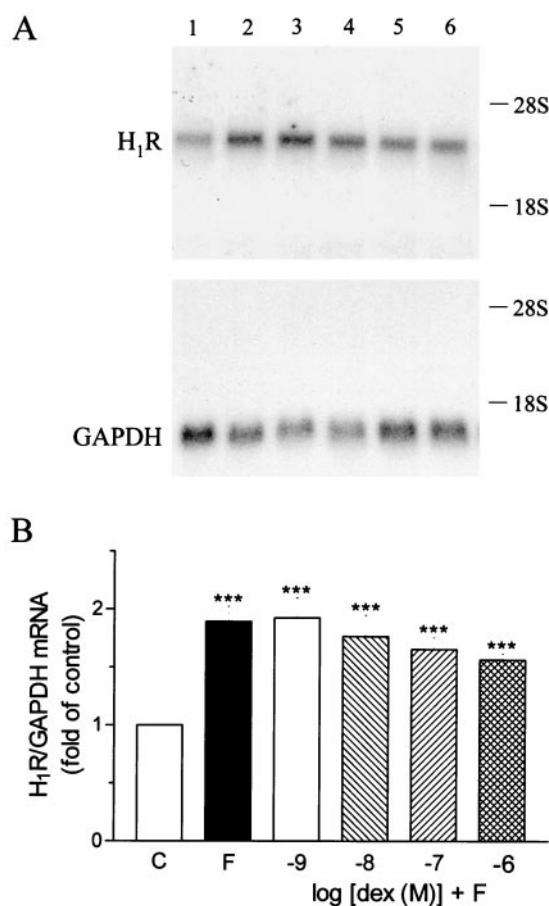
Fig. 10. Concentration-response relationship of the effect of dexamethasone (dex) on fenoterol-induced histamine H_1 receptor mRNA up-regulation in bovine tracheal smooth muscle. A, Northern blot analyses of poly(A)$^+$ RNA isolated after incubation in the absence (C) or in the presence of fenoterol alone or in combination of fenoterol with increasing concentrations of dexamethasone for 2 h. Lane 1, vehicle (control); lane 2, 10^{-7} M fenoterol (F) alone; lane 3, 10^{-9} M dexamethasone plus fenoterol; lane 4, 10^{-8} M dexamethasone plus fenoterol; lane 5, 10^{-7} M dexamethasone plus fenoterol; lane 6, 10^{-6} M dexamethasone plus fenoterol. B, densitometric measurements of H_1 receptor mRNA, means ± S.E. of three separate experiments are shown; significance of differences from control values with Student's t test: ***$P < .001$.

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