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European Journal of Pharmacology 344 (1998) 99–106

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Glucocorticoids reduce tachykinin NK_2 receptor expression in bovine tracheal smooth muscle

Toshio Katsunuma, Judith C.W. Mak, Peter J. Barnes *

Department of Thoracic Medicine, Imperial College School of Medicine, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, UK

Received 5 November 1997; revised 24 November 1997; accepted 28 November 1997

Abstract

Neurokinin A is not only a potent bronchoconstrictor, but also has immuno-modulatory effects in animals and man, mediated via tachykinin NK_2 receptors. We have examined the effect of the glucocorticoid, dexamethasone, on tachykinin NK_2 receptor mRNA and the number of tachykinin NK_2 receptors in bovine tracheal smooth muscle in vitro by Northern blot analysis using a human tachykinin NK_2 receptor cDNA probe and receptor binding assay using [^{3}H]SR48968 {(*S*)-*N*-methyl-*N*[4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl) butyl]benzamide}. Tachykinin NK_2 receptor mRNA showed a time-dependent suppression (62% reduction after 6 h at 10^{-7} M of dexamethasone), as well as a concentration-dependent suppression after the incubation with dexamethasone ($IC_{50} = 1.3 \times 10^{-8}$ M). This suppression was abolished by the glucocorticoid receptor antagonist, mifepristone (RU38486), indicating that dexamethasone acts via the glucocorticoid receptor. It was also abolished by the protein synthesis inhibitor, cycloheximide (10 μg/ml), indicating that new protein synthesis is required on this suppression. Using the RNA polymerase inhibitor actinomycin D (5 μg/ml), we showed that the stability of tachykinin NK_2 receptor mRNA was not affected by dexamethasone ($t_{1/2} = 5$ h). Nuclear run-on assays revealed a 51% reduction in the rate of tachykinin NK_2 receptor gene transcription after treatment with dexamethasone for 6 h. Radioligand binding assay using an selective tachykinin NK_2 receptor antagonist, [^{3}H]SR48968 showed a significant decrease in the number of receptor binding sites after 16 h ($B_{max} = 262 \pm 23$ versus 213 ± 13 fmol/mg protein for vehicle and dexamethasone treatment respectively, $P < 0.05$), with no significant change at the earlier time points. These results suggest that glucocorticoids act on glucocorticoid receptors to decrease tachykinin NK_2 receptor expression by decreasing the rate of tachykinin NK_2 receptor gene transcription.

Keywords: Smooth muscle, airway; Tachykinin NK_2 receptor; Glucocorticoid

1. Introduction

The tachykinins substance P and neurokinin A are present within sensory C-fibers and are released by a variety of physical and chemical stimulants (Solway and Leff, 1991; Joos et al., 1994; Barnes, 1995b). In the airways, substance P and neurokinin A can increase bronchomotor tone and vascular permeability (Barnes, 1991; Barnes et al., 1991). Neurokinin A is more potent in inducing contraction of airway smooth muscle than substance P (Advenier et al., 1987; Martling et al., 1987; Frossard and Advenier, 1991; Sheldrick et al., 1995) and asthmatic patients bronchoconstrict with neurokinin A rather than substance P inhalation (Joos et al., 1987; Cheung et al., 1993). Recently, tachykinins have also been shown to have immuno-modulatory effects. Substance P and neurokinin A induce the synthesis of inflammatory cytokines in human blood monocytes (Lotz et al., 1988), enhance eosinophil chemotaxis induced by platelet-activating factor (PAF) or leukotriene B_4 in asthmatic patients (Numao and Agrawal, 1992), stimulate human lung fibroblast proliferation and chemotaxis (Harrison et al., 1995) and hematopoiesis (Rameshwar and Gascon, 1996). Neurokinin A also increases airway responsiveness to acetylcholine in rats (Chiba and Misawa, 1995) and to specific antigen in guinea pigs (Bertrand et al., 1993; Boichot et al., 1995). These tachykinins exert their effects via specific receptors. There are three types of tachykinin receptor; tachykinin NK_1 receptors are activated preferentially by substance P, tachykinin NK_2 receptors by neurokinin A and tachykinin NK_3 receptors by neurokinin B (Regoli et al., 1987). These three receptors all belong to the superfamily of G protein-coupled receptors and exert their

* Corresponding author. Tel.: +44-171-3518174; fax: +44-171-3515675; e-mail: p.j.barnes@ic.ac.uk

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functions by activating phosphoinositide hydrolysis (Nakanishi et al., 1990). Studies with selective antagonists have demonstrated an important role for tachykinin NK_2 receptors in the airways. SR48968 {(*S*)-*N*-methyl-*N*[4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl) butyl]benzamide}, a potent selective tachykinin NK_2 receptor antagonist, blocked hyperpnea-induced bronchoconstriction in guinea pigs (Solway et al., 1993), inhibited citric acid-induced airway hyperresponsiveness in guinea pigs (Girard et al., 1996) and also suppressed cough induced by citric acid in guinea pigs (Girard et al., 1995). SR48968 also inhibited significantly the maximum bronchoconstriction induced by metabisulfite in guinea pigs (Sakamoto et al., 1994). Tachykinin NK_2 receptor mRNA expression has been shown to be increased fourfold in lung samples from asthmatics compared with nonsmoking controls, although tachykinin NK_1 receptor mRNA levels were similar in the two groups (Bai et al., 1995).

Glucocorticosteroids are highly effective in controlling asthma symptoms and inhibit the inflammatory response in asthmatic airways (Barnes, 1995a, 1996). These effects are due to interaction with glucocorticoid receptors, which, after translocation to the nucleus, induce either activation or suppression of gene transcription (Barnes, 1995a, 1996). Whether glucocorticoids influence tachykinin NK_2 receptor has not yet been investigated.

In this study, we have investigated the effects of a glucocorticoid, dexamethasone, on tachykinin NK_2 receptor expression in bovine tracheal smooth muscle.

2. Material and methods

2.1. Tissue preparation

Fresh bovine trachealis was obtained from the abattoir and the smooth muscle layer was dissected after stripping off epithelium and mucosa. The smooth muscle layer was cut into small pieces in oxygenated Krebs–Henseleit solution ((in 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, 5.6). Incubation was performed in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamate, 100 IU/ml penicillin, 100 mg/ml streptomycin and 2.5 μg/ml amphotericin B at 37°C. The pieces of bovine tracheal smooth muscle were placed in T-75 flasks and incubated in the absence or presence of 10^{-10}–10^{-6} M dexamethasone for the indicated periods of time. The tissue was frozen and kept at −70°C for RNA extraction and membrane preparation. To examine whether the effect of dexamethasone was mediated via receptors, bovine tracheal smooth muscle were pre-incubated with vehicle or 10^{-6} M mifepristone (RU38486), a glucocorticoid receptor antagonist (Moguilewsky and Philibert, 1984), for 1 h before addition of 10^{-7} M dexamethasone for a further 6 h. To estimate the half-life of the tachykinin NK_2 receptor mRNA in control and dexamethasone-treated tissues, bovine tracheal smooth muscle was incubated in the absence and presence of 10^{-7} M DEX for 4 h before the addition 5 μg/ml actinomycin D for various times (Rodgers et al., 1985).

2.2. RNA isolation

A single-step method of RNA isolation using acid guanidinium thiocyanate–phenol–chloroform extraction as described by Chomczynski and Sacchi (1987) was used to isolate total cellular RNA from bovine tracheal smooth muscle. Poly $(A)^+$ RNA was prepared using a PolyATtract mRNA isolation kit system IV (Promega, Southampton, UK) according to the manufacturer's instructions. Samples of mRNA were size-fractionated on a 1% (w/v) agarose/formaldehyde gel containing 20 mM morpholinosulfonic acid (MOPS), 5 mM sodium acetate and 1 mM EDTA, pH 7.0 and blotted onto nylon membrane (Magna, MA) by capillary action using 20 × standard saline citrate (SSC; 1 × SSC, 0.15 mM NaCl and 0.015 M sodium citrate, pH 7.0).

2.3. Northern blot analysis

Random primer labelling was carried out with the 891 kb *Eco*RI and *Sac*I fragment from the human tachykinin NK_2 receptor cDNA (kindly provided by Dr. J.E. Krause, St. Louis, USA) and the 1.3 kb *Pst*1 fragment from the rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA using [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham International, Amersham, UK). The blot was prehybridized for 5 h in 50% formamide, 5 × SSC, 5 × Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), 10 mM NaH_2PO_4 and 100 μg/ml sonicated denatured salmon sperm DNA and then hybridized with ^{32}P-labeled cDNA probes for 12–16 h at 42°C. After hybridization, the filter was washed at high stringency in 0.1 × SSC and 0.1% SDS at 55°C for 30 min. The filter was exposed to Kodak OMAT XS film at −70°C with an intensifying screen for 1–3 days. The nylon membranes were hybridized first to a ^{32}P-labeled human tachykinin NK_2 receptor cDNA probe and subsequently to a GAPDH cDNA probe after stripping. The autoradiograms were scanned with a laser densitometer (New Discovery Series; pdi, Huntingdon Station, NY). The amount of tachykinin NK_2 receptor mRNA was quantified relative to the amount of GAPDH mRNA on the same filter.

2.4. Nuclear run-on transcription assay

To determine whether dexamethasone changed the transcription rate of tachykinin NK_2 receptor mRNA, nuclear run-on transcription assays were performed as described previously (Greenberg and Bender, 1992). Nuclei from

frozen bovine tracheal smooth muscle incubated with or without dexamethasone (10^{-7} M) for 6 h were isolated and stored at −70°C in Keller storage buffer at 25×10^6 nuclei/100 ml. 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_4Cl, 7.5 mM $MgCl_2$, 0.625 mM ATP, 0.313 mM GTP, 0.313 mM CTP (Promega, Southampton, UK), 0.5 mCi [^{32}P]UTP (800 Ci/mmol, Du Pont-New England Nuclear, Stevenage, Herts, UK) 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 (Promega). After DNase and proteinase K treatments, the radiolabelled 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 of which 10 μg of either pGEM-3Z plasmid (as control) or plasmid containing inserts of human tachykinin NK_2 receptor cDNA or rat GAPDH cDNA have been immobilized to a nylon membrane. After hybridization for 72 h at 42°C, the filters were washed at a final stringency of 0.1 × SSC and 0.1% SDS at 55°C, including a 30-min digestion with 1 μg/ml RNase A and 10 units/ml RNase T1 at 37°C to digest any single-stranded RNA not hybridized to DNA. After autoradiography, the film was

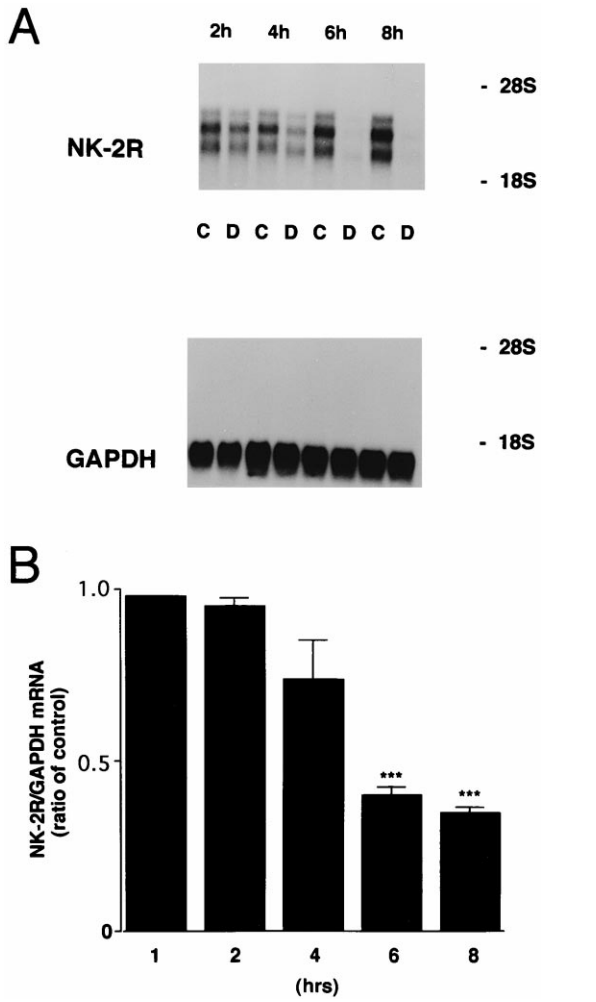

Fig. 1. Time-course of dexamethasone-induced tachykinin NK_2 receptor (NK-2R) mRNA expression in bovine tracheal smooth muscle. Panel A: Northern blot analyses with cDNA for tachykinin NK_2 receptor and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) after isolation of poly(A)$^+$ RNA. Bovine tracheal smooth muscle were incubated in absence (C) and presence of 10^{-7} M dexamethasone (D) for indicated times. Panel B: Quantification of ratio of tachykinin NK-2R mRNA relative to GAPDH mRNA. Mean ± S.E.M. of 4 separate experiments are shown; significance of difference from corresponding control value: $^{***}P < 0.001$.

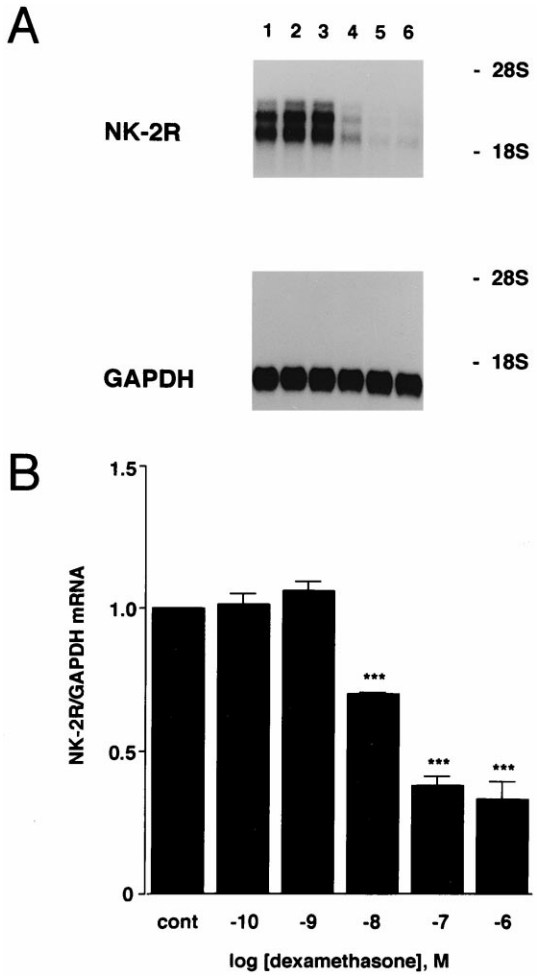

Fig. 2. Dose–response of dexamethasone-induced tachykinin NK_2 receptor (NK-2R) mRNA expression in bovine tracheal smooth muscle. Panel A: Northern blot analyses of isolated poly(A)$^+$ RNA, incubated in the absence (C) or presence of increasing doses of dexamethasone for 6 h. Lane 1, vehicle (control); lane 2, 10^{-10} M dexamethasone; lane 3, 10^{-9} M dexamethasone; lane 4, 10^{-8} M dexamethasone; lane 5, 10^{-7} M dexamethasone; lane 6, 10^{-6} M dexamethasone. Panel B: Densitometric measurements of tachykinin NK-2R mRNA, mean ± S.E.M. of 3 separate experiments are shown; significance of difference from control values: $^{***}P < 0.001$.

scanned with laser densitometry and quantified by calculation of the ratio of tachykinin NK_2 receptor cDNA signal to GAPDH cDNA signal.

2.5. Radioligand binding assay

Frozen bovine tracheal smooth muscle tissues were ground under liquid nitrogen, suspended in 10 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4) containing 0.32 M sucrose and homogenized with a Polytron homogenizer at setting 6 for 30 s bursts. The homogenate was centrifuged at $1000 \times g$ for 10 min at 4°C to remove debris and the supernatant was then centrifuged at $40\,000 \times g$ for 20 min at 4°C. The resulting pellet was washed and recentrifuged at the same speed. The final pellet was resuspended in 50 mM Tris–HCl (pH 7.4). Protein concentration was determined by the method of Lowry et al. (Lowry et al., 1951), using bovine serum albumin as a standard.

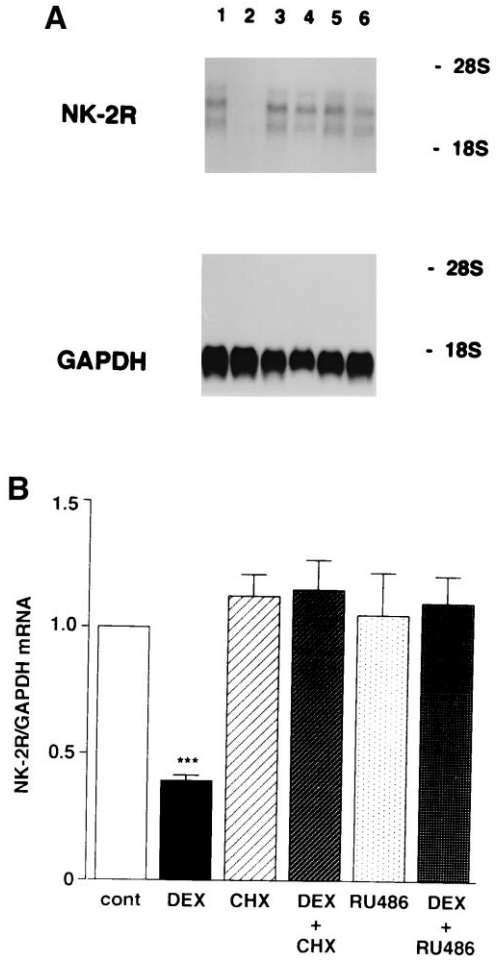

Fig. 3. Effect of the glucocorticoid receptor antagonist mifepristone (RU38486, 10^{-6} M) and the protein synthesis inhibitor cycloheximide (10 μg/ml) on tachykinin NK_2 receptor (NK-2R) mRNA in bovine tracheal smooth muscle. Panel A: Northern blot analysis of isolated poly(A)$^+$ RNA in the absence of dexamethasone (lane 1), 10^{-7} M dexamethasone (DEX) alone for 6 h (lane 2), cycloheximide (CHX) alone (lane 3), combination of dexamethasone and cycloheximide (lane 4), RU38486 (RU486) alone (lane 5) and combination of dexamethasone and RU38486 (lane 6). Panel B: Densitometric measurements of tachykinin NK-2R mRNA, mean ± S.E.M. of 3 separate experiments are shown; significance of difference from control values: $^{***}P < 0.001$.

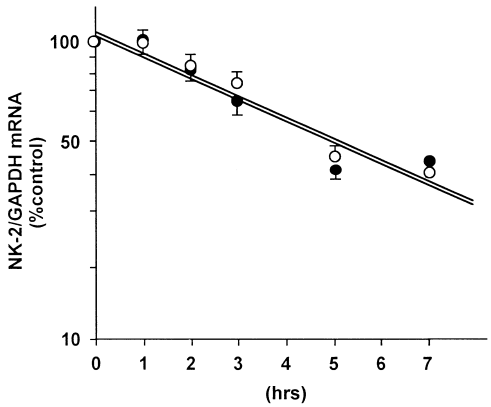

Fig. 4. Effect of dexamethasone on stability of tachykinin NK_2 receptor (NK-2R) mRNA in bovine tracheal smooth muscle. Actinomycin D (5 μg/ml) was added at 0 min to bovine tracheal smooth muscle pretreated for 4 h with vehicle (○) or 10^{-7} M dexamethasone (●) for the times indicated. Mean ± S.E.M. of 3 separate experiments are shown.

The density and affinity of tachykinin NK_2 receptors were assessed from saturation isotherms with the use of 0.5–10 nM [^{3}H]SR48968 (23 Ci/mmol; kindly provided by Dr. Emonds-Alt, Sanofi Recherche, Montpelier, France) in a final volume of 0.5 ml of assay buffer containing approximately 300–400 μg membrane protein in 50 mM Tris–HCl (pH 7.4), 0.4 mg/ml bovine serum albumin, 3 mM $MnCl_2$, 0.04 mg/ml bacitracin and 0.004 mg/ml chymostatin (Emonds-Alt et al., 1993). Non-specific binding was determined in the presence of 10^{-6} M SR48968. After incubation at 25°C for 30 min, bound and free radioactivity were separated through Whatman GF/C glass-fiber filters, presoaked for at least 3 h in a buffer (pH 7.4) containing 50 mM Tris–HCl, 0.02% bovine serum albumin and 0.05% polyethylenimine. Filters were washed 3 times with 5 ml ice-cold buffer, using a Brandel cell harvester and placed in vials with 4 ml of scintillation mixture (Filtron X, National Diagnostics, Manville, NJ) and counted on a Packard liquid scintillation counter (Packard 2200 CA model). Specific binding was determined by substraction of the non-specific binding from total binding and usually accounts for 49–52% of total binding. Maximal binding capacity (B_{max}) and dissociation constant (K_d) were analyzed with the computerized non-linear regression program LIGAND (Munson and Rodbard, 1980).

2.6. Statistical analysis

Data are presented as means ± S.E.M. Groups of data were evaluated by analysis of variance. Data that appeared

statistically significant were compared by paired Student's *t*-test, with Bonferroni correction for comparing the means of multiple groups. Values of $P < 0.05$ were considered to be significant.

3. Results

3.1. Effect of dexamethasone on mRNA

As shown in Fig. 1A, mRNA isolated from bovine tracheal smooth muscle gave rise to three hybridization bands with estimated sizes of tachykinin NK_2 receptor mRNAs of approximately 3.1, 2.7 and 2.3 kb, in both dexamethasone-treated and control bovine tracheal smooth muscle tissues. Two of the bands (3.1 and 2.7 kb) are in agreement with previous reports (Sasai and Nakanishi, 1989), in which the authors concluded that the two tachykinin NK_2 receptor mRNAs differ in the lengths of the extreme 5′ sequence of the 5′-untranslated regions. The time course of the suppression of tachykinin NK_2 receptor mRNA by dexamethasone revealed that a decrease in the tachykinin NK_2 receptor mRNA in all bands could be detected after 4 h and no further decrease could be observed after 8 h (Fig. 1B). Suppression of tachykinin NK_2 receptor mRNA by dexamethasone after 6 h could be detected as low as 10^{-8} M dexamethasone ($IC_{50} = 1.3 \times 10^{-8}$ M, Fig. 2B). Although the glucocorticoid receptor antagonist RU38486 alone had no effect on the expression of tachykinin NK_2 receptor mRNA, it completely blocked the suppressive effect of 10^{-7} M dexamethasone at 6 h (Fig. 3), suggesting that the effect of dexamethasone on tachykinin NK_2 receptor likely mediated by the glucocorticoid receptor.

The protein synthesis inhibitor cycloheximide (10 μg/ml) also completely blocked the suppressive effect of 10^{-7} M dexamethasone at 6 h on the expression of tachykinin NK_2 receptor mRNA (Fig. 3), indicating that new protein synthesis is required on the suppression by dexamethasone.

A change in abundance of the tachykinin NK_2 receptor mRNA could result from either an alteration in the degradation rate or transcription rate of this mRNA. The stability of the tachykinin NK_2 receptor mRNA in the presence of RNA polymerase inhibitor actinomycin D (5 μg/ml) was compared in control and dexamethasone-treated bovine tracheal smooth muscle tissues (Fig. 4). Dexamethasone treatment did not significantly change the degradation rate of the tachykinin NK_2 receptor mRNA; the half-life of tachykinin NK_2 receptor mRNA was approximately 5 h in both control and tissues pretreated with 10^{-7} M dexamethasone for 4 h. These results suggest that the decreased expression of tachykinin NK_2 receptor mRNA in the

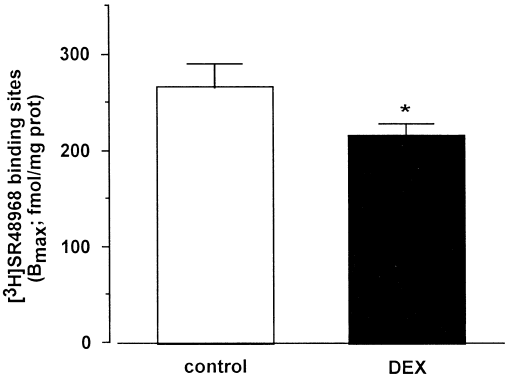

Fig. 6. The effect of 10^{-7} M dexamethasone for 16 h on the number of tachykinin NK_2 receptors, determined by binding of [^{3}H]SR48968 in bovine tracheal smooth muscle. Mean ± S.E.M. of 5 separate experiments are shown; significance of difference from control values: $^*P < 0.05$.

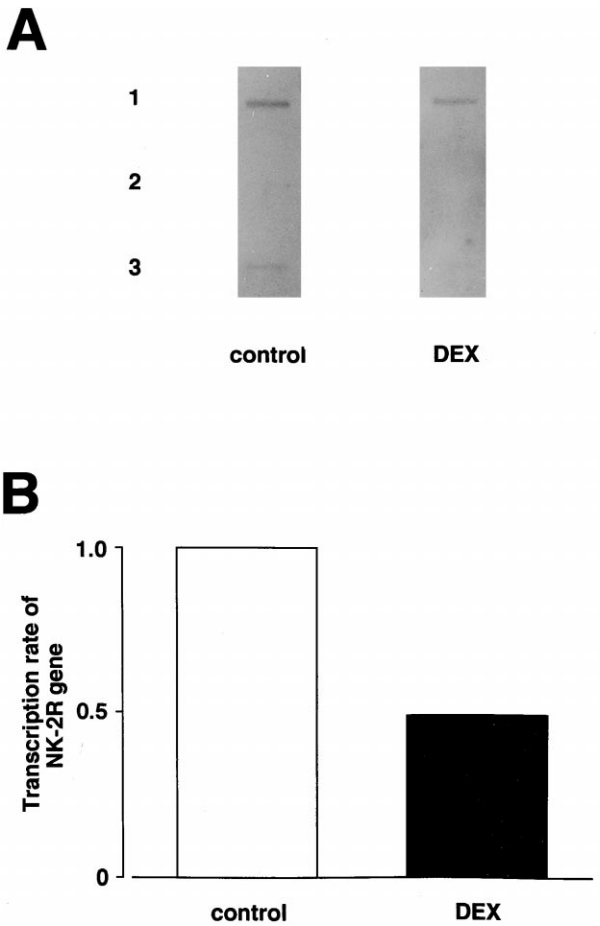

Fig. 5. Effect of dexamethasone on transcription of tachykinin NK_2 receptor (NK-2R) gene in bovine tracheal smooth muscle by nuclear run-on assay in the absence (control) and presence of 10^{-7} M dexamethasone for 6 h. Panel A: A representative autoradiogram showing quantification by GAPDH (1); pGEM-3Z (2); tachykinin NK_2 receptor (3). Panel B: Laser densitometry, showing transcription rate of tachykinin NK-2R gene as the ratio to GAPDH in control and DEX-treated tissues. Average values from 2 separate experiments are shown.

presence of dexamethasone is not due to decreased stability of the tachykinin NK_2 receptor mRNA.

The rate of transcription of newly synthesized tachykinin NK_2 receptor mRNA calculated as a ratio to that of GAPDH mRNA, was reduced by 51% following 10^{-7} M dexamethasone treatment for 6 h, compared with control (Fig. 5).

3.2. Effect of dexamethasone on tachykinin NK_2 receptor binding

[^{3}H]SR48968 binding assay showed a significant decrease in the number of binding sites by dexamethasone after 16 h ($B_{max} = 262 \pm 23$ versus 213 ± 13 fmol/mg protein for vehicle and dexamethasone treatment respectively, $P < 0.05$, Fig. 6), with no significant change at earlier time points. The K_d values did not change significantly in control and dexamethasone-treated tissues.

4. Discussion

We have demonstrated that dexamethasone has a suppressive effect on tachykinin NK_2 receptor gene expression in bovine tracheal smooth muscle. This is the first report on the regulation of tachykinin NK_2 receptor expression by glucocorticoids.

The time-course study showed the decrease of the tachykinin NK_2 receptor mRNA expression by dexamethasone was detected as earlier as 4 h, but a significant reduction in tachykinin NK_2 receptor protein was not seen until 16 h (Figs. 1 and 6). Thus, the decrease in tachykinin NK_2 receptor density was preceded by a decrease in the amount of tachykinin NK_2 receptor mRNA. The glucocorticoid receptor antagonist RU38486 completely blocked the suppressive effect of dexamethasone at 6 h, although RU38486 alone had no effect on the expression of tachykinin NK_2 receptor mRNA (Fig. 3), suggesting that the effect of dexamethasone on tachykinin NK_2 receptor gene acted on glucocorticoid receptor.

To investigate whether there was any change in the stability of the tachykinin NK_2 receptor mRNA in the bovine tracheal smooth muscle tissues treated with dexamethasone, a half-life study was performed, using the RNA polymerase inhibitor actinomycin D (5 μg/ml). The half-life for tachykinin NK_2 receptor mRNA (approximately 5 h) was not affected by dexamethasone, suggesting that a change in the rate of tachykinin NK_2 receptor gene transcription is likely responsible for the reduction in the expression of tachykinin NK_2 receptor mRNA (Fig. 4). This was confirmed by a nuclear run-on assay showing a 51% reduction in tachykinin NK_2 receptor gene transcription after 6 h dexamethasone treatment (Fig. 5). The protein synthesis inhibitor cycloheximide completely blocked the suppressive effect of dexamethasone at 6 h on the expression of tachykinin NK_2 receptor mRNA (Fig. 3), suggesting that the synthesis of a new protein(s) is required for the suppressive effect of glucocorticoids on tachykinin NK_2 receptor gene transcription. This indicates that the effect of glucocorticoid is not mediated directly on the tachykinin NK_2 receptor gene via a glucocorticoid responsive element, but indirectly via an effect on a transcription factor that regulates the tachykinin NK_2 receptor gene promotor region.

In this study, estimated sizes of tachykinin NK_2 receptor mRNAs of approximately 3.1, 2.7 and 2.3 kb appeared in both control and dexamethasone-treated bovine tracheal smooth muscle tissues. In contrast to a previous study using rat tissues, two hybridized bands with estimated sizes of about 3.1 and 2.7 kb were observed (Nakanishi et al., 1990), which were explained by the authors due to the differences in the lengths at the extreme 5′ sequence of the 5′-untranslated regions. We consider all the three bands in our results to be specific, because all three bands never disappear even after the highest stringency washing after hybridization (i.e. $0.1 \times$ SSC and 0.1% SDS at 60°C for 2×30 min). Furthermore, all these bands have been found identically in each independent experiment.

In human bronchus in vitro, it has been shown that the tachykinin-induced contraction appears to be mediated solely by tachykinin NK_2 receptors (Sheldrick et al., 1995), which may be relevant in the pathophysiology of asthma. The relevance of bovine tracheal smooth muscle to human airways is that no detectable level of tachykinin NK_1 receptor mRNA has been found in this tissue. Inhaled neurokinin A induces bronchoconstriction in normal humans (Cheung et al., 1992), and in asthmatic patients (Cheung et al., 1993; Crimi et al., 1993, 1994). Inhaled neurokinin A also induces airway hyperresponsiveness in allergic sheep (Abraham et al., 1991) and enhances airway responsiveness to acetylcholine in rats (Chiba and Misawa, 1995). A selective tachykinin NK_2 antagonist blocks hyperpnea-induced bronchoconstriction in guinea pigs (Solway et al., 1993) and reduced allergen induced bronchoconstriction in guinea pigs (Bertrand et al., 1993; Boichot et al., 1995). These results suggest that neurokinin A may play a role in the bronchial hyperreactivity in asthma, which is an important feature of the disease. Furthermore, neurokinin A induces the release of interleukin-1β, tumour necrosis factor-α (TNF-α) and interleukin-6 from human blood monocytes (Lotz et al., 1988) and enhances PAF-, or leukotriene B_4-induced chemotaxis of eosinophils in vitro (Numao and Agrawal, 1992). These results suggest that neurokinin A may have some inflammatory effects in the pathophysiology of asthma. Inhaled or systemic glucocorticosteroids are an effective therapy for the treatment of asthma. They significantly improve lung function of the patients with chronic asthma and reduce bronchial hyperresponsiveness (Katsunuma et al., 1993). Thus, it is likely that the down-regulation of tachykinin NK_2 receptor ex-

pression by glucocorticoids may be one of the mechanisms involved in controlling airway hyperresponsiveness.

In summary, we have shown the suppression of tachykinin NK_2 receptor mRNA expression as well as a reduction in the receptor density in bovine tracheal smooth muscle by dexamethasone. This is due to a reduced rate of gene transcription and appears to be secondary to the synthesis of an as yet unidentified protein.

Acknowledgements

This work was supported by the National Asthma Campaign, UK.

References

Abraham, W.M., Ahmed, A., Cortes, A., Soler, M., Farmer, S.G., Baugh, L.E., Harbeson, S.L., 1991. Airway effects of inhaled bradykinin, substance P, and neurokinin A in sheep. J. Allergy Clin. Immunol. 87, 557–564.

Advenier, C., Naline, E., Drapeau, G., Regoli, D., 1987. Relative potencies of neurokinins in guinea pig and human bronchus. Eur. J. Pharmacol. 139, 133–137.

Bai, T.R., Zhou, D., Weir, T., Walker, B., Hegele, R., Hayashi, S., McKay, K., Bondy, G.P., Fong, T., 1995. Substance P (NK1)- and neurokinin A (NK2)-receptor gene expression in inflammatory airway diseases. Am. J. Physiol. 269, L309–L317.

Barnes, P.J., 1991. Neuropeptides and asthma. Am. Rev. Respir. Dis. 143, S28–S32.

Barnes, P.J., 1995a. Inhaled glucocorticoids for asthma. N. Engl. J. Med. 332, 868–875.

Barnes, P.J., 1995b. Is asthma a nervous disease?. Chest 107, 119S–125S.

Barnes, P.J., 1996. Molecular mechanisms of steroid action in asthma. J. Allergy Clin. Immunol. 97, 159–168.

Barnes, P.J., Baraniuk, J.N., Belvisi, M.G., 1991. Neuropeptides in the respiratory tract (Part I). Am. Rev. Respir. Dis. 144, 1187–1198.

Bertrand, C., Geppetti, P., Graf, P.D., Foresi, A., Nadel, J.A., 1993. Involvement of neurologic inflammation in antigen-induced bronchoconstriction in guinea pigs. Am. J. Physiol. 265, L507–L511.

Boichot, E., Germain, N., Lagente, V., Advenier, C., 1995. Prevention by the tachykinin NK2 receptor antagonist, SR 48968, of antigen-induced airway hyperresponsiveness in sensitized guinea-pigs. Br. J. Pharmacol. 114, 259–261.

Cheung, D., Bel, E.H., Hartigh, J.D., Dijkman, J.H., Sterk, P.J., 1992. The effect of an inhaled neutral endopeptidase inhibitor, thiorphan, on airway responses to neurokinin A in normal humans in vivo. Am. Rev. Respir. Dis. 145, 1275–1280.

Cheung, D., Timmers, M.C., Zwinderman, A.H., Hartigh, J.D., Dijkman, J.H., Sterk, P.J., 1993. Neutral endopeptidase activity and airway hyperresponsiveness to neurokinin A in asthmatic subjects in vivo. Am. Rev. Respir. Dis. 148, 1467–1473.

Chiba, Y., Misawa, M., 1995. Inhibition of neutral endopeptidase increase airway responsiveness to ACh in nonsensitized normal rats. J. Appl. Physiol. 78, 394–402.

Chomczynski, P., Sacchi, N., 1987. Single-step of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Crimi, N., Oliveri, R., Polosa, R., Pulvirenti, G., Prosperini, G., Palemo, F., Mistretta, A., 1993. Effect of oral terfenadine on bronchoconstrictor response to inhaled neurokinin A and histamine in asthmatic subjects. Eur. Respir. J. 6, 1462–1467.

Crimi, N., Palermo, F., Oliveri, R., Polosa, R., Magri, S., Mistretta, A., 1994. Inhibition of neutral endopeptidase potentiates bronchoconstriction induced by neurokinin A in asthmatic patients. Clin. Exp. Allergy 24, 115–120.

Emonds-Alt, X., Golliot, F., Pointeau, P., Le Fur, G., Breliere, J.C., 1993. Characterization of the binding sites of [^{3}H]SR 48968, a potent nonpeptide radioligand antagonist of the neurokinin-2 receptor. Biochem. Biophys. Res. Commun. 191, 1172–1177.

Frossard, N., Advenier, C., 1991. Tachykinin receptors and the airways. Life Sci. 49, 1941–1953.

Girard, V., Naline, E., Vilain, P., Emonds-Alt, X., Advenier, C., 1995. Effect of the two tachykinin antagonists, SR 48968 and SR 140333, on cough induced by citric acid in the unanaesthetized guinea-pig. Eur. Respir. J. 8, 1110–1114.

Girard, V., Yavo, J.C., Emonds-Alt, X., Advenier, C., 1996. The tachykinin NK2 receptor antagonist SR 48968 inhibits citric acid-induced airway hyperresponsiveness in guinea pigs. Am. J. Respir. Crit. Care Med. 153, 1496–1502.

Greenberg, M.E., Bender, T.P., 1992. Identification of newly transcribed RNA. In: Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K. (Eds.), Current Protocols in Molecular Biology. Wiley, New York, pp. 4.10.1–4.10.9.

Harrison, N.K., Dawes, K.E., Kwon, O.J., Barnes, P.J., Laurent, G.J., Chung, K.F., 1995. Effects of neuropeptides on human lung fibroblast proliferation and chemotaxis. Am. J. Physiol. 268, L278–L283.

Joos, G., Pauwels, R., van der Straeten, M., 1987. Effect of inhaled substance P and neurokinin A on the airways of normal and asthmatic subjects. Thorax 42, 779–783.

Joos, G.F., Germonpre, P.R., Kips, J.C., Peleman, R.A., Pauwels, R.A., 1994. Sensory neuropeptides and the human lower airways: Present state and future directions. Eur. Respir. J. 7, 1161–1171.

Katsunuma, T., Hashimoto, K., Akimoto, K., Ebisawa, M., Iikura, Y., 1993. Effect of inhaled beclomethasone dipropionate on bronchial responsiveness in patients with asthma. Ann. Allergy 70, 165–170.

Lotz, M., Vaughan, J.H., Carson, D.A., 1988. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. Science 241, 1218–1221.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Martling, C.R., Theodorsson-Norheim, E., Lundberg, J.M., 1987. Occurence and effects of multiple tachykinins: Substance P, neurokinin A and neuropeptide K in human lower airways. Life Sci. 40, 1633–1643.

Moguilewsky, M., Philibert, D., 1984. RU 38486: Potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation. J. Steroid Biochem. 20, 271–276.

Munson, P.J., Rodbard, D., 1980. Ligand: A versatile computerized approach for characterization of ligand-binding system. Anal. Biochem. 107, 220–239.

Nakanishi, S., Ohkubo, H., Kakizuka, A., Yokota, Y., Shigemoto, R., Sasai, Y., Takumi, T., 1990. Molecular characterization of mammalian tachykinin receptors and a possible epithelial potassium channel. Recent Prog. Horm. Res. 46, 59–84.

Numao, T., Agrawal, D.K., 1992. Neuropeptides modulate human eosinophil chemotaxis. J. Immunol. 149, 3309–3315.

Rameshwar, P., Gascon, P., 1996. Induction of negative hematopoietic regulators by neurokinin-A in bone marrow stroma. Blood 88, 98–106.

Regoli, D., Drapeau, G., Dion, S., D'Orleans-Juste, P., 1987. Pharmacological receptors for substance P and neurokinins. Life Sci. 40, 109–117.

Rodgers, J.R., Johnson, M.L., Rosen, J.M., 1985. Measurement of mRNA concentration and mRNA half-life as a function of hormonal treatment. Methods Enzymol. 109, 572–592.

Sakamoto, T., Tsukagoshi, H., Barnes, P.J., Chung, K.F., 1994. Involvement of tachykinin receptors (NK1 and NK2) in sodium metabisulfite-induced airway effects. Am. J. Respir. Crit. Care Med. 149, 387–391.

Sasai, Y., Nakanishi, S., 1989. Molecular characterization of rat substance K receptor and its mRNAs. Bichem. Biophys. Res. Commun. 165, 695–702.

Sheldrick, R.L.G., Rabe, K.F., Fischer, A., Magnussen, H., Coleman, R.A., 1995. Further evidence that tachykinin-induced contraction of human isolated bronchus is mediated only by NK2-receptor. Neuropeptides 29, 281–292.

Solway, J., Leff, A.R., 1991. Sensory neuropeptides and airway function. J. Appl. Physiol. 71, 2077–2087.

Solway, J., Kao, B.M., Jordan, J.E., Gitter, B., Rodger, I.W., Howbert, J.J., Alger, L.E., Necheles, J., Leff, A.R., Garland, A., 1993. Tachykinin receptor antagonists inhibit hyperpnea-induced bronchoconstriction in guinea pigs. J. Clin. Invest. 92, 315–323.