J. Pharm. Pharmacol. 1998, 50: 899–905
Received January 13, 1998
Accepted March 10, 1998



# Prejunctional Control of pH 6-Induced Bronchoconstriction by $NK_1$, $NK_2$, $\mu$-Opioid, $\alpha_2$-Adrenoceptor and Glucocorticoid Receptors in Guinea-pig Isolated Perfused Lung

SERGE AUBERSON*†, JEAN S. LACROIX†, ROUZBEH K. KORDESTANI*‡ AND JAN M. LUNDBERG*

*Department of Pharmacology, Karolinska Institute, Box 60400, S-171 77, Stockholm, Sweden, †Department of Oto-Rhino-Laryngology, University Hospital, CH-1211 Geneva, Switzerland and ‡Department of Surgery, Tulane Medical Center, New Orleans, LA 70112, USA

**Abstract**

This study investigated the release of calcitonin-gene related peptide-like (CGRP) immunoreactivity and bronchoconstriction induced by pH 6 buffer in guinea-pig isolated perfused lung.

Both pH 6-induced CGRP-like immunoreactivity and bronchoconstriction were completely abolished after systemic pretreatment with capsaicin. Pretreatment with the $NK_2$ receptor antagonist SR 48968 ($5 \times 10^{-7}$M) completely inhibited bronchoconstriction and significantly reduced the immunoreactivity induced by the pH 6 buffer. The $NK_1$ antagonist SR 140333 ($5 \times 10^{-7}$M) and, to a lesser extent the $NK_1$ antagonist CP 96345, morphine ($5 \times 10^{-6}$M), the $\alpha_2$-adrenoceptor agonist UK 14304 ($10^{-7}$M) and betamethasone ($10^{-6}$M) significantly reduced both pH 6-induced bronchial response and CGRP-like immunoreactivity overflow. The effects of morphine and UK14304 were partially reversed by naloxone ($5 \times 10^{-5}$M) and idazoxan ($5 \times 10^{-5}$M).

Therefore, $NK_1$, $NK_2$, $\mu$-opioid, $\alpha_2$-adrenoceptor and glucocorticoid receptors seemed to have a prejunctional action on pH 6 buffer-induced CGRP-like immunoreactivity and bronchoconstriction.

Development of competitive non-peptide tachykinin receptor antagonists has clarified the role of endogenous tachykinins. In recent studies, potent and selective non-peptide antagonists of the $NK_1$ receptor have been described. They include CP 96345 (2*S*,3*S*-*cis*-2(diphenylmethyl)-*N*-[(2-methoxyphenyl) ethyl]-1-azabicyclo[2.2.2]octam-3-amine) (Snider et al 1991) and SR 140333 (*S*-1-{2-[3-(3,4-dichloro-phenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]-ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]octane, chloride) (Edmond-Alt et al 1993) for the $NK_1$ receptor. The compound SR 48968 (*S*-*N*-methyl-*N*-[4-(4-acethylamino-4-phenylpiperidino)-2-(3,4-dichloro-phenyl)butyl]-benzamide) has antagonist properties for the $NK_2$ receptor (Edmonds-Alt et al 1992).

Low-pH buffer has been shown to induce bronchoconstriction via the $NK_2$ receptor, plasma protein extravasation via the $NK_1$ receptor (Lou & Lundberg 1992; Auberson & Lundberg 1993) and CGRP (calcitonin-gene related peptide) release in guinea-pig isolated perfused heart (Franco-Cereceda & Lundberg 1992).

Neurotransmitter release from sensory nerves can be influenced by opioids and $\alpha_2$-adrenoceptors (Matran et al 1989). Morphine inhibits the non-adrenergic-non-cholinergic (NANC) bronchoconstriction evoked by electric field-stimulation in-vitro (Barthó et al 1987; Matran et al 1989) and in-vivo (Belvisi et al 1988) via a naloxone-sensitive mechanism. Capsaicin-induced bronchoconstriction seems not to be influenced by morphine, suggesting a different site of action (Barthó et al 1987; Matran et al 1989). Bronchoconstriction evoked by electric field-stimulation is markedly reduced in the presence of the $\alpha_2$-adrenoceptor agonist UK 14304 whereas capsaicin-evoked bronchoconstriction is not modified (Matran et al 1989). Lou et al (1992) have demonstrated that bronchoconstriction evoked

Correspondence: S. Auberson, Department of Oto-Rhino-Laryngology, 24 rue Micheli-du-Crest, University Hospital of Geneva, CH-1211 Geneva 14, Switzerland.

by a low concentration of capsaicin ($10^{-8}$M) can be inhibited by $\alpha_2$-adrenoceptor stimulation.

Glucocorticosteroids have anti-inflammatory action and are widely used as anti-asthma drugs. Their mechanism of action is complex and has been partly elucidated (Barnes 1996). Recent studies in-vitro indicate that corticosteroids stimulate the activity of neutral endopeptidase (Borson & Gruenert 1991) and inhibit substance P-receptor expression (Ihara & Nakanishi 1990).

We examined the implication of $NK_1$ and $NK_2$ receptors in pH 6-evoked CGRP-like immunoreactivity and bronchoconstriction in the guinea-pig isolated lung. CGRP-like immunoreactivity release was used as a marker of sensory nerve activation (Kröll et al 1990). The influence of pretreatment with an $\alpha_2$-adrenoceptor agonist, with morphine and with a corticosteroid were also studied.

## Materials and Methods

### *Drugs*

For systemic pretreatment, capsaicin (Fluka, Switzerland) 10mg mL$^{-1}$ was dissolved in 0·9% saline containing 20% Tween 80 and 10% ethanol. For infusion, capsaicin was dissolved in 0·9% saline containing 60% ethanol at a concentration of $10^{-2}$M and then diluted with Krebs buffer. Capsazepine (donated by the Sandoz Institute, London, UK) was dissolved in 100% ethanol to $10^{-1}$M and then diluted in Krebs buffer. Diclofenac (Sigma, St Louis, MO) was dissolved in 100% ethanol to 1M and then diluted in Krebs buffer. Lactic acid and sodium lactate (Sigma) were dissolved in pH 7 stock solution.

CP 96345 and CP 96344 were obtained from Pfizer Central Research (Groton, CT), and SR140333 and SR 48968 from Sanofi Research (Montpellier Cedex, France). They were dissolved in ethanol. Idazoxan was from Synthelabo (France), UK 14304, morphine, naloxone, betamethasone from Sigma, heparin from Kabivitrum (Sweden), terbutaline from Draco (Sweden), theophylline from Kabi Pharmacia (Sweden) and sodium pentobarbital from Apoteksbolaget (Sweden).

### *Experimental procedures*

Dunkin Hartley guinea-pigs (250–400g) of both sexes were used. They were anaesthetized with pentobarbital sodium (Mebumal; 50mg kg$^{-1}$, i.p.). After tracheotomy, a tracheal cannula was inserted and artificial ventilation was maintained by means of an Ealing constant-volume ventilator (Scientific & Research Instruments, Kent, UK) with a tidal volume of approximately 3mL adjusted according to the size of the animal. The cervical vagal nerve beside the trachea was isolated and cut. The chest was opened, heparin (1500Iunits) was injected into the right ventricle and the lung was then perfused, through a cannula inserted in the pulmonary artery, with Krebs-Ringer solution of composition (mM): NaCl 118, $CaCl_2$ 2·5, $MgSO_4$ 1·2, $NaHCO_3$ 24·9, $KHPO_4$ 1·2, KCl 4·7, glucose 5·6 and Hepes 12·6) at 37°C oxygenated with 93·5% $O_2$–6·5% $CO_2$ to give a pH of 7·4. The perfusion solution was collected via a tube introduced into the left atrium. The lung was left in the chest for the duration of the experiment. The lung insufflation pressure was regarded as an indicator of bronchial smooth-muscle tone and was continuously monitored by means of a low-pressure transducer (Statham Instruments, Puerto Rico) and a Grass polygraph (model 7D). The lung was perfused at 25mL min$^{-1}$ using a peristaltic pump (Gilson Medical Electronic, France) and the perfusion pressure was kept between 10 and 12cm water. Body temperature was maintained at 38°C with a heating control device (Elbeco Electronic, Uppsala, Sweden). All experiments were performed after an equilibration period of 15min and in the presence of the $\beta_2$-adrenoceptor agonist terbutaline ($10^{-7}$M).

In the control group pH 6 buffer (composition (mM): NaCl 139·6, $CaCl_2$ 2·5, KCl 0·74, $MgSO_4$ 1·5, $Na_2HPO_4$ 0·8, $KH_2PO_4$ 5·86 and glucose 11) was perfused through the guinea-pig isolated lung for 7·5min. In a separate experiment, 15min before exposure to pH 6 buffer the lung was pretreated with one of several drugs (the $NK_1$ antagonists SR140333 ($5 \times 10^{-7}$M), CP 96345 ($5 \times 10^{-7}$M) or its enantiomer (2*R*,3*R*) CP 96344 ($5 \times 10^{-7}$M), the $NK_2$ antagonist SR 48968 ($5 \times 10^{-7}$M), the $\alpha_2$-adrenoceptor agonist UK 14304 ($10^{-7}$M), UK 14304 ($10^{-7}$M) + idazoxan (an $\alpha_2$ antagonist) ($5 \times 10^{-5}$M), morphine ($5 \times 10^{-6}$M), morphine ($5 \times 10^{-6}$M) + naloxone ($5 \times 10^{-5}$M) or betamethasone ($10^{-6}$M)) by addition of the drug to the perfusate. A separate group received 1mg day$^{-1}$ subcutaneous betamethasone four days before the experiment.

The pH of the perfusate and effluent was measured with a glass pH electrode.

### *Systemic capsaicin pretreatment*

A separate group was pretreated with capsaicin under ketamine (50mg kg$^{-1}$, i.m.) anaesthesia. The bronchodilators terbutaline (50mg kg$^{-1}$, s.c.) and theophyllamine (10mg kg$^{-1}$, i.m.) were given 10min before each injection of capsaicin. The total dose of capsaicin (50mg kg$^{-1}$) was divided into

nine portions ($0{\cdot}25+0{\cdot}25+0{\cdot}5+2+2+5+10+10+20\,mg\,kg^{-1}$) injected subcutaneously over 2 days (Lundberg et al 1983). Experiments on these guinea-pigs were performed three weeks later.

*CGRP-like immunoreactivity release*

The perfusate was collected in 3-min fractions. The volume of each was measured and acetic acid was subsequently added to give a final concentration of 0·2M. Using Sep-Pak $C_{18}$ cartridges, the perfusate samples were desalted, lyophilized and redissolved in buffer before CGRP-like immunoreactivity was determined by radioimmunoassay with an antiserum raised against human CGRP alpha (RAS 6009, Peninsula, Belmont, CA; Franco-Cereceda 1988).

*Data analysis*

Data are reported as means ± s.e.m. Statistical significance was evaluated by analysis of variance (Macintosh computer). $P$ values $< 0{\cdot}05$ were considered to be indicative of significance.

## Results

After introduction of the medium at pH 6 (5·98 ± 0·05) into the isolated perfused lung, the pH in the effluent was higher—6·25 ± 0·03 in the first 3-min fraction and 6·01 ± 0·04 in the second 3-min fraction. Pretreatment with the different drugs did not significantly influence the pH or volume of the effluent (not shown). Buffer at pH 6 resulted in a marked CGRP-like immunoreactivity overflow in the two 3-min fractions, from 8·5 ± 0·6 to 28·6 ± 3·5 and to 72·9 ± 5·9 fmol per fraction in the first and second fractions, respectively (Table 1). The limit of detection for the radioimmunoassay of CGRP immunoreactivity was 7·81 fmol fraction$^{-1}$. Immunoreactivity was significantly higher in the second fraction than in the first. At the same time, the insufflation pressure increased by 100 ± 11·2% (Table 2). Peptide overflow and bronchial response were absent after systemic capsaicin pretreatment (Tables 1 and 2). Pretreatment with the $NK_2$ receptor antagonist SR 48968 ($5 \times 10^{-7}$M) reduced immunoreactivity by 87% in the first fraction and by 80% in the second fraction (Table 1). Pretreatment with SR 48968 completely inhibited pH 6-induced bronchoconstriction (Table 2). The $\alpha_2$-adrenoceptor agonist, UK 14304 ($10^{-7}$M) completely inhibited CGRP-like immunoreactivity induced by pH 6 in the first fraction and reduced it by 70% in the second (Table 1). Simultaneously, there was an 87% attenuation of the insufflation pressure increase (Table 2). We recorded a significant reduction of the onset of maximum bronchoconstriction effect from 236 ± 22·6 s after pH 6-treatment alone to 84 ± 28 s after pretreatment with UK 14304 (not shown). Idazoxan ($5 \times 10^{-5}$M) largely reversed the effects of UK 14304 (Tables 1 and 2).

The $NK_1$ receptor antagonist SR 140333 significantly inhibited both pH 6-induced CGRP-like immunoreactivity overflow in the two fractions and functional response (Tables 1 and 2) whereas CP 96345 significantly reduced immunoreactivity in the second fraction only (Table 1). This reduction of CGRP-like immunoreactivity release was significantly different from the effect of CP 96344, the

Table 1. Calcitonin-gene related peptide-like (CGRP) immunoreactivity release in the first and second 3-min perfusate fractions after exposure to pH 6 in control experiment and after different types of pretreatment.

| Treatment | CGRP (fmol fraction$^{-1}$) | |
|---|---|---|
| | First fraction | Second fraction |
| pH 6 buffer | 28·6 ± 3·5 | 72·9 ± 5·9 |
| pH 6 + SR 48968 ($5 \times 10^{-7}$M) | 15 ± 1·6* | 25·2 ± 2·8*** |
| pH 6 + UK 14304 ($10^{-7}$M) | 8·9 ± 1·1**‡ | 33·6 ± 22·8 |
| pH 6 + UK 14304 ($10^{-7}$M) + idazoxan ($5 \times 10^{-5}$M) | 22·8 ± 2·29 | 55·7 ± 4·4 |
| pH 6 + CP 96345 ($5 \times 10^{-7}$M) | 19·1 ± 1·8† | 34·6 ± 8·9** |
| pH 6 + CP 96344 ($5 \times 10^{-7}$M) | 32·5 ± 3·2 | 46·1 ± 3·9* |
| pH 6 + SR 140333 ($5 \times 10^{-7}$M) | 15·8 ± 2* | 45·9 ± 4·8* |
| pH 6 + betamethasone ($10^{-6}$M) | 33·1 ± 5·7 | 47·1 ± 8·5* |
| pH 6 + morphine ($5 \times 10^{-6}$M) | 11·2 ± 0·9** | 18·6 ± 2·3***§ |
| pH 6 + morphine ($5 \times 10^{-6}$M) + naloxone ($5 \times 10^{-5}$M) | 16·3 ± 1·5* | 35·7 ± 4·9** |
| pH 6 + systemic capsaicin pretreatment | 7·81** | 7·81*** |

Data are means ± s.e.m. (n = 4–9 in each group). *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$, significantly different from result for pH 6 buffer. ‡$P < 0{\cdot}05$, significantly different from result for pH 6 + UK14304 + idazoxan. †$P < 0{\cdot}05$, significantly different from result for pH 6 + CP 96344. §$P < 0{\cdot}05$, significantly different from result for pH 6 + morphine + naloxone.

Table 2. Insufflation pressure changes after exposure to pH 6 in control experiment and after different types of pretreatment.

| Treatment | Insufflation pressure changes (% of basal) |
|---|---|
| pH 6 buffer | 100 ± 11·2 |
| pH 6 + SR 48968 ($5 \times 10^{-7}$ M) | 0*** |
| pH 6 + UK 14304 ($10^{-7}$ M) | 12·7 ± 3·6***† |
| pH 6 + UK 14304 ($10^{-7}$ M) + idazoxan ($5 \times 10^{-5}$ M) | 121·7 ± 38·5 |
| pH 6 + CP 96345 ($5 \times 10^{-7}$ M) | 61·7 ± 12 |
| pH 6 + CP 96344 ($5 \times 10^{-7}$ M) | 15·1 ± 8·2*** |
| pH 6 + SR 140333 ($5 \times 10^{-7}$ M) | 32·1 ± 4·3** |
| pH 6 + betamethasone ($10^{-6}$ M) | 53·5 ± 16·7* |
| pH 6 + morphine ($5 \times 10^{-6}$ M) | 26·1 ± 4·6*** |
| pH 6 + morphine ($5 \times 10^{-6}$ M) + naloxone ($5 \times 10^{-5}$ M) | 37·9 ± 18* |
| pH 6 + systemic capsaicin pretreatment | 0*** |

Data are means ± s.e.m. (n = 4–9 in each group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different from result for pH 6 buffer. † $P < 0.001$, significantly different from result for pH 6 + UK 14304 + idazoxan.

inactive enantiomer of CP 96345, in the first fraction (Table 1). After pretreatment with CP 96345 bronchoconstriction was significantly reduced compared with treatment with CP 96344 (Table 2). Both CP 96344 and CP 96345 reduced the onset of the maximum bronchoconstriction response by 49 and 74%, respectively (not shown). After both types of pretreatment associated with betamethasone there was a significant reduction (48%) of CGRP-like immunoreactivity overflow in the second 3-min fraction (Table 1) with a parallel reduction of the functional response (Table 2). Perfusion with morphine ($5 \times 10^{-6}$ M) inhibited immunoreactivity overflow in the two consecutive fraction by 95% and 87%, respectively (Table 1). This effect was partially reversed by naloxone ($5 \times 10^{-5}$ M). In contrast, the reduction of the bronchoconstriction observed in the presence of morphine was not reversed by naloxone (Table 2).

The basal values of CGRP-like immunoreactivity were statistically higher than controls after pretreatment with SR 140333, betamethasone, morphine + naloxone and UK 14304 + idazoxan.

## Discussion

Treatment at pH 6 induced bronchoconstriction in parallel with CGRP-like immunoreactivity release in the isolated perfused lung. Solutions of pH < 6·2 are known to activate capsaicin-sensitive sensory neurons. (Bevan & Yeats 1991). The involvement of capsaicin-sensitive sensory nerves in the effects of pH 6 treatment was strongly suggested by the abolition of both CGRP-like immunoreactivity overflow and functional response after systemic capsaicin pretreatment.

SR 48968 ($5 \times 10^{-7}$ M) completely abolished the bronchoconstriction and markedly reduced the CGRP-like immunoreactivity induced by pH 6 buffer, confirming the main role of the $NK_2$ receptor in bronchial smooth-muscle contraction. Lou et al (1993) have reported that SR 48968 at the same concentration as in our experiment did not influence CGRP-like immunoreactivity overflow evoked by capsaicin ($10^{-8}$ M) in guinea-pig lung in-vivo and in-vitro. These observations imply selective post-junctional action of SR 48968. The CGRP-like immunoreactivity overflow and bronchoconstriction induced by pH 6 were clearly reduced by SR 48968, suggesting the involvement of prejunctional $NK_2$ receptors. The occurrence of $NK_2$ subtypes, for example the $NK_2A$ receptor, in guinea-pig bronchi has been suggested by Maggi et al (1991), and involvement of prejunctional $NK_2B$ receptors cannot be excluded. Non-specific action of SR 48968 is also possible. Thus, it has been shown that this non-peptide antagonist has a non-specific inhibitory effect on neurotransmission involving blockage of sodium channels by a mechanism similar to that of local anaesthetics (Wang et al 1994).

The involvement of both $NK_1$ and $NK_2$ receptors in bronchoconstriction is supported by the report that these receptor are present in guinea-pig bronchial smooth muscle and that both receptors are involved in NANC bronchoconstriction (Dion et al 1987). However bronchoconstriction seems to be mainly a result of $NK_2$ receptor activation, $NK_1$ receptors being involved to a minor extent only (Lou et al 1993). Consistent with this finding is the observation that pH 6-evoked CGRP-like immunoreactivity release and bronchoconstriction were inhibited by the $NK_1$ antagonists SR 140333 and partially by CP 96345 at the same concentration ($5 \times 10^{-7}$ M). The affinity of CP 96345 seems to be species-dependent—it is approximately two orders

of magnitude higher for the $NK_1$ receptors of man and the guinea-pig than for those of the rat or mouse (Gitter et al 1991). For SR 140333 there seems to be no marked species difference in-vivo or in-vitro (Edmonds-Alt et al 1993). The occurrence of $NK_1$ receptor subtypes cannot, however, be excluded in the guinea-pig ileum (Maggi et al 1994). CP96344, the *2R,3R* enantiomer of CP 96345, lacks $NK_1$ receptor-blocking activity (Snider et al 1991). CP96344 and 96345 have been shown to have L-type calcium-channel antagonism not related to blockade of the $NK_1$ receptor (Constantine et al 1994). This L-type calcium-channel-antagonist action of CP 96344 could explain the effect of this drug on pH 6-induced bronchoconstriction and peptide release, an effect in agreement with the results of Geppetti et al (1991) which showed a reduction of 35% in pH 5-induced CGRP-like immunoreactivity release by nifedipine, a L-type calcium-channel antagonist.

Our current data show the inhibitory action of morphine on sensory peptide release and bronchoconstriction induced by pH 6. These effects were partially reversed by naloxone. Morphine is known to prevent release of substance P from peripheral sensory nerves (Brodin et al 1983). Prejunctional inhibition by morphine of NANC bronchoconstriction by a naloxone-sensitive mechanism has been demonstrated in-vitro (Barthó et al 1987) and in-vivo (Belvisi et al 1988) in guinea-pig bronchi. Conflicting data have been published on the influence of opioids on CGRP-like immunoreactivity release from the spinal cord, depending also on the animal species studied. Opioids reduce CGRP-like immunoreactivity release from the rat spinal cord in-vitro (Pohl et al 1989), whereas the spinal release of CGRP-like immunoreactivity was not affected by morphine in the cat in-vivo (Morton & Hutchinson 1990). Because morphine is preferentially a $\mu$ receptor-selective agonist in-vitro, (Wood et al 1981), our finding with regard to CGRP-like immunoreactivity suggests prejunctional control of $\mu$ receptors by morphine. Participation of other opioid receptors cannot be discounted, because morphine can also bind to $\delta$ and $\kappa$ receptors (Takemori & Portoghese 1987). The reduction of pH 6-induced bronchoconstriction provoked by morphine was not statistically modified by addition of naloxone, whereas CGRP-like immunoreactivity release was partially antagonized. A direct effect of naloxone or morphine on bronchial smooth muscle is less likely, because electrically evoked bronchial contraction is not influence by naloxone (Barthó et al 1987).

$\alpha_2$-Adrenoceptor agonists reduce low-frequency electric field-stimulated bronchoconstriction and low-dose ($10^{-8}$M) capsaicin-induced NANC bronchoconstriction by prejunctional action in the guinea-pig lung (Matran et al 1989; Lou et al 1992). Both CGRP-like immunoreactivity release and bronchoconstriction induced by a high dose of capsaicin ($10^{-6}$M) seem to be less inhibited by an $\alpha$ agonist. In the current study pH 6-induced bronchoconstriction and CGRP-like immunoreactivity release were clearly inhibited by UK 14304 and these effects were reversed by idazoxan. These observations suggest that pH 6 buffer stimulates sensory nerves by a mechanism similar to that of capsaicin at low concentrations. At least two subtypes (a and b) of the $\alpha_2$-adrenoceptor have been described. UK 14304 seemed to have more affinity for the $\alpha_{2a}$-adrenoceptor subtype (Ruffolo et al 1993). Predominant prejunctional control of the sensory nerves by $\alpha_{2a}$ adrenoceptors has been suggested by Matran et al (1989).

In the mouse, dexamethasone was shown to inhibit capsaicin-induced ear oedema, which is primarily mediated by sensory neuropeptides (Inoue et al 1993). In the current study, betamethasone inhibited both pH 6-induced CGRP-like immunoreactivity overflow and bronchoconstriction, indicating probable involvement of prejunctional glucocorticoid receptors. In-vitro studies have demonstrated that betamethasone reduces the expression of the $NK_1$ receptor (Gerard et al 1991). This mechanism of action could explain the reduced bronchoconstriction induced by betamethasone. A betamethasone-induced increase in the activity of neutral endopeptidase, an enzyme involved in CGRP degradation, cannot be excluded (Borson & Gruenert 1991). However, dexamethasone has been shown to reduce the amount of plasma extravasation evoked by tachykinins in the rat trachea via a mechanism unrelated to neutral endopeptidase activation (Brokaw et al 1995).

In conclusion, this study provides evidence that prejunctional $NK_1$, $NK_2$, opioid, $\alpha_2$-adreno- and probably glucocorticoid receptors regulate the local release of CGRP-like immunoreactivity and the bronchoconstriction evoked by pH 6 buffer.

*Acknowledgements*

This study was supported by the Swedish Medical Research Council, the American Council for Tobacco Research, the Swedish Tobacco Company, the Swedish Environmental Protection Board and a grant from the Swiss National fund for Scientific Research.

## References


Auberson, S., Lundberg, J. M. (1993) Lactic acid-induced plasma protein extravasation in rat airways by stimulation of sensory nerves and $NK_1$ receptor activation. Pharmacol. Toxicol. 73: 305–310

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

Barthó, L., Amann, R., Saria, A., Szolcsányi, J., Lembeck, F. (1987) Peripheral effects of opioid drugs on capsaicin-sensitive neurones of the guinea-pig bronchus and rabbit ear. Naunyn Schmiedebergs. Arch. Pharmacol. 336: 316–320

Belvisi, M. G., Chung, K. F., Jackson, D. M., Barnes, P. J. (1988) Opioid modulation of non-cholinergic neural bronchoconstriction in guinea-pig in vivo. Br. J. Pharmacol. 95: 413–418

Bevan, S., Yeats, J. (1991) Protons activate a cation conductance in a subpopulation of rat dorsal root ganglion neurones. J. Physiol. 433: 145–161

Borson, D. B., Gruenert, D. C. (1991) Glucocorticoids induce neutral endopeptidase in transformed human tracheal epithelial cells. Am. J. Physiol. 260: L83–L89

Brodin, E., Gazelius, B., Panopoulos, P., Olgart, L. (1983) Morphine inhibits substance P release from peripheral sensory nerve endings. Acta Physiol. Scand. 117: 567–570

Brokaw, J. J., Werner, L. M., Miller, R., White, W. (1995). Effect of dexamethasone treatment on the regional distribution of neutral endopeptidase activity in the rat trachea. Neuropeptides 29: 273–280

Constantine, J. W., Lebel, W. S., Woody, H. A., Benoit, P. A., Wolfgang, E. A., Knight, D. R. (1994) Cardiovascular effects of CP-96,345, a non-peptide blocker of tachykinin $NK_1$ receptors. Eur. J. Pharmacol. 252: 275–282

Dion, S., D'Orleans-Juste, P., Drapeau, G., Rhaleb, N.-E., Rouissi, N., Tousignant, D., Regoli, D. (1987) Characterization of neurokinin receptors in various isolated organs by the use of selective agonists. Life Sci. 41: 2269–2278

Edmonds-Alt, X., Vilain, P., Goulaouic, P., Proietto, V., Van Broeck, D., Advenier, C., Naline, E., Neliat, G., Le Fur, G., Brelière, J.-C. (1992) A potent and selective non-peptide antagonist of the neurokinin A ($NK_2$) receptor. Life Sci. 50: PL101–PL106

Edmonds-Alt, X., Doutremepuich, J.-D., Heaulme, M., Neliat, G., Santucci, V., Steinberg, R., Vilain, P., Bichon, D., Ducoux, J.-P., Proietto, V., Van Broeck, D., Soubrié, P., Le Fur, G., Brelière, J.-C. (1993) In vitro and in vivo biological activities of SR 140333, a novel potent non-peptide tachykinin $NK_1$ receptor antagonist. Eur. J. Pharmacol. 250: 403–413

Franco-Cereceda, A., Lundberg, J. M. (1992) Capsazepine inhibits low pH- and lactic acid-evoked release of calcitonine gene-related peptide from sensory nerves in guinea-pig heart. Eur. J. Pharmacol. 221: 183–184

Geppetti, P., Del Bianco, E., Patacchini, R., Santicioli, P., Maggi, C. A., Tramontana, M. (1991) Low pH-induced release of calcitonin gene-related peptide from capsaicin-sensitive sensory nerves: mechanism of action and biological response. Neuroscience 41: 295–301

Gerard, N. P., Garraway, L. A., Eddy, R. L., Shows, T. B., Iijima, H., Paquet, J.-L., Gerard, C. (1991) Human substance P receptor (NK-1): organization of the gene, chromosome localization, and functional expression of cDNA clones. Biochemistry 30: 10640–10646

Gitter, B. D., Waters, D. C., Bruns, E. F., Mason, N. R., Nixon, J. A., Howbert, J. J. (1991) Species difference in affinities for substance P receptors. Eur. J. Pharmacol. 197: 237–238

Ihara, H., Nakanishi, S. (1990) Selective inhibition of expression of the substance P receptor mRNA in pancreatic acinar AR42J cells by glucocorticoids. J. Biol. Chem. 265: 22441–22445

Inoue, H., Nagata, N., Koshihara, Y. (1993) Profile of capsaicin-induced mouse ear oedema as neurogenic inflammatory model: comparison with arachidonic acid-induced ear oedema. Br. J. Pharmacol. 110: 1614–1620

Kröll, F., Karlsson, J. A., Lundberg, J. M., Persson, C. G. A. (1990) Capsaicin-induced bronchoconstriction and neuropeptide release in the guinea-pig perfused lung: evidence for local axon reflex. J. Appl. Physiol. 68: 1679–1687

Lou, Y.-P., Lundberg, J. M. (1992) Inhibition of low pH evoked activation of airways sensory nerves by capsazepine, a novel capsaicin-receptor antagonist. Biochem. Biophys. Res. Commun. 189: 537–544

Lou, Y.-P., Franco-Cereceda, A., Lundberg, J. M. (1992) Variable alpha-2 adrenoceptor-mediated inhibition of bronchoconstriction and peptide release upon activation of pulmonary afferents. Eur. J. Pharmacol. 210: 173–181

Lou, Y.-P., Lee, L.-Y., Satoh, H., Lundberg, J. M. (1993) Postjunctional inhibitory effect of the $NK_2$ receptor antagonist SR 48968 on the sensory NANC bronchoconstriction in the guinea-pig. Br. J. Pharmacol. 109: 765–773

Lundberg, J. M., Brodin, E., Saria, A. (1983) Effects and distribution of vagal capsaicin-sensitive substance P neurons with special reference to the trachea and lungs. Acta Physiol. Scand. 119: 243–252

Maggi, C. A., Patacchini, R., Quartara, L., Rovero, P., Santicioli, P. (1991) Tachykinin receptors in the guinea-pig isolated bronchi. Eur. J. Pharmacol. 197: 167–174

Maggi, C. A., Patacchini, R., Meini, S., Quartara, L., Sisto, A., Potier, E., Giuliani, S., Giachetti, A. (1994) Comparison of tachykinin $NK_1$ and $NK_2$ receptors in the circular muscle of the guinea-pig ileum and proximal colon. Br. J. Pharmacol. 112: 150–160

Matran, R., Martling, C. R., Lundberg, J. M. (1989) Inhibition of cholinergic and non-adrenergic, non-cholinergic bronchoconstriction in the guinea-pig mediated by neuropeptide Y and $alpha_2$-adrenoceptor and opiate receptors. Eur. J. Pharmacol. 163: 15–23

Morton, C. R., Hutchison, W. D. (1990) Morphine does not reduce the intraspinal release of calcitonine gene-related peptide in the cat. Neurosci. Lett. 117: 319–324

Pohl, M., Lombard, M. C., Bourgoin, S., Carayon, A., Benoliel, J. J., Mauborgne, A., Besson, J. M., Hamon, M., Cesselin, F. (1989) Opioid control of the in vitro release of calcitonine gene-related peptide from primary afferent fibres projecting in the rat cervical cord. Neuropeptides 14: 151–159

Ruffolo, R. R., Nichols, A. J., Stadel, J. M., Hieble, J. P. (1993) Pharmacologic and therapeutic application of alpha 2-adrenoceptor subtypes. Ann. Rev. Pharmacol. Toxicol. 33: 243–279

Snider, R. M., Constantine, J. W., Lowe III, J. A., Longo, K. P., Lebel, W. S., Woody, H. A., Drozda, S. E., Desai, M. C., Vinick, F. J., Spencer, R. W., Hess, H. J. (1991) A potent nonpeptide antagonist of the substance P ($NK_1$) receptor. Science 251: 435–436

Takemori, A. E., Portoghese, P. S. (1987) Evidence for interaction of morphine with kappa and delta opioid receptors to induce analgesia in beta-funaltrexamine-treated mice. J. Pharmacol. Exp. Ther. 243: 91–94

Wang, Z.-Y., Tung, S. R., Strichartz, G. R., Håkanson, R. (1994) Non-specific actions of the non-peptide tachykinin receptor antagonists, CP-96,345, RP 67580 and SR 48968, on neurotransmission. Br. J. Pharmacol. 111: 179–184

Wood, P. L., Charleson, S., Lane, D., Hudgin, R. L. (1981) Multiple opiate receptors: Differential binding of mu, kappa, and delta agonists. Neuropharmacology 20: 1215–1220