

Local immune response and bronchial reactivity in rats after capsaicin treatment

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The interaction between the nervous system, immune system and bronchial reactivity was studied in rat by using the neurotoxin capsaicin. Rats were treated with capsaicin at 1-2 days of age or at adult age, before or after sensitization by subcutaneous injections with ovalbumin (OA). The levels of the neuropeptides neurokinin A and calcitonin gene-related peptide were decreased in the lung after capsaicin treatment, as determined with radioimmunoassay, whereas the levels of neuropeptide Y were unaffected. The levels of IgA, IgE and IgG in bronchial lavage were also affected by capsaicin treatment; however, the results were heterogeneous. Capsaicin treatment after sensitization reduced the bronchial reactivity to challenge with OA aerosol and serotonin iv. The results demonstrated that reduction of neuropeptide levels with capsaicin affected both bronchial reactivity and the levels of antibodies in bronchial lavage fluid. However, no correlation between these two parameters was seen, demonstrating the complexity of the system.

Key words: bronchial reactivity; capsaicin; immunization; neuropeptides; rats.

Accepted for publication 16 October 1990

The mechanisms underlying bronchial hyper-reactivity in the asthmatic subject still remain to be elucidated. Recently, much interest has been focused on the sensory nervous system (5) and its interaction with the immune system (31, 37). In allergic diseases of the lung and upper respiratory tract, the locally released neuropeptides may be of pathogenetic significance (8). When released, the tachykinins and calcitonin gene-related peptide can induce bronchoconstriction, vasodilatation, edema and mucus secretion (18, 33).

The neurotoxin capsaicin depletes the content of neuropeptides in C-fibre afferent nerves. When administered neonatally to rats, capsaicin causes an irreversible degeneration of the C-fibres, and thus interferes with synthesis and intraneuronal transport of the peptides (12, 13).

Capsaicin treatment of adult rats has been shown to cause a transient decrease of the neuropeptide content in the C-fibres (9). Further, it decreased the bronchoconstriction response to antigen challenge in guinea pigs (18, 24), while it increased the response to iv antigen challenge in rats (3). Capsaicin treatment of immunized rats reduced their lymphocyte proliferation response to mitogen *in vitro* (26) and affected the antibody formation *in vitro* (10, 28).

The purpose of the present study was to examine whether altered immune responses could be responsible for the changed antigen reactivity after capsaicin treatment of sensitized rats. We focused on local levels of total immunoglobulins and antibodies in bronchial lavage. The content of neuropeptides in the lung was also measured.

MATERIAL AND METHODS

Antigen sensitization and capsaicin treatment

Specific pathogen-free hybrid rats of the BN $\times$ Wi/Fu strain of either sex (A-lab, Solentuna, Sweden) were sensitized with ovalbumin (OA) (grade V, Sigma Co., St. Louis, MO) starting at the age of 6 weeks. The antigen was administered by subcutaneous (sc) injections in the neck (300 ng/0.2 ml) once daily, Monday through Friday, for 2 consecutive weeks. The rats were anaesthetized with either ether, for neonatally capsaicin treatment, or with Mebumal® (ACO, Solna, Sweden) 40 mg/kg bw, for treatment at adult age. Capsaicin (50 mg/kg sc, Sigma) was given either in olive oil on the second day of life or in saline with 10% Tween 80 and 10% ethanol at the age of 5 or 8 weeks, i.e. before or after the immunization period.

The rats were bled from the orbital plexus under ether or CO₂ anaesthesia and were then killed by cervical dislocation. The bronchi were washed by injecting and aspirating 2 ml Hank's balanced salt solution (HBSS) containing 0.1% EDTA three times into the surgically exposed trachea. Bronchial lavage fluid was stored at -20°C until analysed. The lung was prepared and immediately frozen at -70°C until analysed.

Neuropeptide analysis

The frozen lung tissue was cut into small pieces and then boiled in acetic acid (for analysis of CGRP and NPY) or in distilled water (NKA), homogenized and centrifuged. The supernatants were lyophilised and stored at -70°C until assayed. The radioimmunoassay was done as previously described (38). The reagents used for the RIA were for NKA: rabbit antibody NKA-5 (6) and NKA iodinated as a tracer (IM 168 Amersham International plc, England); for CGRP: rabbit anti-CGRP (RAS 6009, Peninsula laboratories,

Merseyside, England) and iodinated CGRP (IM 184 Amersham) as a tracer; and, for NPY: rabbit antibody N1 (38) and NPY iodinated (IM 170, Amersham) as a tracer.

Immunoglobulin analysis

The IgE antibody levels to OA were recorded with a radioallergosorbent test (RAST), utilizing rabbit-anti-rat IgE prepared by Dr. T. Karlsson (16) (MIAB, Uppsala, Sweden), as previously described (29). The IgE antibody levels were expressed as percent bound radioactivity of the total radioactivity added.

The IgG and IgA antibody determinations were made utilizing an ELISA, using goat-anti-rat IgG antibodies and goat-anti-rat IgA antibodies (Nordic Labd, Tilbury, The Netherlands) conjugated with alkaline phosphatase. The levels of OA-specific IgG and IgA antibodies were given as the dilution reciprocal giving an OD of 0.10 absorbance units above the background absorbance of a non-immune sample.

The total amount of IgE was recorded with a paper radioimmunosorbent test (PRIST) using rabbit-anti-rat IgE specific antibodies (MIAB). Rat IgE (MIAB) was used as a standard. Concentrations of IgG and IgA isotypes and albumin in BALF were assayed by a competitive, double-antibody radioimmunoassay. For the determination of IgG an affinity purified rabbit-anti-rat IgG (kindly provided by Dr. T. Karlsson, Pharmacia Diagnostics AB) was used. Rat IgG was prepared from serum using a Protein-A-Sepharose column (Pharmacia) and was labelled with ¹²⁵I and used as a tracer and a standard. Rat IgA was determined by using a rabbit-anti-rat IgA serum (Dr. T. Karlsson), adsorbed on a rat-IgG-sepharose column. Rat myeloma IgA protein IR 22 (MIAB), was used as a tracer and a standard. Albumin was measured in the same way, by using a rabbit-anti-rat serum (Dr. T. Karlsson) and rat albumin (Sigma) as a tracer and a standard.

The procedure for the analyses was as follows: 100 μ l purified antibody and 100 μ l

sample were added together with 100 μl ^{125}I labelled tracer into a test tube. Incubation was carried out at room temperature over night on a horizontal shaker. Bound and free protein were separated by addition of 2 ml decanting suspension (Sepharese-sheep-anti-rabbit IgG, Pharmacia) and a second incubation period for 1 h was performed. The tubes were centrifuged and then decanted. The radioactivity in the pellet was measured in a gamma-counter.

Respiratory measurements

The animals were anaesthetized with Inactin® (125 mg/kg ip, Promota, Hamburg, FRG) and placed on a heating pad connected to a thermostat to keep body temperature at 37°C. They were tracheostomized with polyethylene tubing, and polyethylene catheters were inserted into the right common carotid artery and the left external jugular vein for blood pressure recordings and intravenous injections, respectively. The animals were given Pavulon® (0.5 mg/kg iv, Organon, The Netherlands) for muscle relaxation and they were ventilated with a Braun constant volume animal respirator. The respiratory frequency was set to 76–80 breaths/min and the tidal volume was adjusted to give an arterial pCO_2 of 4.7–5.3 kPa measured in an automatic blood gas analyser (Instrumentation Laboratory, model 1312). Ventilation was performed with a positive end-expiratory pressure of 2 cm H_2O to avoid spontaneous atelectasis. The transpulmonary pressure (TPP) was measured by connecting

one outlet of a differential pressure transducer (Gould Statham, PM 131 TC) to a side arm of the tracheal tube (tracheal pressure), and the other to a Braunüle infusion cannula (od 2.0 mm), which was inserted through the 5th or 6th intercostal space (intrapleural pressure). The mean arterial blood pressure (MAP) was measured with a PM 30 transducer (Gould Statham). The animals were challenged with aerosolized OA in sterile distilled H_2O (10 mg/ml) for 10 min using an ultrasonic nebulizer (model 108, Engström Medical AB, Stockholm) connected to the respirator. This resulted in a total delivery of 4 ml antigen solution into the nebulizer. When TPP, MAP and blood gas values had returned to base-line (10–15 min) the animals were challenged by an iv injection of serotonin (Sigma Co.), the main bronchoconstrictive mediator released from rat mast cells, 500 $\mu\text{g/kg}$ dissolved in sterile saline.

Statistical analyses

The Mann-Whitney U-value test was used for statistical analyses of neuropeptide levels in lung tissue, analysis of variance (ANOVA) for immunoglobulins (Ig) in BALF, and Student's t-test for bronchial reactivity. Results were expressed as mean $\pm$ standard error of the mean (SEM).

RESULTS

Neuropeptide levels in the lung

Systemic capsaicin treatment at adult age decreased the levels of neurokinin A-like immunoreactivity (NKA-LI) and calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) in lung tissue, 96% and 64% respectively (Table 1). The effect remained for more than 3 weeks, except for a minor recovery of CGRP-LI levels. The lung level of neuropeptide Y (NPY), mainly found in sympathetic nerves, was not affected by capsaicin pretreatment (Table 1).

Table 1.

Neuropeptide levels in lung after capsaicin pretreatment

Days after CAPS treatment ^b	Neuropeptide ^a		
	NKA	CGRP	NPY
0	1.80 $\pm$ 0.38	2.58 $\pm$ 0.52	4.30 $\pm$ 0.90
7	0.08 $\pm$ 0.02*	1.05 $\pm$ 0.19*	5.82 $\pm$ 0.90
25	0.20 $\pm$ 0.04*	1.45 $\pm$ 0.08*	3.19 $\pm$ 0.60

^a mean pmol/g w.w. $\pm$ SEM

^b separately treated rats which have not been included in other experiments ($n = 4$)

* $P < 0.05$ with Mann-Whitney U-value test

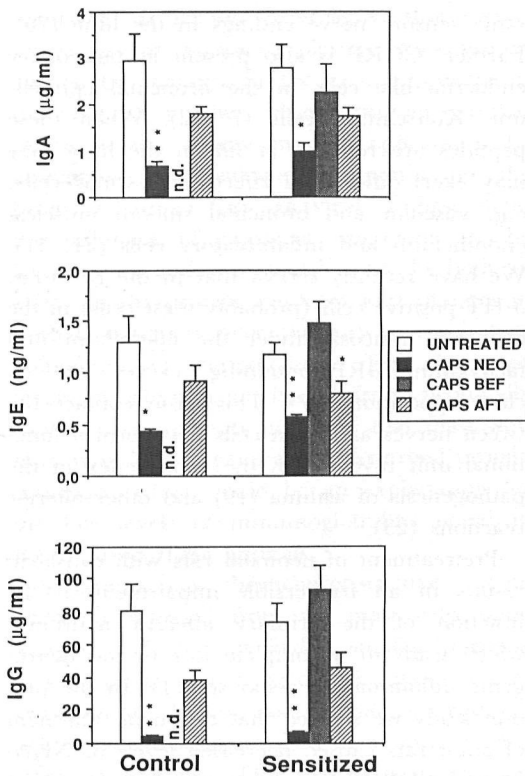


Fig. 1. The total immunoglobulin levels of IgA, IgE and IgG isotypes in bronchial lavage from control and immunized rats. Control rats are indicated by open columns, neonatally capsaicin-treated animals by filled columns, capsaicin-treated before immunization by dotted columns and capsaicin-treated after immunization by hatched columns. * $P < 0.05$ compared with control. $n = 6-20$.

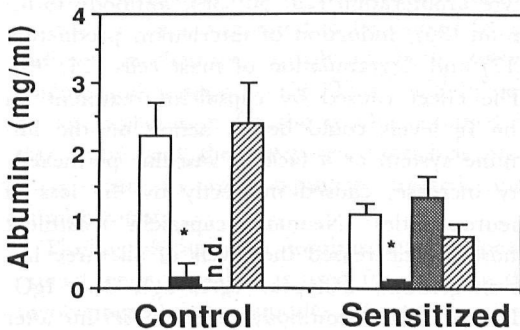


Fig. 2. Albumin in bronchial lavage from control and immunized rats. Designation of columns is as in Fig. 1. * $P < 0.05$ compared with control. $n = 6-17$.

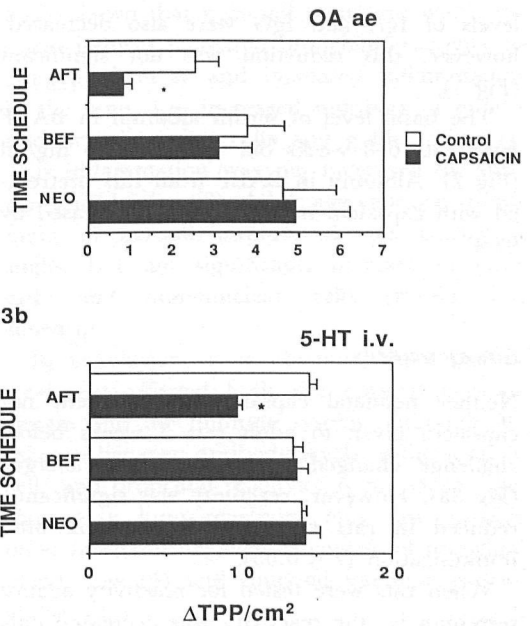


Fig. 3. Bronchial reactivity in subcutaneously sensitized rats, expressed as change in transpulmonary pressure (Δ TPP). Open columns represent untreated and filled columns capsaicin-treated rats, neonatally (NEO) or at adult age (before (BEF) or after (AFT) sensitization). The statistical significance determined with Student's t test is indicated by asterisks. Rats were challenged with OA aerosol (a) or serotonin iv (b). $n = 4-6$.

Immunoglobulins in bronchial lavage fluid

Subcutaneously, sensitization induced a slight production of IgA and IgE antibodies, which was not significantly affected by treatment with capsaicin (results not shown).

Basal immunoglobulin (Ig) levels in bronchial lavage fluid (BALF) from rats 6-8 weeks old were 2.82 ± 0.51 μ g/ml for IgA, 1.29 ± 0.27 ng/ml for IgE and 81.6 ± 15.9 μ g/ml for IgG. Neonatal capsaicin treatment significantly decreased the Ig protein levels in BALF of both IgA, IgE and IgG isotypes (Fig. 1).

When adult rats were treated with capsaicin before immunization the Ig levels were less affected. When capsaicin treatment was performed after immunization, the level of IgE in BALF was decreased ($P < 0.05$) (Fig. 1). The

levels of IgA and IgG were also decreased; however, this reduction was not significant (Fig. 1).

The basal level of serum albumin in BALF from rats 6–8 weeks old was 2.2 ± 0.5 mg/ml (Fig. 2). Albumin in BALF from rats pretreated with capsaicin neonatally was decreased by 93 %

Airway reactivity

Neither neonatal capsaicin pretreatment nor capsaicin given to adult rats 3 weeks before challenge changed the reactivity to allergen (Fig. 3a). However, reactivity was significantly reduced in rats treated with capsaicin after immunization ($P < 0.05$).

When rats were tested for reactivity against serotonin iv, the reactivity was decreased only in the group of sensitized rats, which were capsaicin pretreated at adult age after the immunization (Fig. 3b).

DISCUSSION

The present study showed that bronchial reactivity in sensitized rats can be modulated with capsaicin treatment and that the timing between capsaicin treatment and antigen sensitization is crucial to the outcome. The correlation between IgE, or any other antibody isotype, with bronchial reactivity in rats after sensitization was not seen in this model (2). Neither could we point out any relation between the decreased reactivity in sc sensitized rats to antigen aerosol challenge after capsaicin treatment and the antibody levels in bronchial lavage fluid (BALF).

The role of capsaicin-sensitive nerves in the pathogenesis of allergy in the human skin has been established (23). The etiology of the hyperreactivity of the lung in asthma is unclear, but may be caused by multifactorial inflammation, including both inflammatory cells and their mediators and possibly peptides from the nervous system. Neurokinin A and calcitonin gene-related peptide (CGRP) are co-localized together with substance P in the

same sensory nerve endings in the lung (20). Further, CGRP is also present in pulmonary endocrine-like cells in the bronchial epithelium, Kultschitsky cells (7; 22). When these peptides are released *in situ* in the lung they may exert differential effects on various cells, e.g. vascular and bronchial smooth muscles, endothelium and inflammatory cells (21, 31). We have recently shown that in the rat lung, 5-HT-positive cells (probably mast cells) in the bronchial mucosa under the epithelium and tachykinin/CGRP-containing nerves are in close apposition (27). This close contact between nerves and mast cells may form a functional unit involved in the axon reflex in the pathogenesis of asthma (19) and other allergic reactions (23).

Pretreatment of neonatal rats with capsaicin results in an irreversible impairment of the function of the primary afferent neurones, which leads to a complete loss of the neurogenic inflammatory response (11). In the present study we showed that capsaicin treatment of adult rats caused decreased levels of NKA-LI and CGRP-LI in the lung, which lasted for at least 25 days. The larger reduction of lung NKA-LI than CGRP-LI after capsaicin pretreatment probably reflects that whereas NKA is exclusively present in sensory nerves, CGRP-LI is, in addition, localized in the neuroepithelial bodies (22).

The tachykinins, substance P and NKA, have been shown to affect the activity of immune cells (*in vitro*) in terms of both lymphocyte proliferation (25, 30, 36), antibody formation (36), induction of interleukin production (17) and degranulation of mast cells (14, 35). The effect caused by capsaicin treatment on the Ig levels could be an action on the immune system or a lack of vascular permeability increase, caused indirectly by the loss of neuropeptides. Neonatal capsaicin treatment markedly decreased the levels of all three immunoglobulin isotypes; IgA, IgE and IgG. The pattern of antibody levels in serum after capsaicin treatment are much the same (28). The formation of immunoglobulin *in vitro* of lymphocytes from peripheral blood, spleen and lymph nodes is also affected by capsaicin treat-

ment (28). It has also been shown that capsaicin treatment decreased plasma extravasation evoked by irritants in the airway mucosa of the rat (19). Further, plasma protein extravasation in the airway mucosa appears to be accompanied by leakage of albumin into the bronchial lumen (32). In view of these data, the influence of capsaicin treatment on the levels of antibodies in BALF could be both an effect on the protein synthesis and changes in protein transport over the airway respiratory epithelium or endothelium. The decreased levels of albumin in BALF from neonatally capsaicin treated rats indicated that these animals may have a generalized disturbed protein transport, which could be an explanation for the low levels of immunoglobulins noted in BALF from these animals.

Serotonin, a bronchoconstrictive agent which is released from rat mast cells upon antigen challenge, evoked a smaller response in sc sensitized rats treated with capsaicin after sensitization. In contrast, capsaicin treatment before sensitization resulted in a normal reactivity to serotonin. This may indicate that serotonin, except for its direct effect on smooth muscles, can, like histamine (34), activate sensory nerves to release peptides. Electrophysiological studies have revealed that serotonin activates C-fibre afferents in the airways (39). The results further indicated that there are different effects on the serotonin response depending on the time of capsaicin treatment (before or after sensitization).

The bronchoconstrictive effect of tachykinins in rats has been suggested to be caused by an indirect mechanism, involving both acetylcholine and serotonin (15). This could in part be an explanation for the results obtained in this study and the decreased bronchial reactivity seen in rats capsaicin treated after immunization.

Timing of capsaicin administration appears crucial when capsaicin is used for studying the involvement of the peptide-containing sensory nerves in the immune response and the bronchial reactivity. Neonatal treatment seemed to affect the animal in such a way, that undesirable effects are obtained. Thus, we have previ-

ously shown that neonatal treatment with capsaicin induces increased bronchial reactivity to antigen given iv and increased inflammation in the lung, i.e. increased numbers of mononuclear cells, mast cells and goblet cells (1). This inflammation was not increased by antigen sensitization (3). Adult age capsaicin treatment of aerosol immunized rats caused a slight, but not significant, increase of mast cells and mononuclear cells (results not shown).

In conclusion, it is obvious that capsaicin treatment affected both the sensory nervous system and the immune system, although the relation between antibody levels, infiltration of cells and bronchial reactivity is not clear. The change in lung reactivity may also involve other mechanisms, such as control of bronchial blood flow (4) and mucosal vascular permeability (33).

ACKNOWLEDGEMENTS

The present study was supported by Pharmacia Diagnostics AB, grants from the Swedish Medical Research Council (14X-6554), the Swedish Tobacco Company, the Swedish Work and Environmental Fund and the American Council for Tobacco Research.

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