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Experimentally induced nasal irritation*

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SUMMARY

The aim of this study was to develop a method that is suited for the induction of nasal irritation. For this purpose inflammatory responses were analysed after challenging the nasal mucosa with experimentally induced cold, dry air (8 l/min, 22 °C, 20 %RH). To assess inflammatory effects we determined inflammatory mediators (prostaglandin E2 [PGE2], thromboxane B2 [TXB2], peptide leukotrienes [pLT: LTC4, LTD4, LTE4]) in nasal lavage fluid which was sampled before, immediately after suprathreshold stimulation, and one hour after termination of the stimulation. In addition, subjects estimated the intensity of pain during the stimulation. Cold, dry air produced strong painful sensations which increased throughout the stimulation period. A significant increase of the inflammatory mediator pLT was observed after stimulation; mean concentrations of PGE2 and TXB2 also showed a tendency to increase. One hour after termination of the stimulation the concentration of these inflammatory mediators returned to baseline which indicates the reversibility of the effects of nasal irritation. These data suggest, that this model may be a useful tool in investigations of mucosal irritation as, for example, induced by environmental agents.

Keywords: nasal irritation, inflammatory mediators, nasal lavage, psychophysics, indoor air

INTRODUCTION

The upper respiratory tract, especially the nose is the site of numerous environmentally triggered diseases, e.g. “reactive upper-airways dysfunction syndrome” (RUDS) (Meggs and Cleveland, 1993). Patients with RUDS exhibit a chronic rhinitis after exposure to chemicals. Chemical irritation is also of importance in other syndromes related to the respiratory tract, e.g., “reactive airways dysfunction syndrome” (RADS), “sick-building syndrome” (SBS) or “multiple chemical sensitivities” (MCS) (Brooks et al., 1985; Gots, 1993; Miller, 1994).

Individuals susceptible to irritation induced by environmental agents may become gradually sensitized which may finally result in a chronification of the disease. The pathophysiological mechanisms appear to include inflammation (Meggs, 1993) which is characterized by the release of cytokines and inflammatory mediators, e.g. eicosanoids, bradykinin, or histamine (Ferreira et al., 1974).

To investigate mechanisms of mucosal irritation and of nonallergic rhinitis there is need for models which allow controlled challenge of the mucosa in man. So far, a number of inflammatory models has been proposed. For example, capsaicin or histamine were applied in order to induce neurogenic inflammation (Bedard et al., 1989; Bascom et al., 1991). These and other nonallergic rhinitis models have been reviewed by Philip and Togias (1995). Other researchers used exposition to wood dust and cigarette smoke to assess inflammatory responses (Ahman et al., 1995; Lundberg et al., 1984) which is thought to be more useful to investigate pathological mechanisms that may be caused by these agents.

This study describes the development of a new irritation model induced by intranasal cold, dry air (22 °C, 20 RH, 8 l/min). It has already been applied by Kobal and co-workers to assess the anti-inflammatory and analgesic activity of dipyrone, ibuprofen, ketoprofen and azapropazone (Kobal et al., 1994; Hummel et al.; 1995a, b; Lötsch et al., 1995). Cold, dry air was perceived as less painful after administration of the drugs mentioned above. It is still a matter of debate whether cold, dry air at this temperature, as it is often found in air conditioned buildings, is involved in the generation of inflammatory processes in the nasal cavity. Thus, the aim of the present study was to investigate whether

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intranasal cold, dry air may function as a generator of reversible and controllable nasal irritation in man. Quantitative assessment of inflammatory mediators in nasal fluid was used for this propose.

MATERIALS AND METHODS

Subjects and Experimental Design

Sixteen healthy volunteers (7 females and 9 males between 20 and 34 years of age, mean of 26 years) participated in this study after they had given written informed consent. Each subject took part in one experimental session. In this session their nasal cavity was challenged for 20 minutes with tonic painful stimulation.

To determine the concentration of inflammatory mediators nasal lavage was performed. The nasal cavity was rinsed with 6 ml lactated Ringer's solution before and immediately after the stimulation. In addition, nasal lavage was performed one hour after termination of stimulation.

During the experiment subjects were comfortably seated in an air-conditioned room. White noise (85 dB HL) was used to provide acoustic shielding. In a training session prior to the actual experiment subjects became familiar with the experimental procedures and, specifically, with a breathing technique that avoids all respiratory flow inside the nasal cavity during stimulation (veropharyngeal closure, Kobal and Hummel 1991).

Additionally, subjects were requested to perform a tracking task on a video screen during the experiment (Kobal 1990). Using a joystick they had to keep a small square inside a larger one which moved around randomly. By measuring this tracking performance it was possible to obtain a measure related to vigilance of subjects.

Painful stimulation of the nasal mucosa

To induce inflammatory pain, a constant flow of cold, dry air (8 l/min, 22 °C, 20% RH) was directed into the left nostril for 20 min. This stimulation was perceived as dull and burning pain (Kobal et al., 1994; Hummel et al., 1995a, b). The low humidity (20% RH) was achieved by leading the airstream through a washing bottle filled with silica gel. For thermostabilization at 22 °C the washing bottle and all tubings were located in a thermostat. All materials used were either glass or teflon.

Pain ratings

The perceived intensity of the tonic stimulus was estimated every 5 minutes by means of a visual analogue scale displayed on a computer monitor (Kobal et al., 1990). Subjects reported the actually perceived pain intensity in comparison to a painful phasic stimulus applied at the beginning of the session. This stimulus was induced by 73% v/v CO_2 which excites only nociceptive afferents (Thürauf et al., 1991). The intensity of this standard was defined as 100 Estimation Units (100 EU) symbolized by a 10 cm blue bar on the computer screen. The intensity of the actual stimulus was estimated by adjusting the length of a red bar compared to the blue bar by means of a joystick. For example, if the actually perceived intensity was less than the intensity of the standard, the subjects reduced the length of the red bar accordingly.

NASAL LAVAGE

Nasal lavage is used as a tool in the assessment of acute inflammation in response to irritants (Koren et al., 1990; Naclerio and Togias, 1991). To perform the nasal lavage, subjects tilted their head back 45 degrees with the nasopharynx closed off (velopharyngeal closure). During this process subjects were requested to close the non-stimulated nostril with the finger. Nasal lavage was performed by means of 6 ml lactated Ringer´s solution (pH 5-7), warmed to 37 °C which was instilled with a pipette into the nostril that had been stimulated previously. After 10 sec subjects moved their head back forward and the liquid dropped in a plastic basin. Lavage fluid was centrifuged for 10 minutes (1700 rpm, 4 °C) to separate the cell pellets; it was frozen at -80 °C for further analysis.

Quantification of eicosanoid release

In the present study prostaglandin E2 (PGE2), thromboxane B2 (TXB2) and peptide leukotrienes (pLT: LTC4, LTD4, LTE4) were quantified in parallel with monoclonal antibodies. This was performed twice for each sample by means of highly sensitive and specific competitive enzyme immuno assays using lactated Ringer's solution instead of Hank's balanced salt solution for the dilution of standards and samples (for details see Schaefer et al., 1996).

Statistical Analysis

SPSS for Windows (version 6.1.) was employed for statistical evaluation. To analyse the lavage data, 1-way analyses of variance were performed (MANOVA, repeated measurement design); within subject factor (time: before, immediately after, and 1 h after stimulation). To determine differences between lavage data obtained at the three different times, paired t-tests were employed if the MANOVA yielded a significant result ($p<0.05$).

RESULTS

Pain ratings

Intensity estimates indicated that stimulation with cold and dry air produced already 5 minutes after onset of stimulation strong painful sensations which increased throughout the observation period of 20 minutes (Figure 1).

Inflammatory mediators

In general, the concentrations of the 3 inflammatory mediators increased after intranasal application of cold, dry air. A significant effect was found for peptide leukotrienes (pLT) for the factor time (MANOVA: $F=6.12$, $p=0.006$). The consecutively performed t-tests showed a significant increase of pLT immediately after stimulation compared to baseline (t-test: $p=0.02$; $df=15$) (Fig. 2). In addition, when comparing levels obtained immediately after stimulation and those after one hour pLT concentrations exhibited a significant difference (t-test: $p=0.02$; $df=15$). These results indicate the reversibility of the inflammatory response.

Similarly, the concentrations of PGE2 ($F=2.73$, $p<0.08$) and TXB2 ($F=2.67$, $p<0.08$) exhibited a tendency to increase after application of cold, dry air. As with the pLTs, lavage concentra-

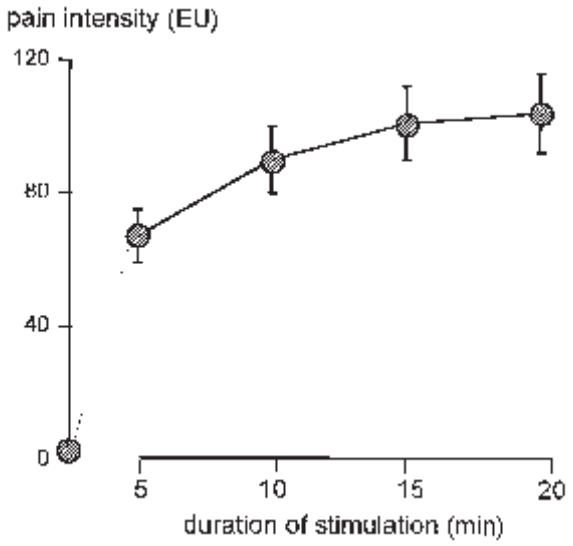

Figure 1. Mean of intensity estimates of tonic pain (SD; n=16) during the course of the experiment (in estimation units, EU). Stimulation with cold and dry air produced strong painful sensations which increased throughout the observation period of 20 minutes.

tions returned to baseline one hour after termination of the stimulation (Figure 2).

DISCUSSION

Experimental approaches in human subjects are needed to study inflammatory responses, especially in environmentally-related diseases, and to assess the anti-inflammatory efficacy of drugs, e.g., aspirin. Beecher described properties of an ideal pain model in man which should also be considered for an experimental model of inflammation (Beecher, 1959). An ideal stimulus should exhibit minimal neurohistological variation between individuals, it should be measureable, closely associated with the changes which produce inflammation, provoke minimal tissue damage, show a relation to pain intensity, provide information about discrimination between stimuli, result in repeatable stimulation without temporal interaction, be applied easily, allow a quantifiable determination of the quality of inflammation, be sensitive to agents of low analgesic power, show dose-related effects of anti-inflammatory drugs, and be applicable for both man and animal.

The present study introduced intranasal cold, dry air as a painful and well controllable and easy-to-use irritant that appears to induce reversible inflammation in man. This was indicated by the increased release of peptide leukotrienes (LTC4, LTD4, LTE4) in response to painful stimulation which returned to baseline one hour after termination of the stimulation. Although the underlying pathophysiological mechanisms have to be investigated in more detail, an inflammatory response may be due to the dehydratation of the respiratory epithelium induced by the low humidity of the stimulatory airstream. In accordance with our results, Cruz and co-workers (1992) found that inhalation of cold dry air increased the level of inflammatory mediators. They also demonstrated that atropine reduced rhinorrhoe, N-alpha-p-tosyl-L-arginine methyl ester (TAME)-esterase activity, and the level of

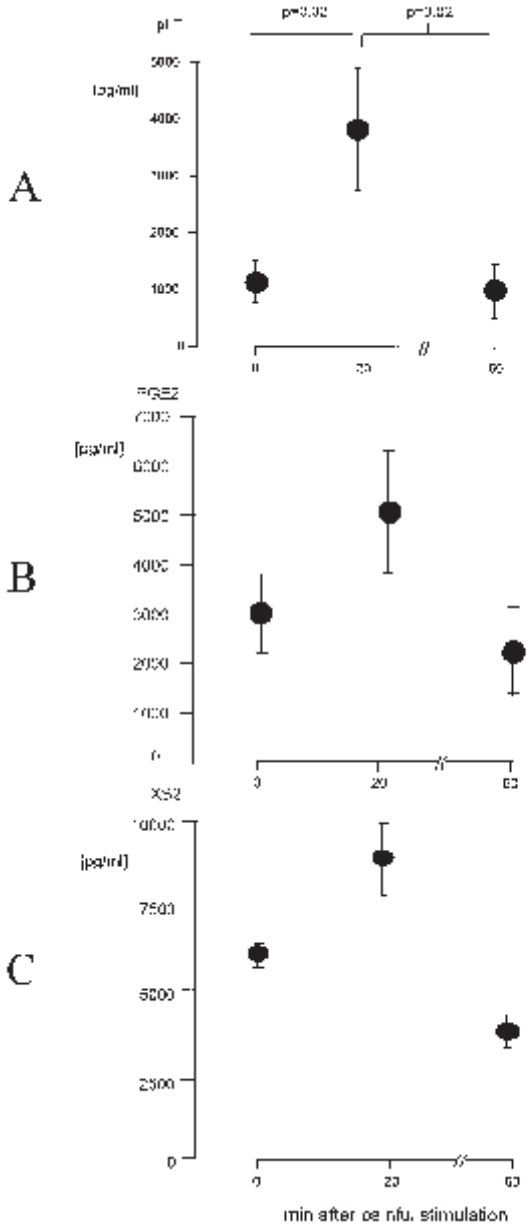

Figure 2. Concentration of peptide leukotrienes (pLT – Fig. 2A), prostaglandin E2 (PGE2 - Fig. 2B), and thromboxane (TXB2 - Fig. 2C) before (baseline), immediately after stimulation and one hour after termination of the stimulation. Concentration of these inflammatory mediators increased in response to painful stimulation; it returned to baseline one hour after termination of the stimulation (means, SEM; n=16).

histamine, however, rhinorrhoe and TAME-esterase activity were still significantly increased compared to baseline. This result suggested partial involvement of parasympathetic activity induced by cold, dry air (Cruz et al., 1992). This parasympathetic activation may also serve as an alternative or additional explanation for the increased levels of peptide leukotrienes and PGE2 (Cruz et al., 1992). As a consequence from this work from Togias and co-workers the assumed presence of an inflammatory response awaits further validation by the demonstration of inflammatory cells in the lavage fluid and/or biopsies taken from the respiratory epithelium.

Today there is only a small number of inflammation models in man; none of these fulfil all of Beecher's requirements. A

model using intranasal neurogenic inflammation was introduced by Bascom and co-workers (1991); it is based on intranasal application of capsaicin, the pungent principle of chilli pepper. Capsaicin predominantly activates nerve endings of unmyelinated nociceptive afferents and causes the release of vasoactive peptides, e.g., substance P, which induces many signs of acute inflammation, e.g., vasodilation or plasma extravasation (Szolcsanyi, 1988). Using a similar concept, Bedard et al. (1989) and Koltzenburg et al., (1992) applied mustard oil or histamine, respectively. This appears to be different with cold, dry air which produced changes compatible with reversible nasal irritation. Hence, the inflammatory effect of this stimulus ended after one hour which results in repeatable application of cold, dry air without temporal interaction. Further, the onset of pain sensation evolved a few minutes after starting the stimulation without showing signs of habituation; this remained constant throughout stimulation. The stimulus is easy to control, i.e. it can be stopped in case subjects find it unbearable.

Another prerequisite of an experimental inflammatory stimulus is its variability (Beecher, 1959). With the presently discussed stimulus, this is easily achieved by either humidity or temperature of the cold, dry air (Mohammadian et al., 1995).

Further, intranasal cold, dry air is suitable to assess the anti-inflammatory efficacy of drugs, e.g., ibuprofen and ketoprofen (Kobal et al., 1994; Hummel et al., 1995a, b).

In conclusion, the present study investigated a model of nasal irritation using intranasal cold, dry air as a tonic inflammatory painful stimulus. Results indicated that the induced response is reversible and well controlled suggesting the usefulness of this method. This model appears to be a promising means to study mechanisms of conditions such as nonallergic rhinitis and environmentally induced diseases.

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