Role of transient receptor potential vanilloid 1 receptors in endotoxin-induced airway inflammation in the mouse

Zsuzsanna Helyes,1* Krisztián Elekes,1† József Németh,2 Gábor Pozsgai,1 Katalin Sándor,1 László Kerékai,3 Rita Bőrzej,1 Erika Pintér,1 Árpád Szabó,1 and János Szolcsányi1,2

1Department of Pharmacology and Pharmacotherapy, 2Neuropharmacology Research Group of the Hungarian Academy of Sciences, and 3Department of Pathology, Faculty of Medicine, University of Pécs, Pécs, Hungary

Submitted 12 October 2006; accepted in final form 15 January 2007

Helyes Z, Elekes K, Németh J, Pozsgai G, Sándor K, Kerékai L, Bőrzej R, Pintér E, Szabó Á, Szolcsányi J. Role of transient receptor potential vanilloid 1 receptors in endotoxin-induced airway inflammation in the mouse. Am J Physiol Lung Cell Mol Physiol 292: L1173–L1181, 2007. First published January 19, 2007; doi: 10.1152/ajplung.00406.2006.—Airways are densely innervated by capsaicin-sensitive sensory nerves expressing transient receptor potential vanilloid 1 (TRPV1) receptors/ion channels, which play an important regulatory role in inflammatory processes via the release of sensory neuropeptides. The aim of the present study was to investigate the role of TRPV1 receptors in endotoxin-induced airway inflammation and consequent bronchial hyperreactivity with functional, morphological, and biochemical techniques using receptor gene-deficient mice. Inflammation was evoked by intranasal administration of Escherichia coli lipopolysaccharide (60 μl, 167 μg/ml) in TRPV1 knockout (TRPV1−/−) mice and their wild-type counterparts (TRPV1+/+) 24 h before measurement. Airway reactivity was assessed by unrestrained whole body plethysmography, and its quantitative indicator, enhanced pause (Penh), was calculated after inhalation of the bronchoconstrictor carbachol. Histological examination and spectrophotometric myeloperoxidase measurement was performed from the lung. Somatostatin concentration was measured in the lung and plasma with radioimmunoassay. Bronchial hyperreactivity, histological lesions (perivascular/peribronchial edema, neutrophil/macrophage infiltration, goblet cell hyperplasia), and myeloperoxidase activity were significantly greater in TRPV1−/− mice. Inflammation markedly elevated lung and plasma somatostatin concentrations in TRPV1+/+ but not TRPV1−/− animals. In TRPV1−/− mice, exogenous administration of somatostatin-14 (4 × 100 μg/kg ip) diminished inflammation and hyperreactivity. Furthermore, in wild-type mice, antagonising somatostatin receptors by cyclo-somatostatin (4 × 250 μg/kg ip) increased these parameters. This study provides the first evidence for a novel counterregulatory mechanism during endotoxin-induced airway inflammation, which is mediated by somatostatin released from sensory nerve terminals in response to activation of TRPV1 receptors of the lung. It reaches the systemic circulation and inhibits inflammation and consequent bronchial hyperreactivity.

capsaicin-sensitive afferents; inflammatory airway hyperreactivity; lipopolysaccharide; myeloperoxidase activity; somatostatin

THE AIRWAYS ARE DENSELY INNERVATED by capsaicin-sensitive sensory nerves (54), which play an important regulatory role in inflammatory processes via the release of sensory neuropeptides. The transient receptor potential vanilloid 1 (TRPV1) receptor, also known as capsaicin receptor, is a nonselective ion channel expressed selectively in the cell membrane of thin afferent (C and Aδ) fibers, which is activated/sensitized by noxious heat and a variety of inflammatory mediators such as protons, lipooxygenase products, bradykinin, or prostaglandins (7, 9, 10, 52). Therefore, its involvement in inflammatory and nociceptive processes has become an issue with pathophysiological relevance and important scopes for drug development (7, 52). The gene of the TRPV1 receptor was successfully deleted in mice (10), which enabled the studying of the functional roles of this ion channel expressed on these sensory fibers in animal models of inflammatory diseases.

TRPV1-expressing afferents are not only involved in sensory input, but these nerve terminals contain several neuropeptides that are released in response to stimulation and exert effector functions (42, 43). Tachykinins (e.g., substance P and neuropeptide K) and calcitonin gene-related peptide (CGRP) elicit neurogenic inflammation and increased microcirculation, respectively, and induce smooth muscle responses in different visceral organs (2, 29, 30, 42).

In the respiratory tract, substance P, neuropeptide K, and CGRP are also localized in a subpopulation of afferent fibers, and capsaicin evokes pronounced airway inflammation and bronchoconstriction by releasing these peptides (2, 29, 30, 42). However, more recently, a surprising counterregulatory humoral function of these nerve endings has been described. Several lines of evidence indicated that somatostatin released from capsaicin-sensitive sensory nerve terminals reaches the circulation and elicits systemic anti-inflammatory (16, 41, 44, 45, 50) and analgesic (5) “sensocrine” effects (41). These systemic inhibitory actions of somatostatin evoked, for example, by antidiromic stimulation of the rat sciatic nerve inhibited plasma protein extravasation in the trachea and the mediastinal connective tissue (45). Furthermore, bilateral stimulation of the peripheral stumps of the cut vagal nerves in rats pretreated with atropine or hexamethonium inhibited neurogenic inflammation in the rat hindpaw skin in addition to inducing neurogenic inflammation locally in the trachea (50).

Endotoxins are constituents of the outer layer of gram negative bacteria and can be found in the surrounding microenvironment. They are significant risk factors for asthma, and asthma severity depends to a great extent on endotoxin concentration of the inhaled air (27, 51). Intranasal administration of lipopolysaccharide (LPS), the main component of endotoxins, to mice is a commonly used confined experimental model to study acute lung inflammation without causing systemic

* Z. Helyes and K. Elekes made equal contributions to the present work.

Address for reprint requests and other correspondence: Z. Helyes, Univ. of Pécs, Faculty of Medicine, Dept. of Pharmacology and Pharmacotherapy, H-7624 Pécs, Szigeti u. 12., Hungary (e-mail: zsuzsanna.helyes@aok.pte.hu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
multiple organ dysfunction (8, 35, 39). Such LPS instillation is known to induce an acute inflammatory response with a huge influx of neutrophils, consequent extravasation of plasma proteins into the airways leading to perivascular/peribronchial edema formation (8, 39), and activation of macrophages (27).

METHODS

Animals. Experiments were performed on female TRPV1 receptor gene knockout mice (TRPV1-/-) and their wild-type counterparts (TRPV1+/+) weighing 20—25 g. Two breeding pairs of TRPV1-/- heterozygote mice were given as generous gifts from Dr. J. B. Davis (GlaxoSmithKline, Harlow, United Kingdom). The genotype of the animals in the first generation was determined by Southern blot analysis and PCR. The TRPV1-/+ and TRPV1-/- were successfully bred on as wild-type and knockout mice in the Laboratory Animal Centre of the University of Pécs under standard pathogen-free conditions at 24—25°C provided with standard chow and water ad libitum, from where they were obtained for the experiments.

Both experimental series presented in this paper were performed in blocks with 10—12 mice per day. Each group presented in one figure ran at the same time. Animals were randomized to the treatments, and mice belonging to every group were assessed for enhanced pause (Penh) simultaneously every day.

Generation of TRPV1 receptor knockout mice. The generation of TRPV1 receptor knockout mice was by homologous recombination in embryonic stem cells (129 ES) to generate a mouse lacking transmembrane domains 2—4 of the murine TRPV1 (mTRPV1) gene. Germline chimeras were crossed onto C57BL/6 females to generate homozygous mutant offspring in the expected Mendelian ratio, as confirmed by PCR and Southern blot analysis. After genotyping by PCR, they were bred from homozygous mutant offspring in the expected Mendelian ratio, as confirmed by PCR and Southern blot analysis.

Determination of airway responsiveness. Airway responsiveness in conscious, spontaneously breathing animals was measured by recording respiratory pressure curves by whole body plethysmography (Buxco Europe, Winchester, United Kingdom) (11, 31). Aerosolized carbachol was administered through bypass tubing via the ultrasonic nebulizer following the same protocol as described for the unrestrained measurement. The concentration of carbachol was increased by each carbachol concentration was calculated and compared with the percentage changes of Penh.

At the end of the measurement, mice were anesthetized with ketamine (100 mg/kg ip; Calypsol, Richter-Gedeon, Budapest, Hungary) and xylazine (10 mg/kg im; Xylavet, Phylaxia-Sanofi, Veterinary Biology, Budapest, Hungary), blood samples were taken by cardiac puncture and then killed by cervical dislocation, and the lungs were excised.

Histological studies and scoring. Lung specimens were fixed in 4% formaldehyde for 8 h, embedded in paraffin, sectioned with microtome (5—7 μm), and stained with hematoxylin (Sigma) and eosin (Molar Chemicals, Budapest, Hungary) or periodic acid–Schiff (Sigma) to more precisely visualize mucus-producing goblet cells. Semiquantitative scoring of the inflammatory changes was performed by an expert pathologist blinded from the study as described by Zeldin et al. (55) on the basis of the presence or abundance of the following: 1) perivascular edema (0: absent; 1: mild to moderate, involving fewer than 25% of the perivascular spaces; 2: moderate to severe, involving more than 25% but less than 75% of perivascular spaces; 3: severe, involving more than 75% of perivascular spaces); 2) perivascular/peribronchial acute inflammation (0: absent; 1: mild acute inflammation in the perivascular edematous space with fewer than 5 neutrophils per high-power field (hpf); 2: moderate acute inflammation in the perivascular spaces extending to involve the peribronchial spaces with more than 5 neutrophils per hpf in these regions; 3: severe acute inflammation in the perivascular and peribronchial spaces with numerous neutrophils encircling most bronchioles); 3) goblet cell metaplasia of the bronchioles (0: absent; 1: few goblet cells present in 1 or 2 bronchioles; 2: large number of goblet cells present); and 4) macrophages/mononuclear cells in the alveolar spaces (0: absent; 1: present in fewer than 25% of alveolar spaces; 2: >25% of alveolar spaces). The score values for these individual parameters were added to form a composite inflammatory score (ranging from 0 to 10). From every specimen (8—10 mice in each group), four to five sections were taken from different depths to give a representative appreciation of the whole lung. Mean scores were determined from the different sections of the individual animals, and composite score values of the different experimental groups were calculated from these mean scores.

Measurement of MPO activity in the lung. Accumulation of granulocytes, especially neutrophils, was determined from the frozen lung samples by assessment of MPO activity. Lung pieces were thawed and chopped into small pieces and then homogenized in 4 ml 20 mM potassium-phosphate buffer (pH 7.4). The homogenate was centrifuged for 10,000 g at 4°C for 10 min, and supernatant was removed.
for somatostatin radioimmunoassay (RIA) (see below). The pellet was resuspended in 4-ml 50 mM potassium-phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (pH 6.0) and centrifuged again. Neutrophil accumulation was assessed by comparing MPO enzyme activity of the samples to a human standard MPO preparation (Sigma). MPO activity was assayed from the supernatant using H₂O₂-3.3′,5.5′-tetramethylbenzidine (TMB/H₂O₂; Sigma). Reactions were performed in 96 well microtiter plates at room temperature. The optical density (OD) at 620 nm was measured at 5-min intervals for 30 min using a microplate reader (Labsystems) and plotted. The reaction rate (AOD/time) was derived from an initial slope of the curve. A calibration curve was then produced, with the rate of reaction plotted against the standard samples (1).

Measurement plasma and lung somatostatin concentrations. Concentration of somatostatin from the plasma and supernatants obtained from lung homogenates was determined with a specific and sensitive RIA technique developed in our laboratory (16, 34). The animals were fasted overnight before the measurement to avoid gastrointestinal somatostatin release. Arterial blood samples (0.8–1 ml per animal) were placed into ice-cold Eppendorf tubes containing EDTA (2 mg) and Trasylol (350 units) at the end of the experiment, 25 h after LPS administration. Following centrifugation (2,200 rpm for 10 min at 4°C), the peptide was extracted from the plasma by addition of three volumes of absolute alcohol. After precipitation and a second centrifugation (2,000 rpm for 10 min at 4°C), the samples were dried under nitrogen flow and then resuspended in assay buffer before RIA determination (34).

Drug treatments. Somatostatin-14 (SOM-14; 100 μg/kg ip; Sigma) was administered to study its actions on the inflammatory response both TRPV1+/− and TRPV1+/+ animals. For examining the functional roles of somatostatin released via TRPV1 activation in LPS-induced airway inflammation, a separate group of TRPV1+/− mice was treated with the somatostatin receptor antagonist cyclo-somatostatin (C-SOM; 250 μg/kg ip; Sigma). Both somatostatin and C-SOM were dissolved freshly in saline and injected 30 min before the experiment. Inhalation of the muscarinic receptor agonist carbachol (5 μg/kg in 50 μl saline, ip; Sigma). Both somatostatin and carbachol inhalation was markedly higher in TRPV1−/− mice than in their wild-type counterparts (Table 1, Fig. 1). Area under the carbachol concentration-response curves were 104.7 ± 9.2 and 179.9 ± 11.4 units in the intact TRPV1+/+ and TRPV1−/− groups, respectively. In comparison, the corresponding data in LPS-treated mice were 503.9 ± 4.6 (P = 0.0074 vs. intact TRPV1+/+ mice) and 1,211.0 ± 19.8 units (P = 0.0002 vs. intact TRPV1−/− mice and P = 0.0085 vs. LPS-treated TRPV1+/+ mice), respectively.

Inflammatory changes in the lung of TRPV1+/+ and TRPV1−/− mice. Histological examination and scoring revealed that, compared with the intact lung structure (Fig. 2A), LPS induced marked peribronchial/perivascula edema formation.

Table 1. LPS-induced increase of Penh and Rl in TRPV1+/+ and TRPV1−/− mice

<table>
<thead>
<tr>
<th>Concentration of Carbachol, nM</th>
<th>Parameters</th>
<th>5.5</th>
<th>11</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% change of Penh</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPV1+/+</td>
<td>Intact</td>
<td>2.7 ± 0.7</td>
<td>4.6 ± 4.1</td>
<td>58.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>LPS-treated</td>
<td>56.1 ± 12.1</td>
<td>99.6 ± 29.4</td>
<td>191.1 ± 33.2</td>
</tr>
<tr>
<td>TRPV1−/−</td>
<td>Intact</td>
<td>24.1 ± 5.4</td>
<td>33.8 ± 13.5</td>
<td>71.0 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>LPS-treated</td>
<td>88.3 ± 20.5</td>
<td>280.0 ± 88.7</td>
<td>527.9 ± 125.2</td>
</tr>
<tr>
<td></td>
<td>% change of Rl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPV1+/+</td>
<td>Intact</td>
<td>3.8 ± 0.9</td>
<td>6.8 ± 1.3</td>
<td>71.7 ± 21.8</td>
</tr>
<tr>
<td></td>
<td>LPS-treated</td>
<td>48.4 ± 3.1</td>
<td>103.3 ± 23.3</td>
<td>210.8 ± 45.5</td>
</tr>
<tr>
<td>TRPV1−/−</td>
<td>Intact</td>
<td>27.2 ± 13.7</td>
<td>52.9 ± 17.0</td>
<td>94.2 ± 30.6</td>
</tr>
<tr>
<td></td>
<td>LPS-treated</td>
<td>96.1 ± 14.3</td>
<td>263.6 ± 67.7</td>
<td>574.4 ± 99.9</td>
</tr>
</tbody>
</table>

Percentage increase of mean enhanced pause (Penh) and mean lung resistance (Rl; ΔcmH₂O·s⁻¹·m⁻¹) values from baseline in response to increasing concentrations of carbachol. Data are normalized to the respective baseline values: [(responses for each carbachol concentration − baseline Penh/baseline Penh) × 100]. Area under these carbachol concentration-response curves (AUC) values were calculated and compared with two-way ANOVA. Histological inflammatory score values were analyzed with Kruskal-Wallis followed by Dunn’s t-test. Data for plasma and lung somatostatin concentrations and MPO activity were evaluated by two-way ANOVA followed by Bonferroni’s modified t-test. In all cases, P < 0.05 was considered to be significant.

Ethics. All experimental procedures were carried out according to the 1998/XXVIII Act of the Hungarian Parliament on Animal Protection and Consideration Decree of Scientific Procedures of Animal Experiments (243/1998) and complied with the recommendations of the Helsinki Declaration. The studies were approved by the Ethics Committee on Animal Research of Pécs University according to the Ethical Codex of Animal Experiments, and license was given (license no. BA 02/200-6-2001).

RESULTS

Inflammatory airway hyperresponsiveness in TRPV1+/+ and TRPV1−/− mice. Baseline Penh and basal lung resistance (Rl = ΔcmH₂O·s⁻¹·m⁻¹) significantly increased after intranasal LPS instillation compared with untreated mice both the TRPV1+/+ (2.42 ± 0.43 vs. 0.85 ± 0.08 and 3.23 ± 0.45 vs. 0.69 ± 0.06, respectively) and TRPV1−/− (2.41 ± 0.67 vs. 0.95 ± 0.15 and 2.69 ± 0.23 vs. 0.86 ± 0.09, respectively) groups. Inhalation of the muscarinic receptor agonist carbachol evoked a concentration-dependent bronchoconstriction shown by the Penh curves as well as the Rl values. Responses demonstrated as percentage increase of Penh and Rl above baseline were markedly enhanced in the LPS-treated groups compared with the respective noninflamed controls pointing out the development of inflammatory bronchial hyperresponsiveness. These results provided evidence that the changes of Penh well correlate with the changes of Rl in the present experimental model (Table 1).

There was no significant difference between the carbachol-induced responses of intact (noninflamed) TRPV1+/+ and TRPV1−/− animals (P = 0.75). In response to LPS-induced inflammation, both maximal Penh values were higher, and the duration of the bronchoconstriction was prolonged in mice lacking the TRPV1 receptor. Therefore, the percentage increase of the mean Penh values evoked by 11, 22, and 44 mM carbachol inhalation was markedly greater in TRPV1−/− mice than in their wild-type counterparts (Table 1, Fig. 1). Area under the carbachol concentration-response curves were 104.7 ± 9.2 and 179.9 ± 11.4 units in the intact TRPV1+/+ and TRPV1−/− groups, respectively. In comparison, the corresponding data in LPS-treated mice were 503.9 ± 4.6 (P = 0.0074 vs. intact TRPV1+/+ mice) and 1,211.0 ± 19.8 units (P = 0.0002 vs. intact TRPV1−/− mice and P = 0.0085 vs. LPS-treated TRPV1+/+ mice), respectively.
Role of somatostatin in endotoxin-evoked airway hyperresponsiveness, inflammatory histopathological changes, and MPO activity. Repeated treatments of TRPV1−/− with SOM-14 (100 μg/kg ip) markedly inhibited bronchoconstriction induced by 11, 22, and 44 mM carbachol with the maximal effect reached at 22 mM concentration. Similarly, the same than in their wild-type counterparts (P = 0.0069 intact vs. LPS-treated TRPV1+/+ mice; P = 0.000078 intact vs. LPS-treated TRPV1−/− mice; P = 0.019 LPS-treated TRPV1+/+ vs. LPS-treated TRPV1−/−) (Fig. 2).

MPO activity in the lung of TRPV1+/+ and TRPV1−/− mice. Endotoxin administration induced about two- and fourfold elevations of MPO activity in the lung of TRPV1+/+ (P = 0.005) and TRPV1−/− mice (P = 0.0002), respectively. This quantitative marker of accumulated granulocytes in the inflamed tissue was significantly greater, more than double in the TRPV1 receptor knockout group (P = 0.007; Fig. 3).

Somatostatin concentration in the lung and plasma of TRPV1+/+ and TRPV1−/− mice. The basal level of somatostatin-like immunoreactivity was about 4- to 5-fold higher in the lung than in the plasma of both wild-type and TRPV1 gene-deleted animals without a significant difference between the two groups (P = 0.75). In response to LPS administration, there was a pronounced increase of somatostatin-like immunoreactivity in the lung and plasma of TRPV1+/+ mice (P = 0.02 and 0.0031), whereas in the TRPV1−/− group, the LPS-induced elevation of somatostatin level was much smaller in the lung (P = 0.16 intact vs. LPS-treated; P = 0.028 LPS-treated TRPV1+/+ vs. TRPV1−/−) and absent in the plasma (P = 0.92; P = 0.002 LPS-treated TRPV1+/+ vs. TRPV1−/−). These results point out a TRPV1 receptor-mediated release of this neuropeptide from the sensory fibers of the lung, which gets into the systemic circulation (Fig. 4).

Fig. 1. Endotoxin-induced bronchial hyperreactivity to inhaled carbachol. Bronchoconstriction induced by increasing concentrations of carbachol was assessed in freely moving, unrestrained mice by whole body plethysmography. Percentage increase of the enhanced pause (Penh) above baseline [(Penh in baseline Penh)/100] was calculated in each 15-min period after respective carbachol stimulations. Data points represent means ± SE of n = 8–14 experiments. Area under these carbachol concentration-response curves (AUC) values were calculated and compared with two-way ANOVA [P = 0.0005] was calculated in each 15-min period after respective carbachol treatment. Each column represents the means ± SE of n = 8–14 mice: *P < 0.05 LPS-treated TRPV1−/− vs. respective intact mice; †P < 0.01 LPS-treated inflamed vs. respective intact mice (Kruskal-Wallis followed by Dunn’s test).

Fig. 2. Histopathological examination of lung samples. LPS-induced inflammatory histopathological changes in lung samples of TRPV1+/+ (B) and TRPV1−/− mice (C) compared with the structure of the intact lung (A). Samples are stained with hematoxylin and eosin and shown at ×200 magnification. a, Alveolar spaces; b, bronchi; v, vessels. D: semiquantitative evaluation and scoring of the lung samples on the basis of perivascular edema formation, perivascular/peribronchial granulocyte accumulation, goblet cell hyperplasia, and alveolar mononuclear cell infiltration. Each column represents the means ± SE of n = 8–14 mice: †P < 0.05 LPS-treated TRPV1−/− vs. respective intact mice; #P < 0.01 LPS-treated inflamed vs. respective intact mice; *P < 0.05 LPS-treated TRPV1+/+ vs. LPS-treated TRPV1−/− group (Kruskal-Wallis followed by Dunn’s test).
doses of somatostatin also significantly diminished inflammatory airway hyperreactivity in TRPV1<sup>+/+</sup> mice, although the degree of the inhibitory effect was smaller. Meanwhile, C-SOM (250 μg/kg ip), which antagonizes the effects of somatostatin in all 5 receptor subtypes, significantly enhanced bronchial hyperreactivity in TRPV1<sup>−/−</sup> mice. In the LPS-treated TRPV1<sup>+/+</sup> group, AUC for the carbachol concentration-response curves was 503.9 ± 4.6 units, which was significantly smaller after repeated SOM-14 injections (323.5 ± 2.6 units; P = 0.012) and greater following C-SOM administrations (1,676.5 ± 12.4 units; P = 0.0062). The corresponding AUC values in LPS-treated TRPV1<sup>−/−</sup> mice was 1,211.0 ± 19.8 units (P = 0.0085 vs. LPS-treated TRPV1<sup>+/+</sup> mice), and it was also markedly decreased by somatostatin injections (370.8 ± 3.4 units; P = 0.0014) (Fig. 5).

Somatostatin administration diminished endotoxin-induced inflammatory changes both in knockout mice (P = 0.02) and their wild-type counterparts (P = 0.04). C-SOM injection in the TRPV1<sup>+/+</sup> group aggravated these parameters, especially peribronchial edema formation, granulocyte infiltration, and goblet cell hyperplasia (P = 0.027; Figs. 6 and 7). In accordance with these histological findings, somatostatin significantly decreased LPS-evoked MPO activity in the lung of TRPV1<sup>−/−</sup> mice (P = 0.007) as well as in TRPV1<sup>+/+</sup> animals (P = 0.037). Administration of the antagonist induced a more than twofold elevation in the TRPV1<sup>+/+</sup> group compared with their controls (P = 0.0082; Fig. 8).

Administration of C-SOM (250 μg/kg ip, 4 times) to TRPV1<sup>−/−</sup> mice in LPS-induced inflammation did not alter bronchial hyperreactivity and the inflammatory changes. No change was observed in carbachol-induced bronchoconstriction, histological parameters, and MPO activity after repeated administration of the same doses of the antagonist, as well as

---

**Fig. 3.** Myeloperoxidase (MPO) activity of lung samples. MPO activity, as a quantitative indicator of the number of accumulated granulocytes determined from homogenized lung samples of TRPV1<sup>+/+</sup> and TRPV1<sup>−/−</sup> mice 25 h after intranasal administration of LPS, was compared with the respective intact animals. Results are means ± SE of n = 8–14 mice; *P < 0.01 LPS-treated inflamed vs. respective intact mice and +P < 0.05, **P < 0.01 LPS-treated inflamed vs. the respective intact group, and +P < 0.05, +++P < 0.01 TRPV1<sup>−/−</sup> vs. TRPV1<sup>+/+</sup> group (two-way ANOVA followed by Bonferroni’s modified t-test).

**Fig. 4.** Somatostatin concentrations. Concentrations of somatostatin measured with radioimmunoassay in the lung (A) and plasma (B) of TRPV1<sup>+/+</sup> and TRPV1<sup>−/−</sup> mice. Columns show means ± SE of n = 8–14 mice; *P < 0.05, **P < 0.01 LPS-treated inflamed vs. the respective intact group, and +P < 0.05, +++P < 0.01 TRPV1<sup>−/−</sup> vs. TRPV1<sup>+/+</sup> mice (two-way ANOVA followed by Bonferroni’s modified t-test).

**Fig. 5.** Role of somatostatin in endotoxin-induced bronchial hyperreactivity. Effect of somatostatin-14 (SOM-14) and the somatostatin receptor antagonist cyclo-somatostatin (C-SOM) on LPS-induced inflammatory bronchial hyperreactivity in TRPV1<sup>−/−</sup> and TRPV1<sup>+/+</sup> mice, respectively. Bronchoconstriction induced by increasing concentrations of carbachol was assessed in freely moving, unrestrained mice by whole body plethysmography. Percentage increase of the Penh above baseline [(Penh in response to the respective carbachol concentration – baseline Penh/baseline Penh) × 100] was calculated in each 15-min period after respective carbachol stimulations. Data points represent means ± SE of 8–14 mice; *P < 0.01 LPS-treated inflamed vs. the respective intact group, and +P < 0.05, **P < 0.01 TRPV1<sup>−/−</sup> vs. TRPV1<sup>+/+</sup> group (two-way ANOVA followed by Bonferroni’s modified t-test).
SOM-14 (100 µg/kg ip, 4 times) to intact TRPV1\(^{+/+}\) and TRPV1\(^{-/-}\) mice compared with the respective intact animals.

**DISCUSSION**

The present results demonstrate that endotoxin-induced airway inflammation, as revealed by MPO activity measurement and histological assessments, as well as consequent bronchial hyperreactivity are enhanced in TRPV1 gene-deleted mice compared with their wild-type counterparts. Activation of the TRPV1 capsaicin receptor in different tissues elicits neurogenic inflammation and nociception, which are absent in TRPV1\(^{-/-}\) animals (6). On the contrary, inflammatory and pain responses evoked by several other agents are differently influenced by the TRPV1 receptor. In TRPV1-null mutant mice, neurogenic inflammation induced by mustard oil (1) and edema evoked by carrageenin or mechanical hyperalgesia 1 day after the injection of complete Freund’s adjuvant into the hindpaw were similar to the TRPV1\(^{+/+}\) controls (6). Meanwhile, thermal hyperalgesia in these acute inflammatory models (10) and adjuvant-induced chronic arthritis and consequent mechanical hyperalgesia (40) were markedly decreased in TRPV1 receptor knockout mice. On the contrary, this receptor proved to have a protective role against the development of mechanical allodynia under diabetic and toxic polyneuropathy.
conditions (5). Based on these data, TRPV1 can be considered as an integrator target molecule for a variety of noxious stimuli and inflammatory mediators (21, 22, 52), and its function seems to depend on the pathomechanism of the disease.

In the present series of experiments, lung function in unrestrained mice was assessed by whole body plethysmography, and bronchoconstriction was determined on the basis of Penh. Although this calculated parameter as an appropriate indicator of bronchoconstriction has been questioned by some authors (31), several data in literature suggest that it is acceptable for the evaluation of airway reactivity (11, 14, 15). The reason for this contradiction might be differences in species, experimental models, and severity/type of the inflammation. Our results provided evidence that in the presently used LPS-induced lung inflammation model, there is a relatively good correlation between carbachol-induced Penh responses and lung resistance measured in tracheostomized, mechanically ventilated animals, which is supported by other authors using these parameters to determine airway reactivity (11, 14, 15, 39).

Endotoxin (LPS), a constituent of gram negative bacteria found in the environment including house dust (51), induces powerful inflammatory effects on the murine airways accompanied by bronchopulmonary hyperreactivity and increased responsiveness to bronchoconstrictor agents, e.g., muscarinic receptor agonists (28). Intranasal endotoxin administration in the present experimental model evokes a well-defined acute airway inflammation involving infiltration of granulocytes and cytokine (mainly TNF-α and IL-6) production (38, 39). Neutrophil activation, as opposed to recruitment alone, probably accounts for LPS-induced inflammation and consequent bronchial hyperreactivity, since their depletion is fully suppressive (39). They are recruited to the airway epithelial/subepithelial regions and cause tissue damage via the production and release of oxygen radicals, proteases, cytokines, and chemokines (25), which attract and stimulate mononuclear cells and lymphocytes. All these inflammatory and immune cells release several other inflammatory mediators like leukotrienes, prostaglandins, bradykinin, etc., which can activate a variety of receptors including TRPV1 on the sensory nerves in the airways (3, 29).

The released neuropeptides, in turn, influence the inflammatory process by acting at receptors localized on these peripheral nerve terminals themselves, vascular endothelial, bronchial epithelial, and inflammatory cells (53). There is increasing evidence that neuropeptides are synthesized in nonneural sources as well, e.g., mononuclear cells and lymphocytes, and they can be released in a TRPV1-independent manner, especially under inflammatory conditions. Proinflammatory neuropeptides like substance P, neurokinin A, and CGRP are localized in capsaicin-sensitive unmyelinated and thinly myelinated sensory fibers (C and Aδ fibers) in the airways of several species (24), their release from these nerve terminals and participation in the enhancement of both vascular and cellular phases of several inflammatory reactions being well established (4, 29, 53). Besides these proinflammatory neuropeptides, somatostatin is also present in the capsaicin-sensitive afferents of the lung (19). However, immunohistochemical studies have provided evidence that somatostatin and substance P/CGRP are localized in distinct subpopulations of primary sensory neurons (19, 32).

This study revealed that intranasal endotoxin administration increased somatostatin concentration in the lung and also the plasma of wild-type mice. In the TRPV1 gene-deleted group, LPS-induced pronounced rise of somatostatin concentration was abolished in the plasma and markedly inhibited in the lung. These results indicate that inflammatory mediators stimulate the TRPV1 receptor on sensory nerve terminals and elicit the release of somatostatin from these afferents. The increase of plasma somatostatin level was similar to that observed in response to topical application of mustard oil on the rat hindpaw skin, which was also sufficient to evoke systemic anti-inflammatory actions (46). In accordance with these findings in TRPV1 knockout mice, enhanced inflammatory reaction was observed in the lung in response to endotoxin administration. This is likely to be explained by LPS-induced production of an “inflammatory mixture” containing lipoxygenase products, protons, prostaglandins, bradykinin, etc., which all activate/sensitize the integrative TRPV1 ion channel localized on somatostatin-containing capsaicin-sensitive fibers in the airways (40, 44), although even in this model some involvement of decreased tachykinin release cannot be ruled out in TRPV1+/− mice.

In LPS-treated TRPV1+/− mice, exogenous administration of SOM-14 prevented both increased bronchoconstriction and enhanced inflammatory reaction determined by MPO and histology. On the other hand, inhibitory function of the released somatostatin in the development airway inflammation and hyperresponsiveness was further evidenced in wild-type mice treated with the somatostatin receptor antagonist C-SOM. This compound, which inhibits the actions of somatostatin at all five receptor subtypes (sstr1–sstr5; Refs, 20, 36), enhanced both inflammatory responses and bronchial hyperreactivity.

Somatostatin effectively inhibits the release of proinflammatory neuropeptides (36, 37) and modulates the immune system by inhibiting monocyte/macrophage functions (26), B lymphocyte immunoglobulin production, T lymphocyte proliferation, and cytokine production (23, 36). All the five different receptor subtypes (sstr1–sstr5) belong to the G protein-coupled receptor family (20). In the lung, they have been detected on lymphocytes and also on the sensory nerve terminals (13). Somatostatin receptor autoradiography has shown upregulation of somatostatin binding sites in several immune-mediated diseases (36, 48, 49). Furthermore, plasma somatostatin level has been shown to significantly increase during the development of adjuvant-induced arthritis in the rat (16) and, according to the present results, also during endotoxin-induced airway inflammation in the mouse.

Our data obtained in the same LPS-induced airway inflammation model suggest that tachykinins (e.g., substance P) and CGRP participate in neutrophil accumulation and in the production of the inflammatory cytokine IL-1β but do not affect the overall severity of this type of inflammatory reaction (12). However, in the present model, these inflammatory actions of the released proinflammatory sensory neuropeptides are counteracted by the anti-inflammatory somatostatin also derived from the C-fibers in response to TRPV1 receptor activation. Since somatostatin and its synthetic agonists have previously been proved to inhibit the outflow of these neuropeptides from isolated rat tracheae (17, 18), the ability of somatostatin released from sensory nerves to diminish the release of substance P and CGRP might be involved in its inhibitory effect.

These data provide the first evidence for a TRPV1 receptor-dependent novel type of counterregulatory mechanism devel-
opining during airway inflammation, since endotoxin-induced inflammation and consequent bronchial hyperresponsiveness were enhanced in TRPV1 gene-deficient mice. In this model, somatostatin released from sensory fibers of the lung via TRPV1 receptor stimulation mediates these inhibitory actions at the site of activation and also reaches the systemic circulation. The somatostatin receptor antagonist C-SOM prevents this counterregulatory effect and aggravates endotoxin-induced responses indicating that synthetic somatostatin receptor agonists might be potential novel agents for the treatment of airway inflammation.

ACKNOWLEDGMENTS

We acknowledge Dr. J. B. Davis (GlaxoSmithKline, Research and Development, Harlow, Essex, United Kingdom) for the generous gift of TRPV1 knockout mice. We thank Aníkó Perécz for the histological slides.

GRANTS

This work was supported by Hungarian Grants OTKA F-046635, T-040927, T-046729, T-043467, RET-008/2005, ETT-284/2006, and NRPDP/005/2004. Buxco whole body plethysmograph was purchased with the financial support of GVOP-3.2.1-2004-0420/3.0.

REFERENCES


20. Nemeth J, Helyes Z, Gorcs T, Gardi J, Pinter E, Szolcsanyi J. Development of somatostatin radioimmunoassay for the measurement of...


