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 Kereskai,3 Rita Bőrsei,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

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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 cation 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 neurokinin A) 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, neurokinin A, 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, Szégrét u. 12., Hungary (e-mail: zsuzsanna.helyes@aok.pte.hu).
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).

The aim of the present study was to analyze the modulatory role of the TRPV1 capsacin receptor channel in endotoxin-induced airway inflammation and consequent bronchial hyper-reactivity using TRPV1 gene-deleted mice and participation of the anti-inflammatory sensory neuropeptide somatostatin in these responses.

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. Germine chimeras were crossed onto C57BL/6 females to generate heterozygotes, which were intercrossed, giving rise to healthy homozygous mutant offspring in the expected Mendelian ratio, as described by Davis and coworkers (10). Successful targeting of the locus and germine transmission was confirmed by PCR and Southern blot analysis. After genotyping by PCR, they were bred from homozygous knockout breeding pairs so that all offspring were also homozygous knockouts (TRPV1$^{-/-}$) as described.

Induction of airway inflammation. Subacute airway inflammation was evoked by 60 µl of Escherichia coli (serotype 083) LPS (167 µg/ml dissolved in sterile PBS; Sigma, St. Louis, MO) applied intranasally 24 h prior to measurement (35). Data showing that intranasal administration of this LPS dose evoked maximal inflammation (neutrophil accumulation and inflammatory cytokine production) 24 h after its instillation served as basis for choosing this time point. The same volume of sterile PBS was administered to control mice regarded as intact (noninflamed) animals.

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 (Buxco Europe) (11, 31). Aerosolized carbachol was administered through bypass tubing via the ultrasonic nebulizer following the same protocol as described for the unrestrained measurement. The percentage changes of $R_L$ above baseline induced 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/nomonuclear 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 (ΔOD/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 both TRPV1 knockout mice (Sigma). 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 (ΔOD/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).

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 measurement to avoid gastrointestinal absorption.

Concentration of somatostatin from the plasma and supernatants obtained both TRPV1 knockout mice (Sigma). 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 (ΔOD/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).

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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 (243/1988) 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 (Rₐ = ΔcmH₂O·s⁻¹·m⁻¹) significantly increased after intra-nasal 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 Rₐ values. Responses demonstrated as percentage increase of Penh and Rₐ 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 Rₐ 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, though 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 1211.0 ± 19.8 units (P = 0.0002 vs. intact TRPV1−/− mice and P = 0.0005 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/perivascular edema forma-

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

<table>
<thead>
<tr>
<th>Concentration of Carbachol, mM</th>
<th>Penh (mmH₂O/cmH₂O·s⁻¹·m⁻¹)</th>
<th>Rₐ (cmH₂O·s⁻¹·m⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>5.5</td>
<td>2.7 ± 0.7</td>
<td>19.1 ± 3.2</td>
</tr>
<tr>
<td>11</td>
<td>4.6 ± 4.1</td>
<td>33.8 ± 13.5</td>
</tr>
<tr>
<td>22</td>
<td>58.9 ± 1.0</td>
<td>71.0 ± 2.5</td>
</tr>
<tr>
<td>% change of Penh</td>
<td>56.1 ± 12.1</td>
<td>191.1 ± 33.2</td>
</tr>
<tr>
<td>TRPV1+/+ Intact</td>
<td>21.4 ± 5.4</td>
<td>527.9 ± 125.2</td>
</tr>
<tr>
<td>% change of Rₐ</td>
<td>88.3 ± 20.5</td>
<td>280.0 ± 88.7</td>
</tr>
<tr>
<td>TRPV1+/+ LPS-treated</td>
<td>3.8 ± 0.9</td>
<td>6.8 ± 1.3</td>
</tr>
<tr>
<td>TRPV1−/− Intact</td>
<td>48.4 ± 3.1</td>
<td>103.2 ± 23.3</td>
</tr>
<tr>
<td>% change of Penh</td>
<td>27.2 ± 13.7</td>
<td>210.8 ± 45.5</td>
</tr>
<tr>
<td>TRPV1−/− LPS-treated</td>
<td>52.9 ± 17.0</td>
<td>94.2 ± 30.6</td>
</tr>
<tr>
<td>LPS-treated</td>
<td>96.1 ± 14.3</td>
<td>574.4 ± 99.9</td>
</tr>
</tbody>
</table>

Percentage increase of mean enhanced pause (Penh) and mean lung resistance (Rₐ = 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 − respective baseline responses)/baseline response] × 100 and presented as means ± SE of n = 8 – 14 experiments. Penh was determined with unrestrained whole body plethysmography in conscious mice, and lung resistance was measured directly in tracheotomized, mechanically ventilated animals to compare these 2 ways of airway function measurements and to prove that Penh well correlates with bronchoconstriction in the present endotoxin-induced inflammation model. TRPV1+/+, transient receptor potential vanilloid 1 wild-type; TRPV1−/−, TRPV1 knockout.
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 response to the respective carbachol concentration − baseline Penh/baseline Penh) × 100] was calculated and compared with two-way ANOVA \([P = 0.0074 \text{ LPS-treated TRPV1 knockout (TRPV1}^{-/-}) \text{ mice vs. intact group; } P = 0.0002 \text{ LPS-treated TRPV1 knockout (TRPV1}^{-/-}) \text{ mice vs. respective intact group and } P = 0.0085 \text{ LPS-treated TRPV1}^{+/+} \text{ vs. LPS-treated TRPV1}^{-/-} \text{ mice}].\)

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 \(\times200\) 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 \(\pm\) SE of \(n = 8–14\) 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).

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stimulations. Data points represent means \(\pm\) SE of \(n = 8–14\) mice; * \(P < 0.05\) LPS-treated TRPV1\(^{-/-}\) vs. respective intact mice; ** \(P < 0.005\) LPS-treated TRPV1\(^{-/-}\) vs. 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).

Role of somatostatin in endotoxin-evoked airway hyperresponsiveness, inflammatory histopathological changes, and MPO activity. Repeated treatments of TRPV1\(^{-/-}\) with SOM-14 (100 \(\mu g/\text{kg ip}\) 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).
doses of somatostatin also significantly diminished inflammatory airway hyperreactivity in TRPV1+/− mice, although the degree of the inhibitory effect was smaller. Meanwhile, cyclo-somatostatin (C-SOM) on LPS-induced inflammatory bronchial hyperreactivity in TRPV1−/− mice, and the somatostatin receptor antagonist SOM-14; and the somatostatin receptor antagonist cyclo-somatostatin (C-SOM) on LPS-induced inflammatory bronchial hyperreactivity in TRPV1−/− and TRPV1+/− 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 n = 8–14 experiments. AUC values were calculated and compared with two-way ANOVA (P = 0.0085 LPS-treated TRPV1+/− vs. LPS-treated TRPV1−/− mice; P = 0.012 TRPV1+/−, LPS vs. TRPV1−/−, LPS + SOM-14; P = 0.0062 TRPV1−/−, LPS vs. TRPV1+/−, LPS + C-SOM; and P = 0.0014 TRPV1−/−, LPS vs. TRPV1+/−, LPS + SOM-14).

TRPV1+/− 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−/− mice was 1,211.0 ± 19.8 units (P = 0.0085 vs. LPS-treated TRPV1+/− 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+/− 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−/− mice (P = 0.007) as well as in TRPV1+/− animals (P = 0.037). Administration of the antagonist induced a more than twofold elevation in the TRPV1+/− group compared with their controls (P = 0.0082; Fig. 8).

Administration of C-SOM (250 μg/kg ip, 4 times) to TRPV1−/− 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
SOM-14 (100 μg/kg ip, 4 times) to intact TRPV1<sup>+/+</sup> and TRPV1<sup>−/−</sup> 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<sup>−/−</sup> 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<sup>+/+</sup> 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.

**Fig. 6.** Role of somatostatin in endotoxin-induced inflammatory histological changes. LPS-induced inflammatory changes in lung samples obtained from TRPV1<sup>+/+</sup> (A), TRPV1<sup>−/−</sup> (B), C-SOM-treated TRPV1<sup>+/+</sup> (C), and SOM-14-treated TRPV1<sup>−/−</sup> (D) mice. Staining was performed with periodic acid-Schiff for better identification of mucus-producing bronchial goblet cells and shown at ×200 magnification. a, Alveolar spaces; b, bronchi; v, vessels.

**Fig. 8.** Role of somatostatin in endotoxin-induced granulocyte accumulation in the lung MPO activity, as a quantitative indicator of the number of accumulated granulocytes, determined from homogenized lung samples 25 h after intranasal administration of LPS. The effect of C-SOM treatment in TRPV1<sup>+/+</sup> mice and SOM-14 administration in both TRPV1<sup>+/+</sup> and TRPV1<sup>−/−</sup> animals are shown. Results are means ± SE of n = 8–14 mice; *P < 0.05 TRPV1<sup>−/−</sup> vs. TRPV1<sup>+/+</sup> group; #P < 0.05 (C-SOM-treated vs. TRPV1<sup>+/+</sup>); +P < 0.05 SOM-14-treated vs. respective control group (Kruskal-Wallis followed by Dunn’s test).
cytokine (mainly TNF-α) and IL-6) production (38, 39). Neuropeptides, somatostatin is also present in the capsaicin-sensitive unmyelinated and thinly myelinated sensory fibers (C and Aδ fibers) in the airways of 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.

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.

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