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

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Helyes Z, Elekes K, Németh J, Pozsgai G, Sándor K, Kereskai L, Börzsei 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 receptor gene-deficient 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 indicate 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 antidromic 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 reaction 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.

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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 capsaicin 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<sup>−/−</sup>) and their wild-type counterparts (TRPV1<sup>+/−</sup>) weighing 20–25 g. Two breeding pairs of TRPV1<sup>−/−</sup>-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<sup>+/−</sup> and TRPV1<sup>−/−</sup> 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 healthy heterozygotes, which were intercrossed, giving rise to homozygous knockout breeding pairs so that all offspring were also homozygous for this genotype. Successful targeting of the mTRPV1 membrane domains 2–4 of the murine TRPV1 (mTRPV1) gene. Germline chimeras were crossed onto C57BL/6 females to generate healthy heterozygotes, which were intercrossed, giving rise to homozygous knockout breeding pairs so that all offspring were also homozygous for this genotype.

Determination of airway responsiveness. Airway responsiveness was measured in conscious, spontaneously breathing animals by recording respiratory pressure curves by whole body plethysmography (Buxco Europe, Winchester, United Kingdom) (11, 31). Aerosolized saline and then the muscarinic acetylcholine receptor agonist carbachol (carbamoyl-β-methylcholine, Sigma) in increasing concentrations (50 µl per mouse for 1.5 min in 5.5, 11, 22, and 44 mM concentrations) were nebulized through an inlet of the main chamber for 50 s to induce bronchoconstriction 24 h after LPS administration, and readings were taken and averaged for 15 min following each nebulization. Baseline values usually returned at the end of this period. Penh was measured as an indicator of bronchoconstriction and consequent increase of airway resistance. Penh is a complex, calculated parameter [(expiratory time/relaxation time) − 1](max. expiratory flow max/inspiratory flow), which closely correlates with airway resistance as measured by traditional invasive techniques using ventilated animals (11, 32, 33). 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.

For examining if Penh correlates with lung resistance (R<sub>L</sub>; ∆cmH<sub>2</sub>O·s<sup>−1</sup>·ml<sup>−1</sup>) in the present model, direct measurement of R<sub>L</sub> was also performed in tracheostomized and mechanically ventilated mice. Animals were anesthetized with ketamine and xylazine, and a stainless 18-gauge tube was inserted into the trachea and tied into place. The tracheostomy cannula was passed through a hole in the whole body plethysmograph (Buxco Europe). A connector was attached to this tube with two ports connected to the inspiratory and expiratory sides of a ventilator (MiniVent 845; Hugo Sachs Electronic-Harvard Apparatus, Germany). Ventilation was achieved at 150 breaths/min and a tidal volume of 0.2 ml with a positive end-expiratory pressure of 2–4 cmH<sub>2</sub>O. The flow was measured by digital differentiation of the volume signal, and the R<sub>L</sub> was continuously computed by fitting flow, volume, and pressure to an equation of motion using a least square algorithm (47). 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<sub>L</sub> 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. Semi-quantitative 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; 4: severe and marked edema);
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; 4: 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);
3. 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 (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 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 administration of LPS and every 6 h during the 24-h experimental period. Mice in the control groups received the same volume of saline. There were 6–12 animals in each experimental group.

Statistical analysis. Values for all measurements were expressed as the means ± SE of n = 8–14 mice in each group. Penh data were expressed as % increase above baseline [(Penh in response to the respective 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 test. Data for plasma and lung somatostatin concentrations and lung 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 Pecs 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 (Rt = LmH2O ·s−1 ·mL−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 Rt values. Responses demonstrated as percentage increase of Penh and Rt 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 Rt 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 = 0.0005 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-
stimulations. Data points represent means vs. respective intact mice; * indicates significant difference between the groups (P < 0.05 LPS-treated TRPV1−/− vs. intact mice). Each column represents the 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 < 0.002 LPS-treated TRPV1 knockout (TRPV1−/−) mice vs. respective intact group and P = 0.0085 LPS-treated TRPV1+/+ vs. LPS-treated TRPV1−/− mice).

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 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 = 0.0074 LPS-treated transient receptor potential vanilloid 1 wild-type (TRPV1+/+) mice vs. respective intact group; P = 0.0002 LPS-treated TRPV1 knockout (TRPV1−/−) mice vs. respective intact group and P = 0.0085 LPS-treated TRPV1+/+ vs. LPS-treated TRPV1−/− 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 ×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 < 0.01 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).

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
doses of somatostatin also significantly diminished inflammatory airway hyperreactivity in TRPV1++/ 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++/ mice. In the LPS-treated 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

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+/− and TRPV1−/− 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 < 0.01 TRPV1−/− vs. TRPV1+/− 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+/− and TRPV1−/− 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−/− vs. TRPV1+/− 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+/− 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).
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.

Fig. 6. Role of somatostatin in endotoxin-induced inflammatory histological changes. LPS-induced inflammatory changes in lung samples obtained from TRPV1+/+ (A), TRPV1−/− (B), C-SOM-treated TRPV1+/+ (C), and SOM-14-treated TRPV1−/− (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. 7. Composite histopathological scores demonstrating the role of somatostatin in endotoxin-induced inflammation. 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 TRPV1−/− vs. TRPV1+/+ group; #P < 0.05 C-SOM-treated vs. TRPV1+/+ group; ++P < 0.05 SOM-14-treated vs. respective control group (Kruskal-Wallis followed by Dunn’s test).

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+/+ mice and SOM-14 administration in both TRPV1+/+ and TRPV1−/− animals are shown. Results are means ± SE of n = 8–14 mice; *P < 0.01 TRPV1+/+ vs. TRPV1−/− group; *P < 0.01 C-SOM-treated vs. TRPV1+/+ group; #P < 0.01 SOM-14-treated vs. TRPV1+/+ group; ++P < 0.05 SOM-14-treated vs. respective control group (Kruskal-Wallis followed by Dunn’s test).
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 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). The reason for this correlation is supported by other authors using these parameters to determine airway reactivity (11, 14, 15).

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 inflammatory reaction model, which is supported by other authors using these parameters to determine airway reactivity (11, 14, 15). The reason for this correlation is supported by other authors using these parameters to determine airway reactivity (11, 14, 15). The reason for this correlation is supported by other authors using these parameters to determine airway reactivity (11, 14, 15).

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 of adjuvant-induced arthritis in the rat (16) and, according to the present results, also during 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.

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 (sst1-sst5; Refs. 20, 36), enhanced both inflammatory responses and bronchial hyperreactivity.

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-
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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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