Ethanol Causes Inflammation in the Airways by a Neurogenic and TRPV1-Dependent Mechanism

Marcello Trevisani,[1] David Gazzieri,[1] Francesca Benvenuti, Barbara Campi, Q. Thai Dinh, David A. Groneberg, Michela Rigoni, Xavier Emonds-Alt, Christophe Creminon, Axel Fischer, Pierangelo Geppetti, and Selena Harrison

Center of Excellence for the Study of Inflammation, University of Ferrara, Ferrara, Italy (M.T., D.G., F.B., B.C., M.R., S.H.); Clinical Research Unit of Allergy, Charité School of Medicine, Berlin, Germany (Q.T.D., D.A.G., A.F.); Sanofi Recherche, Montpellier, France (X.E.-A.); CEA, Service de Pharmacologie et d'Immunologie, CEA-Saclay, Gif sur Yvette, France (C.C.); and Department of Critical Care Medicine and Surgery, University of Florence, Florence, Italy (P.G.)

ABSTRACT

Ethanol (EtOH) stimulates peptidergic primary sensory neurons via the activation of the transient receptor potential vanilloid-1 (TRPV1). EtOH is also known to trigger attacks of asthma in susceptible individuals. Our aim was to investigate whether EtOH produces airway inflammation via a TRPV1-dependent mechanism and to verify whether this effect is produced via a mechanism distinct from that of acetaldehyde (AcH). EtOH caused a Ca^{2+}-dependent release of neuropeptides from guinea pigs airways, an effect that was inhibited by both capsaicin pretreatment and the TRPV1 antagonist capsazepine (CPZ). Furthermore, EtOH contracted isolated guinea pig bronchi, showing efficacy similar to that of carbachol: this effect of EtOH was sensitive to capsaicin pretreatment, tachykinin receptor blockade, and TRPV1 antagonism. The EtOH metabolite AcH also contracted isolated guinea pig bronchi, but this action was not affected by capsaicin pretreatment, tachykinin receptor, or TRPV1 antagonism. EtOH by intravenous or intragastric route of administration caused bronchoconstriction and increased plasma extravasation in the guinea pig airways, effects that were abolished selectively by CPZ. In conclusion, we have demonstrated that EtOH stimulates peptidergic primary sensory neurons in the guinea pig airways by TRPV1 activation. This excitatory effect of EtOH, distinct from that of AcH, results in neurogenic inflammatory responses that may contribute to the mechanism of EtOH-induced asthma.

Ethanol (EtOH) is known as an inhibitor of neural function, seemingly via a GABAergic mechanism and by facilitating the opening of GABA-A receptor-chloride channels (Ma et al., 2001). We recently demonstrated a novel excitatory activity of EtOH (Trevisani et al., 2002) in a specific subset of neurons that are sensitive to the excitatory action of capsaicin and that contain neuropeptides, including the calcitonin gene-related peptide (CGRP) and the tachykinins, substance P (SP) and neurokinin A (Holzer, 1991). Stimulation of these peptidergic C- and Aδ-fibers results in the activation of nociceptive and protective reflex responses, as well as the release of neuropeptides from their peripheral endings. This latter effect causes a series of local inflammatory responses collectively referred to as "neurogenic inflammation," which in the airways include plasma extravasation and bronchoconstriction (Holzer, 1991; Geppetti and Holzer, 1996).

The unique sensitivity of primary sensory neurons to capsaicin and other vanilloid molecules (Szallasi and Blumberg, 1999) is conferred by the expression on the neuronal plasma membrane of the vanilloid receptor-1 (Caterina et al., 1997), a nonselective cation channel belonging to the transient receptor potential (TRP) family, and for this reason reclassified as transient receptor potential vanilloid-1 (TRPV1) (Gunthorpe et al., 2002). Additional stimuli that can activate TRPV1 are temperature (>43°C) (Caterina et al., 1997), low pH (pH 6–5) (Geppetti et al., 1991), and lipid derivatives, including anandamide (AEA) (Zygmunt et al., 1999; Smart et al., 2000; Tognetto et al., 2000), 12-hydroperoxyeicosatetraenoic acid, leukotriene B_4 (Hwang et al., 2000) and N-arachidonyldopamine (Huang et al., 2002). EtOH has been found to

ABBREVIATIONS: EtOH; ethanol; CGRP, calcitonin gene-related peptide; SP, substance P; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid-1; AcH, acetaldehyde; CPZ, capsazepine; CCh, carbachol; L-NAME, N$^\omega$-nitro-L-arginine methyl ester; NK, neurokinin; DMSO, dimethyl sulfoxide; PIP, pulmonary insufflation pressure; SR 140333, nolpitantium; SR 48968, (S)-N-methyl-N-[4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl]benzamide.

potentiate dramatically the action of various agents known to stimulate the TRPV1, including protons and AEA (Trevisani et al., 2002). More importantly, EtOH lowers the threshold of TRPV1 activation by temperature, so that at the physiological temperature (37°C) exposure to EtOH (0.3–3%) activates a TRPV1-dependent current, increasing intracellular Ca^{2+} mobilization, inducing release of sensory neuropeptides from dorsal spinal cord, and causing neurogenic plasma extravasation in the esophagus (Trevisani et al., 2002).

EtOH ingestion is considered to trigger asthma attacks in susceptible individuals (Myou et al., 1996; Saito et al., 2001). The EtOH metabolite acetaldehyde (AcH) is considered to play a major role in this effect of EtOH, evoking bronchoconstriction both in rodents (Bianchi et al., 1998; Koivisto et al., 1999) and humans (Fujimura et al., 1999), through the release of histamine from mast cells (Myou et al., 1995; Shimoda et al., 1996). There is evidence that a minor component of bronchoconstriction and plasma extravasation caused by AcH is due to a neurogenic inflammatory mechanism, e.g., sensory nerve terminal stimulation and the subsequent release of SP and neurokinin A (Berti et al., 1994).

The novel findings reported above (Trevisani et al., 2002) that EtOH stimulates sensory nerves via TRPV1 activation suggests that EtOH might per se cause neurogenic inflammatory responses in the airways. Thus, in the present study we have explored the possibility that EtOH activates TRPV1 in guinea pig airway sensory nerves and in this manner causes inflammatory neurogenic responses. To accomplish this purpose, we investigated the ability of EtOH, in airway tissue, to cause via TRPV1 activation: release of CGRP and SP, contraction of isolated airways in vitro, bronchoconstriction, and plasma protein extravasation in vivo.

Materials and Methods

Animals and Reagents. Male albino Dunkin Hartley guinea pigs were used (Charles River Italica, Calco, Italy). All experiments complied with the national guidelines and were approved by the regional ethical committee.

Release Assay. Guinea pigs were terminally anesthetized and decapitated. The airways (including trachea and bronchus) were prepared at 4°C using a tissue slicer (McIlwain tissue chopper; Mickle Laboratory Engineering Co. Ltd, Surrey, UK). Slices (~100 mg) were placed in 2-ml chambers and superfused at 0.4 ml/min with a Krebs' solution of the following composition: 119 mM NaCl, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.5 mM $MgSO_4$, 2.5 mM $CaCl_2$, 4.7 mM KCl, and 11 mM D-glucose. To the basic Krebs' solution the following agents were added: 0.1% bovine serum albumin and 1 μM phosphoramidon, maintained at 37°C, and gassed with 95% O_2 and 5% CO_2. After a 90-min stabilization period, 10-min fractions were collected into acetic acid (final solution 2 N). Two prestimuli samples were taken at 10-min intervals followed by a third set of samples during stimulation. A final poststimulus 10-min sample was also collected. At the end of the experiment, tissues were blotted and weighed. Fractions were freeze-dried, reconstituted with assay buffer, and analyzed by enzyme immunoassays for CGRP-LI and SP-LI according to the methods reported previously (Frobert et al., 1999; Ricciardolo et al., 2000). The detection limits of the assays were 5 pg/ml CGRP and 2 pg/ml SP. The level of release of CGRP-LI and SP-LI were calculated by subtracting the mean prestimulus value from those values obtained during and poststimulation. The results are expressed as femtomoles of peptide per gram per tissue per 20 min. The highest concentration of EtOH (3%), capsaicin (10 μM), and CPZ (10 μM) did not show any significant cross-reactivity with CGRP and SP antisera.

Organ Bath Studies. Guinea pigs were sacrificed by cervical dislocation, and the lungs were removed and rings from the main bronchi (approximately 2 mm in width) were suspended with a resting tension of 1.5 g. The tissues were bathed and aerated (95% O_2 and 5% CO_2) with Krebs' solution (see above), which was maintained at 37°C and contained phosphoramidon (1 μM) to minimize peptide degradation. Tissues were allowed to equilibrate for 60 min before the beginning of and between each set of experiments (washed every 5 min). In all experiments, the tissues were first contracted with carbachol (CCh; 1 μM).

Cumulative concentration-response curves were performed with EtOH (0.01–10%), AcH (0.01–3%), capsaicin (0.01 nM–1 μM), and SP (0.01 nM–1 μM), either in the presence of the selective TRPV1 antagonist CPZ (10 μM), the combination of the selective tachykinin NK_1 (SR 140333; 1 μM) (Emonds-Alt et al., 1993) and NK_2 (SR 48968; 1 μM) (Emonds-Alt et al., 1992) receptor antagonists, nonselective muscarinic antagonist atropine (1 μM), the cyclooxygenase inhibitor indomethacin (5 μM), nitric-oxide synthase inhibitor N^ω-nitro-L-arginine methyl ester (L-NAME; 100 μM), or their respective vehicles.

In another set of experiments, bronchial preparations were preincubated twice for 20 min with a capsaicin (10 μM) concentration known to desensitize the sensory nerve endings (Szallasi and Blumberg, 1999). The bathing fluid was then changed repeatedly (every 5 min over a period of 1 h) until the contractile response had returned to baseline, and cumulative concentration-response curves were performed with EtOH or the other agents.

In Vivo Bronchoconstriction. Guinea pigs were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and ventilated artificially through a tracheal cannula at a frequency of 60 breaths/min. Airflow was monitored continuously with a pneumotachograph (A Fleisch Medical Inc., Richmond, VA) connected to a differential pressure transducer (model DP45; Validyne, Northridge, CA), according to the method reported previously (Ricciardolo et al., 2000). The right jugular vein was cannulated for drug administration. The values are expressed as the percentage increase over the basal level. Bronchoconstriction was provoked by a single administration of EtOH (221 mg/kg i.v. or 790 mg/kg intragastric), capsaicin (25 nmol/kg i.v.) or SP (0.1 nmol/kg i.v.). These experiments were also performed in the presence of CPZ (10 μmol/kg i.p.) given 10 min before the stimulus.

Plasma Extravasation. Guinea pigs were anesthetized with sodium pentobarbital (60 mg/kg i.p.). Evans blue (30 mg/kg) was injected into the jugular vein and 1 min later an instillation of EtOH was performed. After 5 additional minutes, animals were transcardially perfused. Pretreatment with SR 140333 (1 mg/kg i.v.), CPZ (0.1 mM, 100 μl i.t.), or their respective vehicles were given 15 min before the injection of the dye. The trachea was removed, weighed, and incubated in 1 ml of formamide for 24 h in the dark, at room temperature. The amount of extravasated Evans blue was measured spectrophotometrically at 620 nm.

Chemical Reagents. Drugs and reagents were obtained from the indicated companies: EtOH, AcH (Carlo Erba Reagent, Milano, Italy); atropine, bovine serum albumin, capsaicin, capsazepine, CCh, indomethacin, L-NAME, phosphoramidon, SP (Sigma-Aldrich, Milan, Italy); SR 140333 and SR 48968 were synthesized at Sanofi-Synthelabo (Montpellier, France). All drugs were dissolved in saline with the exception of capsaicin, CPZ, and indomethacin that were stocked at a concentration of 1 mM in DMSO (100%) with further solution dissolved in saline.

Statistical Analysis. All data are mean ± standard error of the mean. Contractile responses in vitro are expressed as a percentage of the response to CCh (1 μM). Statistical analysis was performed by means of the Student's t test or analysis of variance and the Dunnett's test when required (Glantz, 1992). If $P < 0.05$, the results were considered significant.

Results

CGRP-LI and SP-LI Release from Isolated Guinea Pig Airways. We examined the effect of EtOH on the outflow of CGRP-LI and SP-LI on superfused slices of guinea pig airways. EtOH (3%) produced a statistically significant increase in CGRP-LI and SP-LI outflow (48 ± 11 fmol/g/20 min, $n = 8$ and 13.0 ± 2.2 fmol/g/20 min, $n = 8$, respectively). In experiments performed in a Ca^+-free medium or with the pretreatment of capsaicin, the increase in the outflow of CGRP-LI or SP-LI evoked by EtOH was practically abolished (Fig. 1). Pretreatment with capsaicin (10 μM for 30 min) or in the presence of CPZ (10 μM) had no significant effect on basal CGRP-LI or SP-LI outflow (not shown). CPZ (10 μM) markedly reduced the increase in CGRP-LI and SP-LI outflow induced by EtOH (Fig. 1) and capsaicin (0.1) μM (not shown), whereas it left unchanged the released induced by a high KCl (80 mM) concentration (not shown).

In Vitro Airway Constriction Induced by EtOH, AcH, Capsaicin, CCh, and SP. Exposure to EtOH (0.01–10%) on guinea pig isolated bronchi caused a concentration-related contraction. Threshold concentration to cause a visible contractile effect was 0.1 to 0.3% and maximum response (97 ± 7% of CCh) was obtained with 3% EtOH (Figs. 2A and 3A). The contractile effect of EtOH was significantly attenuated by capsaicin pretreatment (preincubated twice for 20 min with a capsaicin concentration of 10 μM) or by a combination of tachykinin NK_1 and NK_2 (SR 140333 and SR 48968 both at 1 μM, respectively) receptor antagonists (Figs. 2B and 3A). A small component of the contraction resistant to both capsaicin pretreatment and tachykinin antagonists was seen at 3% EtOH. Thus, this residual component of the contraction is probably not mediated by sensory nerve activation (Fig. 3A). CPZ (10 μM) shifted to the right the concentration-response curve to EtOH (Fig. 3A): the response to 1% EtOH was practically abolished and the response to 3% EtOH was halved by CPZ. Capsaicin (0.01 nM-1 μM), SP (0.01 nM-1 μM), and CCh (0.01 nM-100 μM) induced a concentration-related contraction of isolated guinea pig bronchi (Fig. 2C). The concentration-response curve to capsaicin was shifted to the right by CPZ, whereas the one to SP was not (data not shown), indicating specificity by CPZ. Pretreatment with EtOH (3%) caused a marginally and not significant reduction in the contractile response to capsaicin (not shown). The possible role of prostanoids, cholinergic, and nitrergic mechanisms on airway constriction produced by EtOH was ruled out, because indomethacin (5 μM), atropine (1 μM), and L-NAME (1 μM) did not affect the contractile response to EtOH (not shown).

AcH (0.01–3%) contracted the isolated guinea pig bronchus in a concentration-related manner (Figs. 2A and 3B). Threshold concentration of AcH to produce this effect was 0.03% and maximum response was obtained with 1%. AcH was about 3 times more potent than EtOH to contract the guinea pig isolated bronchus. However, in contrast with EtOH, airway constriction in response to AcH was marginally reduced by capsaicin pretreatment only when the highest concentration of AcH was used (Figs. 2B and 3B), thus indicating that sensory nerve activation plays, if any, a minor role in the motor response to AcH. Tachykinin receptor antagonists (1 μM) and CPZ (10 μM) were also ineffective in reducing the airway constriction evoked by AcH (Fig. 3B), thus strengthening the proposed hypothesis.

In Vivo Bronchoconstriction. After a stabilization period of 30 min, baseline bronchoconstriction remained stable for at least 2 h. Injections (1 ml/kg i.v. or intragastric) of the vehicle of EtOH (0.9% NaCl) did not change the baseline value of bronchoconstriction. The intravenous injection of 221 mg/kg EtOH produces a statistically significant increase in pulmonary insufflation pressure (PIP) (Fig. 4A). This response to EtOH (389 ± 55% of baseline value; $n = 5$) was markedly reduced by a dose of CPZ (10 μmol/kg i.p., 149 ± 32% of baseline value; $n = 6$, $P < 0.05$) (Fig. 4A) that inhibited the bronchoconstrictor response to capsaicin (25 nmol/kg i.v.) but did not alter the response to SP (0.1 nmol/kg i.v.) (Fig. 4A). These finding clearly indicated selectivity of this dose of CPZ for capsaicin-induced response and hence for the TRPV1.

In a second series of experiments, EtOH was given by an intragastric route of administration to mimic the route through which alcoholic beverages are used. EtOH (790 mg/kg, administered via a cannula that reached the gastric cavity) significantly increased PIP (104 ± 25%; $n = 6$) compared with the injection of its vehicle (0.9% saline, 1 ml/kg, 5 ± 3%; $n = 5$, $P < 0.01$). The bronchomotor response to intragastric EtOH (790 mg/kg) was observed in the presence of CPZ vehicle (108 ± 24%; $n = 5$) was markedly decreased by pretreatment with CPZ (10 μmol/kg i.p, 24 ± 8%; $n = 5$, $P < 0.05$) (Fig. 4B).

Plasma Extravasation. Baseline Evans blue extravasation in the guinea pig trachea after the injection of vehicle of EtOH (280 μl/kg of 0.9% saline) was 14 ± 2 ng/g tissue. The injection of 221 mg/kg EtOH produce a statistically signifi-
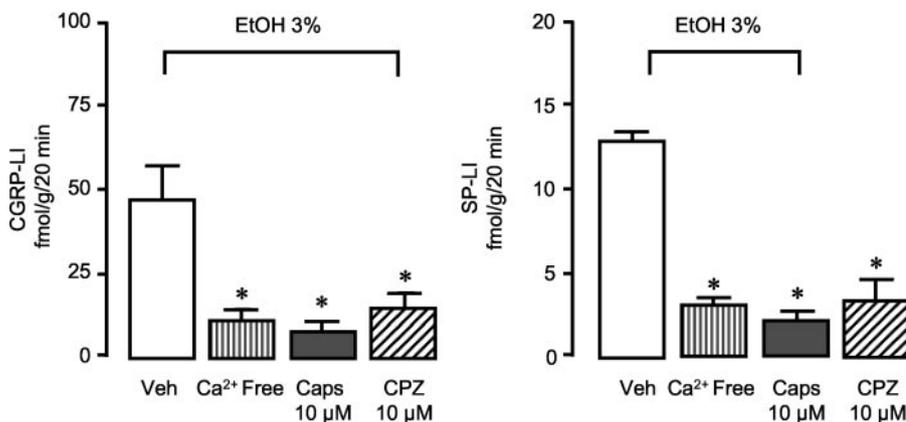
Fig. 1. Increase in CGRP-LI and SP-LI outflow induced by EtOH from slices of guinea pig airways in a Ca^{2+}-free medium (Ca^{2+} Free), after capsaicin (Caps) desensitization or in the presence of CPZ. Each point represents the mean $\pm$ S.E.M. value of at least six experiments ($*$, $P < 0.05$).
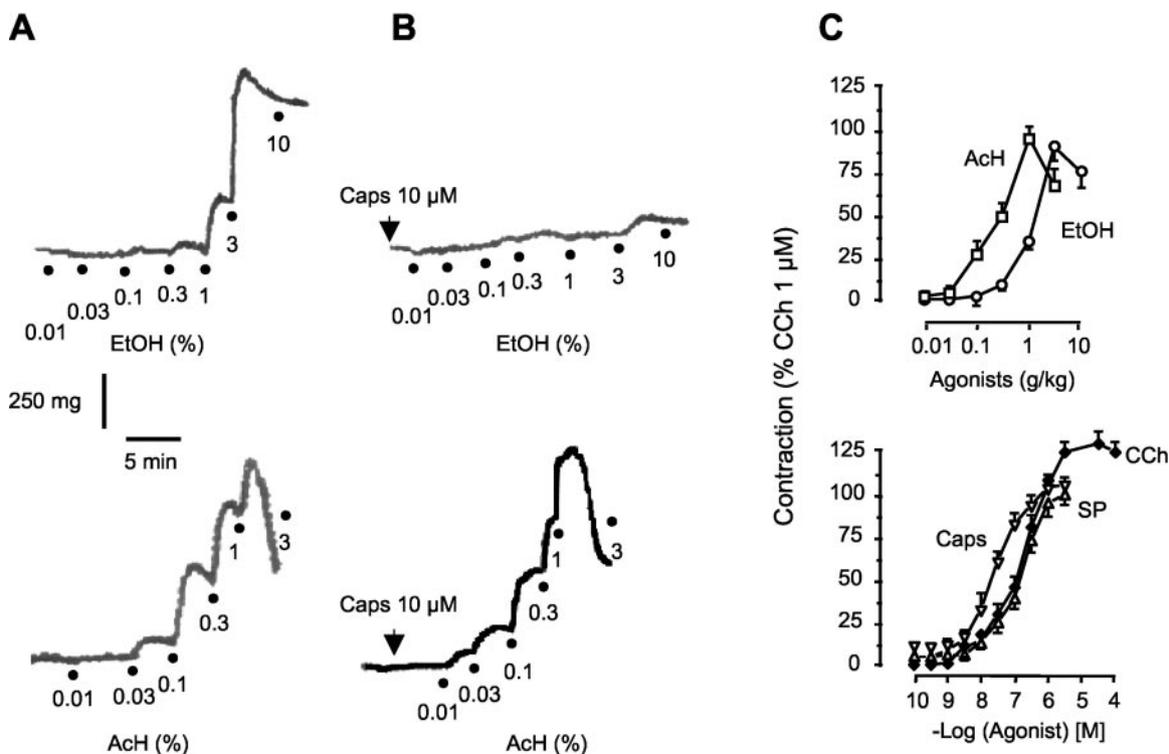
Fig. 2. Typical traces taken from guinea pig isolated bronchus representing cumulative concentration-response curves to EtOH and AcH after pretreatment with (B) capsaicin (Caps) or its vehicle (A). Cumulative concentration-response curves to EtOH and AcH (C) compared with contractile response to CCh, Caps, and SP. Each point represents the mean $\pm$ S.E.M. value of at least six experiments.
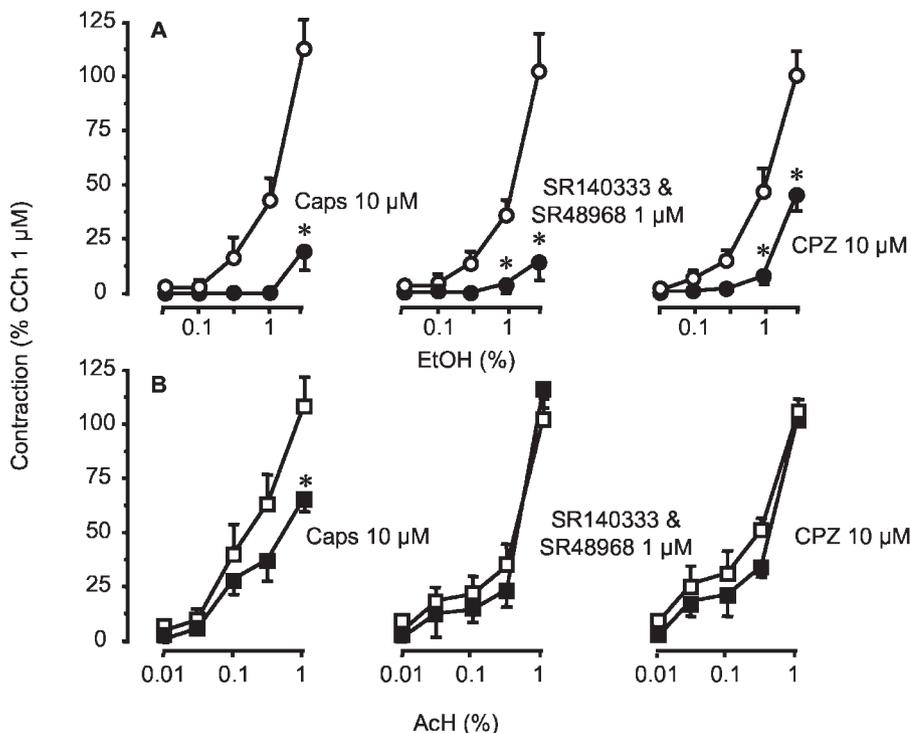

Fig. 3. Cumulative concentration-response curves to EtOH (A) or AcH (B) after pretreatment with capsaicin (Caps), in the presence of the combination of the tachykinin NK_1 (SR 140333) and NK_2 (SR 48968) receptor antagonists or the TRPV1 antagonist CPZ, or their respective vehicles. Each entry represents the mean $\pm$ S.E.M. value of at least six experiments (∗, $P < 0.05$).

cant increase in Evans blue extravasation ($39 \pm 7\%$; $n = 6$) (Fig. 5A), a response that was abated by CPZ (10μmol/kg i.p., $18 \pm 5\%$; $n = 6$). This dose of CPZ blocked the increase in Evans blue caused by capsaicin (25 nmol/kg i.v.) but did not alter the response to SP (0.1 nmol/kg i.v.) (Fig. 5A). As in the case of the bronchoconstriction studies, these finding indi-

cated selectivity of CPZ for capsaicin-induced responses and hence for the TRPV1.

Intragastric administration of EtOH vehicle (1 ml/kg of 0.9% saline, administered via a cannula that reached the gastric cavity) caused an extravasation of the Evans blue dye that was 16 ± 4 ng/g of tissue ($n = 4$) in the guinea pig

A

B
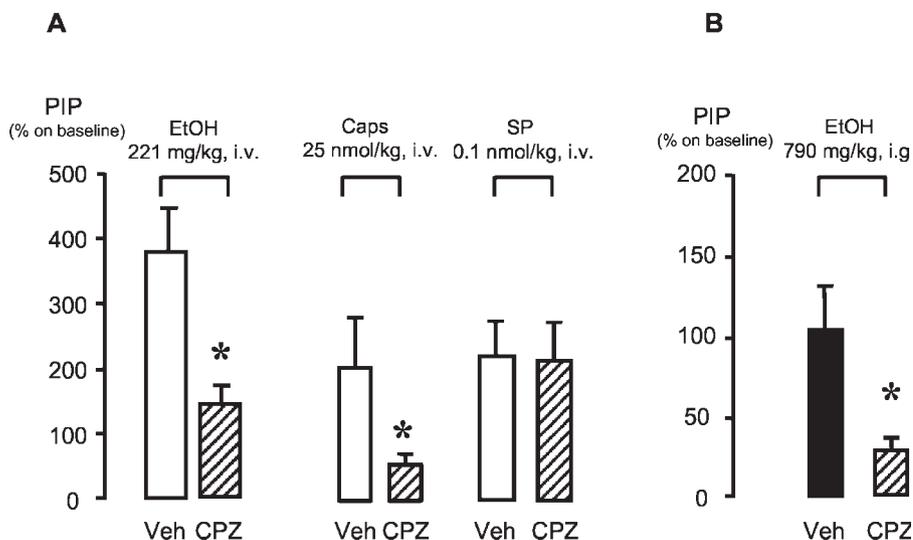
Fig. 4. Effect of CPZ or its vehicle [Veh; Tween 80, 10% DMSO, and saline 1:1:8 (v/v/v)] on the increase in PIP produced by intravenous (A) or intragastric (B) administration of EtOH in anesthetized guinea pigs. Each entry represents the mean $\pm$ S.E.M. value of a least six experiments ($*$, $P < 0.05$).

A

B
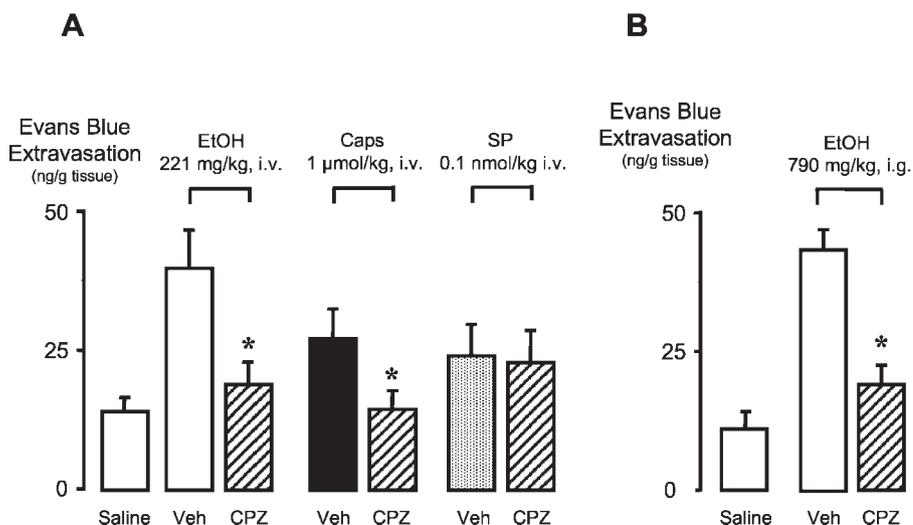
Fig. 5. Effect of CPZ or its vehicle [Veh; Tween 80, 10% DMSO, and saline 1:1:8 (v/v/v)] on the increase in plasma extravasation produced by intravenous (A) or intragastric (B) administration of EtOH in anesthetized guinea pigs. Each entry represents the mean $\pm$ S.E.M. value of a least six experiments ($*$, $P < 0.05$).

trachea. Intragastric EtOH (790 mg/kg) administration in the presence of CPZ vehicle significantly increased the extravasation of the Evans blue dye ($41.1 \pm 4.9\%$; $n = 10$), an effect that was markedly decreased in the presence of CPZ ($20.9 \pm 4\%$; $n = 7$) (Fig. 5B).

Discussion

In this study, we have shown that EtOH is able to stimulate airway sensory nerves by a TRPV1-dependent mechanism, thus causing the release of sensory neuropeptides, and the consequent activation of inflammatory responses in airways of guinea pigs. Release experiments showed that EtOH-induced increase in neuropeptides outflow was abolished in a Ca^{2+}-free medium and after desensitization to capsaicin, indicating that EtOH induced a neurosecretory process exclusively from terminals of airways primary sensory neurons. Because CPZ abolished EtOH-induced neuropeptide release, TRPV1 can be considered the mediator of this effect of EtOH.

However, the most remarkable observations of the present study are those related to functional in vitro and in vivo experiments. Concentrations of EtOH identical to those used in the peptide release experiments produced a marked contraction of isolated guinea pig bronchi. It is worth mentioning

that the efficacy of EtOH to contract the isolated guinea pig bronchi paralleled those of powerful spasmogenic substances, such as CCh or SP. Several lines of evidence show that the mechanism of EtOH-induced bronchoconstriction is neurogenic. First, the response to EtOH was abolished by pretreatment with an elevated concentration of capsaicin, a procedure known to selectively defunctionalize sensory nerves (Szallasi and Blumberg, 1999). Second, this response was abolished by a combination of NK_1 and NK_2 receptor antagonists, indicating that tachykinins release from sensory nerves were involved. Also, in the case of in vitro bronchoconstriction experiments, a large part of the excitatory effect of EtOH was due to TRPV1 activation because the response to EtOH was diminished by CPZ, at a concentration that left unchanged the contraction produced by SP, thus indicating selectivity.

AcH is a known bronchoconstrictor agent for rodents (Bianchi et al., 1998; Koivisto et al., 1999) and humans (Fujimura et al., 1999). This effect of AcH is apparently mediated by histamine release from mast cells (Myou et al., 1995; Shimoda et al., 1996). There is evidence (Berti et al., 1994) that at least a minor part of AcH proinflammatory action in the airways is mediated by sensory nerve activation. In the

present in vitro experiments, AcH produced a remarkable bronchial contraction, being as efficacious as, and slightly more potent than, EtOH. These findings suggest that the contraction produced by EtOH could result from the bronchoconstrictor action of AcH produced locally from the metabolism of EtOH. However, different observations reject this hypothesis. In contrast to EtOH, the contractile effect of AcH was marginally reduced by capsaicin desensitization and unaffected by tachykinin receptor antagonists and CPZ, indicating that this action was non-neurogenic and TRPV1-independent and that the mechanism of AcH differs from that of EtOH. Although specific investigation in the guinea pig airways is lacking, a recent study in the dog showed that, contrary to the liver parenchyma, in the trachea no alcohol dehydrogenase could be found (Maier et al., 1999), thus strengthening the hypothesis that AcH is not produced from EtOH in the airways.

Finally, we showed that two major in vivo proinflammatory responses, such as bronchoconstriction and plasma extravasation, are produced in the guinea pig airways by EtOH via a neurogenic mechanism. The role of TRPV1 in EtOH-induced bronchoconstriction and plasma extravasation is indicated by the ability of CPZ to selectively abolish these responses. Thus, if sufficient amounts of EtOH are present in the airways they may activate TRPV1 on terminals of primary sensory neurons and produce neurogenic inflammatory responses.

There is a report showing a minor fall in sGaw and increase in pulse rate in a few asthma patients (Ayres and Clark, 1982). These effects were possibly ascribed to stimulation of irritant receptors in the upper airways (Ayres and Clark, 1982). In contrast, another study did not demonstrate any significant effect on upper airway reflex sensitivity (Erskine et al., 1994). Irrespective of these findings, the majority of studies point to the fact that alcoholic beverages on average exacerbate asthma (Cuddy and Li, 2001). Furthermore, alcoholic beverages are known to trigger a wide range of allergic responses (rhinitis, headache, cough, and asthma) (Vally and Thompson, 2002). The sensitivity of EtOH to trigger those adverse effects (particularly among the Asian population) is partly due to a reduced capacity to metabolize AcH (Vally and Thompson, 2003). In addition, other molecules contained in certain alcoholic beverages, such as sulfite additives and histamine (a by-product of the fermentation process of red wine) are thought to be contributing factors in wine-based alcohol-induced asthma (Vally and Thompson, 2002). Nevertheless, the present observation that EtOH per se is able to induce inflammatory responses in a rodent airway model relevant for human asthma suggests that this novel mechanism may also contribute to alcohol-induced asthma. Of particular interest is the observation that in guinea pigs an intragastric dose of EtOH as low as 790 mg/kg can cause two effects, bronchoconstriction and airway edema, which are particularly relevant for asthma. It is worth mentioning that 790 mg/kg EtOH corresponds to amounts of alcohol not infrequently assumed for alimentary or recreational uses, such as a half liter of wine or two to three glasses of whiskey.

The clinical finding that the threshold concentration of capsaicin to induce cough is lowered in asthma (Doherty et al., 2000; Barber et al., 2001) suggests the hypothesis that chronic inflammation may up-regulate or "sensitize" TRPV1.

In a patient with a chronic inflammatory process in the airways, a possible sensitized TRPV1 could be more responsive to endogenous and exogenous stimuli, including EtOH and could cause exaggerated inflammatory airway responses. In human embryonic kidney cells transfected with the human TRPV1, relatively moderate concentrations of EtOH have been demonstrated to potentiate the response of TRPV1 to stimuli such as capsaicin, protons, and heat (Trevisani et al., 2002). Much uncertainty, however, exists regarding the mechanisms that eventually result in TRPV1 "up-regulation/sensitization". Bradykinin B_2 receptor activation have been proposed to activate TRPV1 via 1) protein kinase C-ϵ and channel phosphorylation (Cesare et al., 1999); 2) a phospholipase C-dependent displacement of inositol bisphosphates from TRPV1 binding (Chuang et al., 2001); or 3) the release of 12-hydroperoxyeicosatetraenoic acid (Hwang et al., 2000). It is worth mentioning that both kinins (Bertrand and Geppetti, 1996; Proud, 1998) and nerve growth factor in the serum (Bonini et al., 1999) and airways (Kassel et al., 2001) are increased in asthma and seem to play a major role in this disease. It would be of interest to explore the role of these molecular mechanisms in the regulation of TRPV1 in in vivo conditions and especially in asthma.

A better understanding of the mechanisms by which inflammatory mediators regulate intracellular pathways in sensory neurons, and possibly TRPV1 sensitization, may also clarify the concentrations of EtOH required to trigger neurogenic inflammatory responses in different organs including the airways.

References

Ayres J and Clark TJ (1982) Alcohol in asthma and the bronchoconstrictor effect of chlorpropamide. *Br J Dis Chest* **76**:79–87.

Barber CM, Gordon SB, Curran AD, and Fishwick D (2001) The use of capsaicin cough provocation in the investigation of patients with asthma-like symptoms. *Allergy* **56**:78–79.

Berti F, Rossoni G, Buschi A, Villa LM, Trento F, Della Bella D, and Bagolan M (1994) Influence of theophylline on both bronchoconstriction and plasma extravasation induced by acetaldehyde in guinea-pigs. *Arzneim-Forsch/Drug Res* **44**:323–326.

Bertrand C and Geppetti P (1996) Tachykinin and kinin receptor antagonists: therapeutic perspectives in allergic airway disease. *Trends Pharmacol Sci* **17**:255–259.

Bianchi M, Rossoni G, Maggi R, Panerai AE, and Berti F (1998) Effects of carbamazepine on plasma extravasation and bronchoconstriction induced by substance P, capsaicin, acetaldehyde and histamine in guinea-pig lower airways. *Fundam Clin Pharmacol* **12**:58–63.

Bonini S, Lambiase A, Levi-Schaffer F, and Aloe L (1999) Nerve growth factor: an important molecule in allergic inflammation and tissue remodelling. *Int Arch Allergy Immunol* **118**:159–162.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, and Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature (Lond)* **389**:816–824.

Cesare P, Moriondo A, Vellani V, and McNaughton PA (1999) Ion channels gated by heat. *Proc Natl Acad Sci USA* **96**:7658–7663.

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, and Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature (Lond)* **411**:957–962.

Cuddy R and Li G (2001) The role of alcohol in asthma: a review of clinical and experimental studies. *Am J Emerg Med* **19**:501–503.

Doherty MJ, Mister R, Pearson MG, and Calverley PM (2000) Capsaicin responsiveness and cough in asthma and chronic obstructive pulmonary disease. *Thorax* **55**:643–649.

Emonds-Alt X, Doutremepuich JD, Heaulme M, Neliat G, Santucci V, Steinberg R, Vilain P, Bichon D, Ducoux JP, Proietto V, et al. (1993) In vitro and in vivo biological activities of SR140333, a novel potent non-peptide tachykinin NK1 receptor antagonist. *Eur J Pharmacol* **250**:403–413.

Emonds-Alt X, Vilain P, Goulaouic P, Proietto V, Van Broeck D, Advenier C, Naline E, Neliat G, Le Fur G, and Breliere JC (1992) A potent and selective non-peptide antagonist of the neurokinin A (NK2) receptor. *Life Sci* **50**:L101–L106.

Erskine R, Murphy P, and Langton JA (1994) The effect of ethyl alcohol on the sensitivity of upper airway reflexes. *Alcohol Alcohol* **29**:425–431.

Frobert Y, Nevers MC, Amadesi S, Volland H, Brune P, Geppetti P, Grassi J, and Creminon C (1999) A sensitive sandwich enzyme immunoassay for calcitonin gene-related peptide (CGRP): characterization and application. *Peptides* **20**:275–284.

Fujimura M, Myou S, Kamio Y, Ishiura Y, Iwasa K, Hashimoto T, and Matsuda T (1999) Increased airway responsiveness to acetaldehyde in asthmatic subjects with alcohol-induced bronchoconstriction. *Eur Respir J* **14:**19–22.

Geppetti P, Del Bianco E, Patacchini R, Santicioli P, Maggi CA, and Tramontana M (1991) Low pH-induced release of calcitonin gene-related peptide from capsaicin-sensitive sensory nerves: mechanism of action and biological response. *Neuroscience* **41:**295–301.

Geppetti P and Holzer P (1996) *Neurogenic Inflammation*, CRC Press, Boca Raton, FL.

Glantz SA (1992) *Primer of Biostatistics*, McGraw-Hill Book Companies, New York, NY.

Gunthorpe MJ, Benham CD, Randall A, and Davis JB (2002) The diversity in the vanilloid (TRPV) receptor family of ion channels. *Trends Pharmacol Sci* **23:**183–191.

Holzer P (1991) Capsaicin: cellular targets, mechanisms of action and selectivity for thin sensory neurons. *Pharmacol Rev* **43:**143–201.

Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, et al. (2002) An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci USA* **99:**8400–8405.

Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J, Cho S, Min KH, Suh YG, Kim D, and Oh U (2000) Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc Natl Acad Sci USA* **97:**6155–6160.

Kassel O, de Blay F, Duvernelle C, Olgart C, Israel-Biet D, Krieger P, Moreau L, Muller C, Pauli G, and Frossard N (2001) Local increase in the number of mast cells and expression of nerve growth factor in the bronchus of asthmatic patients after repeated inhalation of allergen at low-dose. *Clin Exp Allergy* **31:**1432–1440.

Koivisto T, Kaihovaara P, and Salaspuro M (1999) Acetaldehyde induces histamine release from purified rat peritoneal mast cells. *Life Sci* **64:**183–190.

Ma W, Pancrazio JJ, Andreadis JD, Shaffer KM, Stenger DA, Li BS, Zhang L, Barker JL, and Maric D (2001) Ethanol blocks cytosolic Ca^{2+} responses triggered by activation of $GABA_A$ receptor/Cl^- channels in cultured proliferating rat neuroepithelial cells. *Neuroscience* **104:**913–922.

Maier KL, Wippermann U, Leuschel L, Josten M, Pflugmacher S, Schroder P, Sandermann H Jr, Takenaka S, Ziesenis A, and Heyder J (1999) Xenobiotic-metabolizing enzymes in the canine respiratory tract. *Inhal Toxicol* **11:**19–35.

Myou S, Fujimura M, Kamio Y, Bando T, Nakatsumi Y, and Matsuda T (1995) Repeated inhalation challenge with exogenous and endogenous histamine released by acetaldehyde inhalation in asthmatic patients. *Am J Respir Crit Care Med* **152:**456–460.

Myou S, Fujimura M, Nishi K, Watanabe K, Matsuda M, Ohka T, and Matsuda T (1996) Effect of ethanol on airway caliber and nonspecific bronchial responsiveness in patients with alcohol-induced asthma. *Allergy* **51:**52–55.

Proud D (1998) The kinin system in rhinitis and asthma. *Clin Rev Allergy Immunol* **16:**351–364.

Ricciardolo FL, Steinhoff M, Amadesi S, Guerrini R, Tognetto M, Trevisani M, Creminon C, Bertrand C, Bunnett NW, Fabbri LM, et al. (2000) Presence and bronchomotor activity of protease-activated receptor-2 in guinea pig airways. *Am J Respir Crit Care Med* **161:**1672–1680.

Saito Y, Sasaki F, Tanaka I, Sato M, Okazawa M, Sakakibara H, and Suetsugu S (2001) Acute severe alcohol-induced bronchial asthma. *Intern Med* **40:**643–645.

Shimoda T, Kohno S, Takao A, Fujiwara C, Matsuse H, Sakai H, Watanabe T, Hara K, and Asai S (1996) Investigation of the mechanism of alcohol-induced bronchial asthma. *J Allergy Clin Immunol* **97:**74–84.

Smart D, Gunthorpe MJ, Jerman JC, Nasir S, Gray J, Muir AI, Chambers JK, Randall AD, and Davis JB (2000) The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *Br J Pharmacol* **129:**227–230.

Szallasi A and Blumberg PM (1999) Vanilloid (capsaicin) receptors and mechanisms. *Pharmacol Rev* **51:**159–212.

Tognetto M, Amadesi S, Harrison S, Creminon C, Trevisani M, Carreras M, Matera M, Geppetti P, and Bianchi A (2000) Anandamide excites central terminals of dorsal root ganglion neurons via vanilloid receptor-1 (VR-1) activation. *J Neurosci* **21:**1104–1109.

Trevisani M, Smart D, Tognetto M, Gunthorpe M, Campi B, Amadesi S, Gray J, Jerman J, Owen D, Smith G, et al. (2002) Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. *Nat Neurosci* **5:**546–551.

Vally H and Thompson PJ (2002) Alcoholic drinks and asthma. *Clin Exp Allergy* **32:**186–191.

Vally H and Thompson PJ (2003) Allergic and asthmatic reactions to alcoholic drinks. *Addict Biol* **8:**3–11.

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, Julius D, and Hogestatt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature (Lond)* **400:**452–457.

Address correspondence to: Dr. Selena Harrison, Center of Excellence for the Study of Inflammation, University of Ferrara, 44100 Ferrara, Italy. E-mail: selena.harrison@unife.it