Ethanol dilates coronary arteries and increases coronary flow via transient receptor potential vanilloid 1 and calcitonin gene-related peptide

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Abstract

Objectives: Consumption of alcoholic beverages reduces the risk of coronary artery disease (CAD), and epidemiological studies have shown that ethanol per se is protective. However, the mechanism by which ethanol exerts protection is not fully known. Ethanol can stimulate neuropeptide-containing primary sensory neurons via the activation of transient receptor potential vanilloid 1 (TRPV1). Here, we have studied whether ethanol-mediated TRPV1 activation causes the release of calcitonin gene-related peptide (CGRP) that, via dilatation of coronary arteries and other mechanisms, may protect the heart from CAD.

Methods and results: Ethanol caused a marked relaxation of small-sized porcine isolated coronary (0.008–2.37\%, w/v) and human isolated gastro-epiploic (0.0008–2.37\%, w/v) arteries in vitro, an effect that was abolished by capsaicin-desensitization, the TRPV1 antagonist capsazepine, and the CGRP receptor antagonist, CGRP(8–37). In guinea-pig isolated and perfused hearts, ethanol (0.079–0.79\%, w/v) increased baseline coronary flow in a concentration-dependent manner: 0.237\% ethanol doubled baseline coronary flow. This effect was also abolished by capsaicin-desensitization, capsazepine, and CGRP(8–37). Finally, the ethanol-induced increase in CGRP release from guinea-pig isolated and perfused hearts and from slices of porcine coronary arteries was abolished by capsaicin-desensitization and by capsazepine. Similar functional and neurochemical results were obtained in all preparations with capsaicin.

Conclusions: Ethanol, at low concentrations not dissimilar from those found in blood following low to moderate consumption of alcoholic beverages, releases CGRP within coronary arteries via stimulation of TRPV1 on perivascular sensory nerve terminals. Ethanol-induced release of CGRP may contribute to the reduction in the risk of CAD associated with alcohol consumption by various mechanisms, including the increase in coronary flow and arterial dilatation.

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1. Introduction

There have been numerous proposals for the so-called “French paradox”, i.e. a reduced risk of coronary artery disease (CAD) in spite of elevated fat and red wine consumption [1,2]. The initial proposal that flavonoids, polyphenols and other non-alcoholic components of wine could be the major protective agents has been challenged by
previous [3], and more recent [4], epidemiological studies that, unequivocally, found an inverse association between the consumption of alcoholic beverages of any type with the risk of CAD. These findings indicate that alcohol per se is protective. A variety of diverse effects produced by ethanol have been claimed to reduce CAD risk, including an increase in high-density lipoproteins [5,6] as well as other mechanisms [7,8]. However, the exact mechanism(s) by which ethanol reduces the risk of CAD is still to be completely clarified.

The transient receptor potential vanilloid 1 (TRPV1) is a non-selective cation channel abundantly, and rather selectively, expressed in a subpopulation of nociceptive sensory neurons. TRPV1 is operated by vanilloid molecules naturally (43–52°C), extracellular acidosis (pH 6–5) [10], and diverse lipid derivatives [11]. Sensory neurons expressing TRPV1 contain neuropeptides, including the calcitonin gene-related peptide (CGRP) and the tachykinins, substance P (SP) and neurokinin A, which, when released from peripheral endings, induce proinflammatory responses that are referred to as ‘neurogenic inflammation’ [12]. At the vascular level, neurogenic inflammation includes CGRP-mediated arterial dilatation, as well as tachykinin-mediated plasma extravasation and leukocyte adhesion to the venule endothelium [12]. Indeed, it has been known for at least two decades that CGRP is one of the most potent vasodilating agents ever described in man [13] and there is evidence that CGRP protects the heart during ischemic events in experimental animals and in man [14–16].

Recently, ethanol has been shown to stimulate TRPV1 by lowering the threshold temperature for channel activation from 43°C to below 37°C [17]. This action of ethanol, in addition to contributing to the burning pain associated with exposure of wounds and mucosal surfaces to alcohol, also results in a series of local responses [17] that are mediated by neuropeptide release from peripheral terminals of sensory neurons. Thus, tachykinins released from airway sensory nerves by TRPV1 activation may contribute to the symptoms of alcohol-induced asthma [18] or esophagitis [18,19].

The present study aims to evaluate whether ethanol is able to release CGRP from arterial and coronary vessels. To investigate this hypothesis we have directly studied the ability of ethanol to produce a TRPV1-dependent neurosecretion of CGRP from guinea pig isolated hearts and from slices of porcine coronary arteries. This issue has also been addressed indirectly, by testing the ability of ethanol to cause TRPV1- and CGRP-dependent (i) dilatation of porcine isolated coronary arteries and human isolated gastro-epiploic arteries and (ii) increase in coronary flow in guinea-pig isolated hearts. Present results, showing that ethanol, by a TRPV1-dependent mechanism, releases CGRP that dilates coronary arteries and increases coronary flow, suggest that this neurogenic pathway may contribute to the ability of alcoholic beverages to reduce the risk of CAD.

2. Materials and methods

Porcine coronary arteries (male, \(n=12\)) were obtained from the local slaughterhouse and 90 male Dunkin Hartley guinea-pigs (250–300 g) were purchased from Harlan, Italy. All experimental procedures complied with and were approved by the Local Ethical Committee and conformed to NIH guidelines [20]. Human right gastro-epiploic arteries were obtained from patients undergoing segmental gastric resection for malignancy. Informed consent was obtained from each patient, and the study was approved by the Local Ethical Committee and conformed to the principles outlined in the Declaration of Helsinki [21].

2.1. Porcine isolated coronary arteries

Intramyocardial (outer diameter <1 mm, small) segments of the left anterior descending porcine coronary artery were placed in ice-cold Krebs–Henseleit solution (mM: NaCl 119, NaHCO3 25, KH2PO4 1.2, MgSO4 1.5, CaCl2 2.5, KCl 4.7 and D-glucose 11). Arterial rings (length 4–5 mm) were mounted in organ baths maintained at 37°C, filled with Krebs–Henseleit solution and gassed with 95% O2/5% CO2 to pH 7.4. Preliminary experiments showed that optimal tensions for small arteries was 0.5 g (60 min). Rings were pre-contracted by adding the thromboxane mimetic, U46619 (10 nM). When the contractile response reached a stable tone, cumulative concentration–response curves were generated with the test agents. Relaxation or contraction were measured with an isometric force transducer (model 7003, Ugo Basile, Italy) and recorded on a polygraph (Unirecord 7050, Ugo Basile, Italy) and expressed as a percentage of the contractile response to U46619 (1.82±0.30 g, \(n=18\)). Epicardial (outer diameter >2 mm, large) arteries were also studied. However, since these vessels produced only small and variable responses to both ethanol (0.079–2.37%) and capsaicin (0.1–10 μM), data originated from these experiments have not been considered in the text.

The TRPV1 antagonist, capsazepine (10 μM), and the CGRP receptor antagonist, CGRP(8–37)(10 μM) were added 20 min before the stimulus. To desensitize sensory nerve terminals, capsaicin (10 μM) was added for 20 min, and after an interval of 20 min, the same procedure was repeated. Additional experiments were performed in the presence of the nitric oxide synthase inhibitor, \(\text{L-arginine (L-NMMA, } 100 \mu\text{M)}\) and the cyclooxygenase inhibitor, indomethacin (10μM).

2.2. Guinea-pig isolated and perfused heart

Guinea-pigs were killed by cervical dislocation and the hearts rapidly removed. A cannula was inserted into the proximal ascending aorta and the heart was perfused (Langendorff’s preparation) at a constant pressure of 38 cm H2O with modified Krebs–Henseleit solution (as above...
with exception of CaCl₂ which was reduced to 1.7 mM) maintained at 37°C, and gassed with 95% O₂/5% CO₂ to pH 7.4. Following a 60-min equilibration period, coronary flow (ml/min) was determined by collecting and weighing 1-min samples of the heart effluent, from 5-min prior to, up to 15-min post-stimuli. Heart rate and contractile force were measured continuously, by means of a hook inserted into the cardiac apex and connected to an isometric transducer (model 7003, Ugo Basile, Italy) under a resting tension of 2.5 g, and recorded on a polygraph (Unirecord 7050, Ugo Basile, Italy). Drugs were injected through a side arm of the perfusion apparatus. One milliliter of the Krebs–Henseleit solution, containing ethanol (0.079–0.791%), capsaicin (0.1–10 µM) or their vehicles, was slowly injected over 2 min. Preliminary experiments indicated that cardiac and coronary parameters were unchanged after the administration of 1 ml of physiologic salt solution. Capsazepine and CGRP(8–37) (1 µM) were given and capsaicin desensitization was performed as described above.

2.3. CGRP release

In experiments using the Langendorff’s preparation, perfusate fractions (~3 ml, basal conditions) were taken every minute from guinea-pig isolated hearts, from 5-min prior to, up to 10 min after stimuli. Ethanol and capsaicin were administered after a 60-min equilibration period. In addition, slices (~0.4 mm, ~100 mg) of small-size porcine coronary arteries were superfused in 2-ml chambers at 0.4 ml/min with a Krebs–Henseleit solution (as above, plus bovine serum albumin, 0.1%) maintained at 37°C, and gassed with 95% O₂/5% CO₂. Following equilibration for 90-min two pre-stimuli perfusate samples were taken at 10-min intervals followed by a third sample during stimulation and a final post-stimulus sample. CGRP release above baseline was calculated as the sum of values of stimulus and post-stimulus samples, each subtracted by the mean baseline value. Capsazepine and capsaicin desensitization was performed as described above. In experiments with a Ca²⁺-free medium, CaCl₂ was not included, and 1 mM EDTA was added to the solution.

Fractions were freeze-dried and re-constituted with 100 mM of phosphate buffer (pH 7.4). CGRP-like immunoreactivity (CGRP-LI) was evaluated as reported previously [22] with a two-site immunometric assay using the monoclonal antibody (mAb) CGRP-83 as capture antibody, whereas mAb CGRP-72 acts as tracer, covalently labelled with the enzyme acetylcholinesterase. Less than 0.1% cross reactivity was observed with human and rat amylin, rat and human calcitonin, ethanol (2.37%), capsaicin (10 µM) or capsazepine (10 µM). Coefficient of variation between-assay was below 10% for 400, 150 and 50 pg/ml of rat α-CGRP. The detection limit of the assay was 2 pg/ml.

2.4. Human isolated gastro-epiploic arteries

Human gastro-epiploic arteries (outer diameter <2 mm) with macroscopically normal appearance were obtained from 12 patients (7 men and 5 women, 73 ± 2 years). Experiments were performed in an identical manner as for the porcine arteries (see above). For each set of experiments, a variable number of rings were used and taken from, at least, 4 different patients. Preliminary experiments indicated 1 g as the optimal tension to produce maximal contraction of arterial rings. Under these experimental conditions, contraction in response to 10 nM U46619 averaged 2.66 ± 0.74 g (n = 16 rings). In preliminary experiments we also found that pre-exposure to 30 µM capsaicin produced a complete desensitization of sensory nerve terminals in human arterial rings, as suggested by a failure to produce any relaxation by a further challenge with 10 µM capsaicin. A 30 µM concentration of capsazepine was required to abate responses to 10 µM capsaicin. Accordingly, 30 µM capsazepine and 30 µM capsaicin (given twice for 20 min, at a 20-min interval) and 10 µM CGRP(8–37) were used in this series of experiments.

2.5. Materials

All agents were provided by Sigma, Italy. Stock solutions of capsaicin (10 mM) and capsazepine (10 mM) were prepared in 100% DMSO and serial dilutions were made in distilled water or Krebs–Henseleit solution. Ethanol (98% v/v) was diluted with Krebs–Henseleit solution to obtain solutions from 0.0008 to 2.37% (w/v).

2.6. Statistical analysis

Data are reported as mean ± standard error (s.e.m.). Changes in tone (contraction or relaxation) of porcine coronary arteries are expressed as percent values of U46619 (10 nM)-induced contraction. E₅₀ denotes the maximal response achieved, and EC₅₀ the concentration of test agent required to elicit a 50% relaxation. Differences between the groups were analysed (GraphPad Prism version 2.01, San Diego, USA) by one- or two-way analysis of variance (ANOVA), followed by post hoc Dunnett’s test for multiple comparisons, where appropriate, with the significance determined as p < 0.05.

3. Results

3.1. Porcine isolated coronary

In the majority of small-size porcine coronary arteries pre-contracted with U46619, ethanol (0.079–2.37%) induced a concentration-dependent relaxation (Fig. 1a) that was, as those produced by capsaicin and CGRP, endothe-
Both treatment with L-NMMA or indomethacin did not affect the relaxation produced by ethanol (data not shown), thus excluding a possible role of nitric oxide and prostanoids in this effect of ethanol. Occasionally (in 34% of the cases), ethanol produced a biphasic response, consisting of an early contractile component (up to 15% of U46619), followed by a marked relaxation. The robust relaxation produced by 2.37% ethanol was converted into a moderate contraction in preparations desensitized to capsaicin (Figs. 1b and 2a). The relaxant response to ethanol was markedly reduced in the presence of the TRPV1 antagonist, capsazepine (Figs. 1c and 2c), or was converted into a contraction by the CGRP receptor antagonist, CGRP(8–37) (Figs. 1g and 2e).

Capsaicin (0.1—10μM) also induced a concentration-dependent relaxation (Fig. 1e–h and Table 1) in pre-contracted (U46619) coronary arteries which peaked at 10μM concentration. In addition, responses to capsaicin (1μM) or 2.37% ethanol after a first exposure to capsaicin (1μM over 20 min, followed by 20 min of washout) (52±7%, n=5) or ethanol (2.37%, over 20 min, followed by 20 min of washout) (59±8%, n=5) or their vehicle (0.9% saline, 57±9% and 67±4%, n=5, respectively) were not different, indicating that both capsaicin and ethanol, at these concentrations, do not cause desensitization. However, the relaxation to capsaicin (10μM) was significantly reduced in preparations desensitized to capsaicin (Figs. 1f and 2d), or in the presence of capsazepine (Figs. 1g and 2e) and CGRP(8–37) (Figs. 1h and 2f). CGRP was very effective in relaxing rings of small-size pig coronary arteries, with concentrations as low as 30pM already producing significant dilatation (12±2% vs. 4±1% of the vehicle, p<0.05; two-way ANOVA, n=6).

The selectivity of these pharmacological interventions was tested through the administration of isoproterenol, which is considered to act directly on vascular smooth muscle. Isoproterenol (1μM) induced 88±8% (n=9) relaxation to U46619 pre-contracted pig coronary arteries. No significant difference was observed in the presence of endothelium (Fig. 1a), or in the presence of endothelium and capsaicin des, or in the presence of endothelium and CGRP (Fig. 1e–h).

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(+) Endothelium</th>
<th>(-) Endothelium</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>E_max (E% of U46619)</td>
<td>EC_50 (μM)</td>
</tr>
<tr>
<td>Ethanol (0.079–2.37%, w/v)</td>
<td>68±8%</td>
<td>0.85±0.12%</td>
</tr>
<tr>
<td>Capsaicin (0.1–10μM)</td>
<td>54±6%</td>
<td>4.44±0.52μM</td>
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<tr>
<td>CGRP (0.001–100nM)</td>
<td>87±14%</td>
<td>1.25±0.16nM</td>
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Values represent the maximum effect (E_max) and the concentration required to elicit 50% relaxation (EC_50) (% of U46619, 10nM) with the agent in the presence (+) or (−) absence of endothelium. Each value represents the mean±S.E.M. of a least 6 experiments.
difference was observed between the control group and those tissues desensitized with capsaicin (Fig. 3a), or pre-treated with capsazepine (Fig. 3b) or CGRP(8–37) (Fig. 3c), thus indicating the selectivity of these interventions.

3.2. Guinea-pig isolated and perfused heart

Following 60-min of equilibration, ethanol (0.079–0.79%, 1 ml solution given over 2 min) evoked a prompt and rapid increase in coronary flow, peaking at about 4 min and returning to baseline within ~10 min of administration (Fig. 4a,b). Significant responses were already detectable at ethanol concentrations as low as 0.079%. Ethanol (0.237%) almost doubled the coronary flow (Fig. 3a), and caused a significant increase in heart rate and a reduction in contractile strength (see Table 2). As 0.237% ethanol produced sub-maximal effect, this concentration was selected to further investigate its mechanism of action.

Furthermore, we observed that injections of capsaicin (0.1–10 μM, 1 ml solution given over 2 min) also produced a...
prompt and rapid increase in coronary flow (Fig. 4c,d), similar to that observed with ethanol. Capsaicin (1 μM) also caused a marked increase in heart rate coupled with a significant reduction in contractile strength (Table 2). The coronary and myocardial effects peaked at about 4 min following capsaicin administration, and returned to baseline within ~10 min.

In hearts desensitized to capsaicin, or in the presence of capsazepine or CGRP(8–37), both 0.237% ethanol (Fig. 5a,b,c and Table 2) and 1 μM capsaicin (Fig. 5d,e,f and Table 2) failed to produce significant changes in baseline coronary flow, heart rate and contractile force. As reported previously [23], SP (1 μM) increased coronary flow (from 3.2 ± 0.3 to 6.1 ± 0.3 ml/min, p < 0.05 vs. vehicle; one-way ANOVA, n = 6), but did not affect heart rate or contractile force (data not shown). In contrast with responses produced by capsaicin or ethanol, the increase in coronary flow induced by SP was not affected by capsaicin desensitization, capsazepine or CGRP(8–37) (data not shown), indicating the selectivity of these pharmacological interventions.

3.3. CGRP release

In guinea-pig isolated and perfused hearts, injection (1 ml solution given over 2 min) of either 0.237% ethanol (Fig. 6a,b) or 1 μM capsaicin (1052 ± 153 fmol/10-min, n = 4) significantly increased the outflow of CGRP-LI (one-way ANOVA, p < 0.05 vs. vehicle, 32 ± 8 fmol/10-min, n = 5). Desensitization to capsaicin or the presence of capsazepine, reduced the amount of CGRP-LI released by 0.237%
ethanol (Fig. 6b) and by 1 μM capsaicin (92 ± 11% and 83 ± 9% reduction, respectively, \( p < 0.05 \); one-way ANOVA, \( n = 6 \)).

Exposure to ethanol (0.079–0.79%) produced a concentration-dependent increase in CGRP-LI outflow from slices of small size porcine coronary arteries. The CGRP-LI outflow induced by ethanol from guinea-pig isolated and perfused hearts (Fig. 6b, c, f) (* \( p < 0.001 \) treated groups vs. vehicle, two-way ANOVA, \( n = 6 \)).

**Fig. 6.** Panel a shows the time-course of the outflow of CGRP-like immunoreactivity (CGRP-LI) from guinea-pig isolated and perfused hearts following administration (arrow) of ethanol (0.237%) or capsaicin (1 μM) following capsaicin desensitization (Capsaicin des, a, d), and in the presence of capsaicin (10 μM) (b, e), CGRP(8-37) (1 μM) (c, f), or their vehicles (-), or one-way ANOVA, \( n = 6 \)).
outflow produced by 0.079%, 0.237% and 0.79% ethanol was 40 ± 8 fmol/g/20 min (n = 4), 83 ± 12 fmol/g/20 min (n = 4) and 94 ± 7 fmol/g/20 min (n = 4) (one-way ANOVA; p < 0.05 vs. vehicle, 6 ± 3 fmol/g/20 min, n = 4), respectively. The CGRP-LI release produced by 0.237% ethanol was significantly reduced after capsaicin desensitization, in a Ca²⁺-free medium, or in the presence of capsazepine (Fig. 6c). Capsaicin (1μM) produced an increase in CGRP-LI outflow (112 ± 12 fmol/g/20 min, n = 4) that was reduced by capsaicin desensitization and in the presence capsazepine (84 ± 10% and 76 ± 9% reduction, p < 0.05; one-way ANOVA, n = 5, respectively).

3.4. Human isolated gastro-epiploic arteries

In U46619 pre-contracted human isolated gastro-epiploic arterial rings ethanol (0.0008–2.37%) produced a concentration-dependent relaxation (Fig. 7a) that was practically abolished by capsaicin desensitization (Fig. 7b,e), in the presence of capsazepine (Fig. 7c,f) or CGRP(8-37) (Fig. 7d,g). In some instances, a small contractile response (always <20% of the subsequent relaxation) preceded the relaxant effect of ethanol. In other cases, following capsaicin desensitization, ethanol produced a contractile response instead of an attenuated relaxation (Fig. 7b). Ethanol concentrations, below the blood alcohol concent-

trations, legally accepted for driving in different countries (open bars, Fig. 7e–g), already produced a sensory nerve-, CGRP- and TRPV1-dependent relaxation. Removal of endothelium did not affect the relaxant response to ethanol (data not shown). Results similar to those produced by ethanol were also observed with capsaicin (1–30μM) (data not shown). Slightly higher concentrations of capsaicin (30μM) to desensitize nerve terminals and capsazepine (30μM) to block the channel could indicate a lower sensitivity to exogeneous agonists/antagonists of the human, compared to pig and guinea-pig TRPV1.

4. Discussion

The present study demonstrates that ethanol induces a remarkable dilatation in porcine small size coronary arteries, and increases coronary flow in guinea-pig isolated hearts, and that these effects are mediated by the release of the sensory neuropeptide, CGRP, via activation of TRPV1 located on terminals of primary sensory neurons. The ability of ethanol to release CGRP was demonstrated by neurochemical experiments. Ca²⁺-dependency of ethanol-induced increase in CGRP-LI outflow from porcine coronary arteries suggests that this effect resulted from a neurosecretory process. Capsaicin desensitization causes complete unre-

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**Fig. 7.** Typical tracings and pooled data of the motor effect of ethanol in rings of human isolated gastro-epiploic arteries pre-contracted with U46619 (10nM) following desensitization to capsaicin (Capsaicin des, 30μM twice for 20 min, 20 min before the stimulus, b, c), in the presence of capsazepine (30μM, c, f), CGRP(8-37) (10μM, d, g) (+●+) or their respective vehicles (-○-, a). The open bars represent the range of legal limits of blood alcohol concentration in different countries for driving (p < 0.001 treated groups vs. vehicle; two-way ANOVA, n = 6).
sponsiveness of sensory nerve terminals to capsaicin itself and to any other excitatory stimulus \[9,24\]. Thus, the observation that ethanol-induced CGRP release from porcine coronary arteries and guinea-pig hearts was abolished by capsaicin-desensitization, suggests that the neuropeptide originates exclusively from capsaicin-sensitive nerve terminals. Finally, inhibition of ethanol-induced CGRP release by the TRPV1 antagonist, capsazepine \[25\], indicates a primary role of TRPV1. The mechanism of ethanol-induced CGRP release was identical to that of capsaicin in terms of \(\text{Ca}^{2+}\)-dependency, and capsaicin- and capsazepine-sensitivity \[24,25\]. A large variety of peptides are expressed in perivascular sensory neurons \[26\]. However, biological functions of the released peptides have been demonstrated only for tachykinins and CGRP. This observation and the finding that a CGRP-receptor antagonist blocked ethanol-induced coronary artery vasodilation exclude the hypothesis that neuropeptides other than CGRP contribute to the effect of ethanol.

Our findings confirm previous data \[23,27\] that capsaicin causes coronary vasodilatation both in pigs and guinea-pigs. In guinea-pig isolated hearts, capsaicin was also found to increase heart rate \[23\]. In our experiments, ethanol produced similar effects that, as for capsaicin, were abolished by capsaicin desensitization, capsaepzine and CGRP \[8–37\]. In the guinea-pig isolated heart, or in isolated papillary muscles, capsaicin and CGRP induce a negative inotropic effect \[23,28\]. Similarly, ethanol decreased the contractile force, in a capsaicin and capsazepine-sensitive manner. Thus, all functional effects of ethanol mimic the responses evoked by capsaicin, and appear to result from a TRPV1-dependent, neurogenic mechanism. Although CGRP released from sensory nerve endings contributes to neurogenic inflammation, its protective action has been shown in different injured tissues, including the gastric mucosa \[29\] or the heart \[14\]. Our findings further reinforce the concept that endogenous mediators known for their proinflammatory action, including, CGRP, prostaglandins or bradykinin, under specific circumstances, can exert a protective role.

Ethanol-induced dilatation of porcine small size coronary arteries was remarkable, and reversed the powerful contraction produced by the thromboxane mimetic, U46619. Occasionally, a biphasic response (transient contractile component, readily overridden by a relaxation) was observed following ethanol administration in small coronary arteries. The ability of ethanol to contract, or to not dilate, large-size arterial vessels, including large epicardial coronary arteries, and to dilate small resistance canine and human coronary arteries, has been reported previously \[30–33\]. It is worth mentioning that, when the ability of sensory nerve terminals to release CGRP was abolished by capsaicin desensitization, ethanol-induced dilatation was changed into a moderate contraction also in small size porcine coronary arteries.

Our present results showing that TRPV1 stimulation and CGRP release from sensory nerve endings mediates ethanol-induced vasodilatation in pig and guinea pig coronary arteries and in human gastro-epiploic arteries, suggest that an identical mechanism might exist in human coronary arteries. This hypothesis is corroborated by the observation that CGRP-positive nerve fibres are abundant in human coronary arteries \[34,35\], and CGRP is released in vitro by capsaicin from human tissues, including the coronary arteries, in a \(\text{Ca}^{2+}\)-dependent and capsaicin-sensitive manner \[34,36\]. Finally, CGRP release is apparently the mechanism responsible for the dilatation produced by the stimulation of sensory nerve terminals by capsaicin in human coronary arteries \[34\]. In the present study we have shown that neurogenic relaxation mediated by CGRP in human arteries was already produced by an ethanol concentration as low as 0.008%, a concentration that is much below the level attained after low to moderate drinking (~1 glasses of wine in a 70kg man). Although the mechanism is identical in the three mammal species, sensitivity of human arteries to ethanol-induced neurogenic vasodilatation, and possibly CGRP release, is higher than those of pig and guinea pig arteries. The reason for the difference in potency of ethanol to cause neurogenic vasodilatation in pig vs. human arteries is unknown. Several factors may contribute, including species related differences, the use of different vessels in the two species, and the use of apparently healthy vessels, that, however, were taken in the vicinity of a tumour.

Ethanol exerts a series of detrimental effects in diverse organs and tissues including direct toxicity to the myocardium \[37\] and these effects contribute to the overall mortality associated to alcohol consumption \[38\]. However, in the case of CAD epidemiological evidence \[3,4\] indicates that alcohol, irrespective of the type of alcoholic beverage and of the amount of the consumption (up to 50 g/day) is associated to a protective effect \[4\]. Thus, alcohol per se, rather than the non-alcoholic constituents of alcoholic beverages, seems to exert a protective effect. Although several hypotheses have been proposed, including effects on lipoproteins \[6\], platelet aggregation \[7\], or tissue plasminogen activator \[8\] and other effects, the mechanism by which ethanol reduces the risk of CAD is, at the best, only partially known.

Increase in myocardial blood flow results from dilatation of coronary arteries and arterioles, a phenomenon mediated by diverse mechanisms, including neurogenic stimuli. In man, intracoronary ethanol or CGRP increase coronary blood flow and decreases resistance in subjects with or without CAD \[33,39\]. CGRP produces a marked dilatation of coronary arteries at the site of atheromatous stenoses, delays the onset of myocardial ischaemia and increases the work tolerance during treadmill exercise in patients with stable angina pectoris \[40\]. Protective actions of CGRP released from C-fibres following myocardial ischemia \[14\] include an increase in coronary blood flow, protein kinase C activation, antiarrhythmogenic and other effects \[14–16\]. The present neurochemical and functional evidence show-
ing that, via TRPV1 activation, ethanol releases CGRP within the coronary arterial wall, suggests that this neurogenic mechanism may contribute to the reduction in the risk of CAD by alcoholic beverages. The extraordinary ability of human periartrial sensory nerve endings to release CGRP in response to ethanol, as indicated by the peculiar sensitivity of these vessels to produce a neurogenic vasodilatation, further supports the hypothesis that low-moderate drinking results in a reduced risk of CAD because, among other factors, this habit causes a neurogenic, TRPV1-dependent release of CGRP.

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References


