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Ethanol potentiates heat response in the carotid artery via TRPV1

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ABSTRACT

Aim: Ethanol is one of the most widely used recreational drugs in the world. At high concentrations, it can induce carotid artery vasoconstriction. Hyperthermia potentiates its effects resulting in carotid artery vasoconstriction at any concentration. The aim of this study is to investigate the interaction between ethanol and heating and to understand the underlying mechanisms leading to their synergistic effect.

Materials and methods: Isometric tension of rabbit carotid artery ring segments suspended in organ baths filled with Krebs solution was recorded. Different concentrations of ethanol were examined at 37 °C and during temperature elevation to39–43 °C. Capsaicin and capsazepine were used to examine the mechanism of action of ethanol.

Key findings: Ethanol induced contraction at 37 °C when the concentration reached 100 mM. Contraction was observed at any concentration at higher temperatures. Ethanol potentiated heat-induced contraction. Capsaicin, the vanilloid receptor subtype1 (TRPV1) agonist, potentiated the vasoconstriction due to heating. While capsazepine, TRPV1 antagonist, abolished the effect of ethanol and its potentiation of heating-induced contraction, but it did not abolish the heating effect.

Significance: Ethanol's mechanism of action and its effect on heating induced-vasoconstriction of the carotid artery is being mediated by TRPV1. The combination of ethanol and hyperthermia can lead to a synergistic effect on carotid vasoconstriction. This effect may induce brain damage and heat stroke. Development of new drugs act as TRPV1 antagonist can be used to prevent these fatal effects.

1. Introduction

Alcohol is one of the most widely used recreational drugs in the world. Its main active ingredient is ethanol. Many studies have demonstrated the beneficial and harmful effects of drinking alcohol [1–7]. Mustafa et al., 2007b [8] showed that ethanol, at body temperature, elicited carotid artery vasoconstriction at high concentrations (toxic levels). This effect was also demonstrated at any concentration when the body's temperature is elevated. The synergistic effect of ethanol and hyperthermia has been proven [8]. It has been shown that ethanol reduces cerebral perfusion and potentiates the effect of hyperthermia-induced vasoconstriction which may lead to heat stroke [8–10]. The mechanism of action of ethanol is not fully understood. Some studies have shown that ethanol stimulates transient receptor potential vanilloid 1 (TRPV1) channels on primary sensory neurons [11] and potentiates a nociceptor response through vanilloid receptor-1 [12]. Other studies have shown that ethanol withdrawal can contribute to hyperalgesia in the peripheral TRPV1during deep pain conditions [13], and stated that TRPV1 is important for specific behavioral actions of ethanol [14]. Transient receptor potential channel (TRP) superfamily responds to stimuli from inflammatory agents, pressure, temperature and receptor activation. TRP includes TRPV which has receptors in different organs in the body. There are several types of TRPV ion channels. They cover a range of temperatures. TRPV1 is activated by heat and also activated by capsaicin which is the active ingredient of hot chili peppers. Capsaicin produces a sensation of burning pain. The cloned capsaicin receptor is stimulated by heat. It is considered TRPV1 agonist. Capsaicin receptor can be also stimulated by increases in temperature in the noxious range [15]. While capsazepine is a synthetic analog to capsaicin, it blocks the painful effect of heat due to capsaicin, accordingly considered to be a TRPV1 antagonist [16]. Most of the previous studies examined the effect of ethanol and heating in neurological research but this work focuses on their effect on arterial smooth muscle.

The aim of this study is to investigate the mechanism of action of ethanol and its synergistic effect on heating-induced carotid artery vasoconstriction using capsaicin and capsazepine receptors TRPV1.

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2. Materials and methods

2.1. Experimental animals

Twenty male New Zealand White rabbits of the same age (10 weeks) weighing 3.5–4 kg were used in this study. Experiments were done in accordance with guidelines approved by the Institutional Animal Care and Use Committee of Kuwait University. Sodium pentobarbital (120 mg/kg; IP) was used to anaesthetize the rabbits. The carotid arteries were isolated and mounted in Krebs' solution, (NaCl 118, $MgSO_4$ 1.2, KCl 5.9, glucose 11.1, NaHCO3 26, KH_2PO_4 1.2, and $CaCl_2$ 2.2 in mM concentration) at pH 7.4. The arteries were cleaned from fat and connective tissues. They were cut into 5 mm in length as ring segments with care to avoid endothelium injury. Then were suspended in 10-ml organ baths filled with Krebs' solution, at 37 °C and continuously gassed with a 95% O_2 and 5% CO_2 mixture. The preparations were allowed to equilibrate under optimal resting tension of 2 g for up to 60 min. The bath fluid was changed twice before starting the experiment. Isometric contractions were recorded by computerized, automated isometric transducer system (Schuler organ bath 809; Hugo Sachs Electronik, March-Hugstetten, Germany) which connected to a Gould recorder (Gould Instrument Inc., Cleveland, OH, USA). After the period of equilibration, KCl (30 mM) was added to the bath to test for tissue viability. The tissues were washed immediately after the peak concentration has been obtained. The contraction to KCl was repeated every 30 min until two consecutive contractions did not differ by > 5%. The last contraction to KCl was used as reference against which contractions were expressed. Thereafter, ethanol (1, 10, 50, 100, 300, 500, 1000 mM) was added cumulatively to the bath and the contractions were recorded. The contraction was expressed as a percentage of KCl-induced contraction. In experiments conducted with capsaicin or capsazepine, the appropriate concentration, which was commonly used in many previous studies, of each chemical was added to the organ baths and allowed to equilibrate with the tissues for 30 min raising the temperature.

2.2. Heating protocol

The temperature of the Krebs solution was elevated using a circulator bath (Haake F3; Fisons, Germany) from 37 °C to 45 °C in 2 °C increments (37, 39, 41, 43, and 45 °C). The desired temperature took 2–3 min to be reached. Each heating period was maintained until a peak response had leveled off before further temperature elevation.

2.3. Drugs

Capsaicin and capsazepine were obtained from Tocris International (Avonmouth, UK). They were dissolved in ethanol.

2.4. Calculations

Data are calculated as the mean of (n) experiments ± SEM. The differences between two mean values were analyzed using Student's-*t*-test paired. One-way or two-ways analysis of variance (ANOVA) as appropriate was used. A one-way ANOVA was used with a Kruskal-Wallis test for individual comparisons. Two-ways ANOVA was used with the Holm-Sidak test for individual comparisons. The difference was considered significant at $p < 0.05$.

3. Results

3.1. Heating-induced contraction

All preparations maintained a stable baseline before starting to change the temperature. Elevation of the organ bath temperature from 37 °C to 39, 41, 43, 45 °C induced a rapid and reproducible stepwise increase in tension that was proportional to heating. When the temperature was returned to 37 °C, the tone rapidly went back to the basal level.

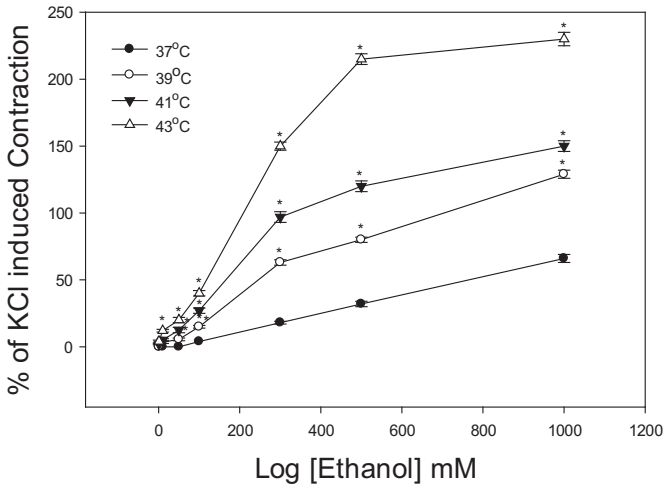

Fig. 1. Effect of heating on concentration-response curves to Ethanol (10, 50, 100, 300, 500, 1000 mM) at 37, 39, 41, 43 °C, of isolated carotid artery segments. Values are means ± SE of 4 experiments. *$p < 0.05$.

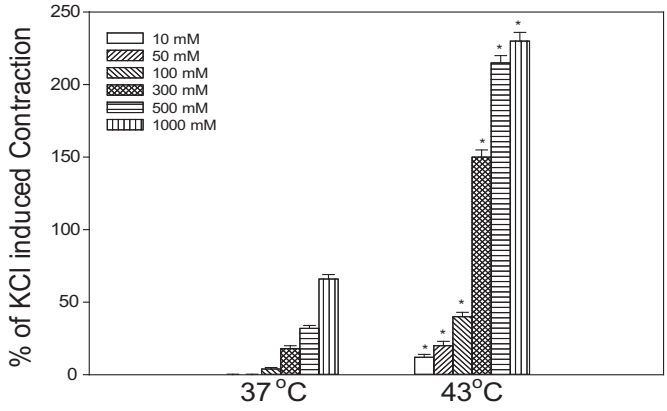

Fig. 2. Effect of Ethanol (10, 50, 100, 300, 500, 1000 mM) at 37 and 43 °C, of isolated carotid artery segments. Values are means ± SE of 4 experiments. *$p < 0.05$.
(Notice the absence of ethanol effects at lower concentration than 100 mM at 37 °C and their appearance at 43 °C, also the great potentiation of Ethanol responses at all its concentrations at higher temperature than 37 °C).

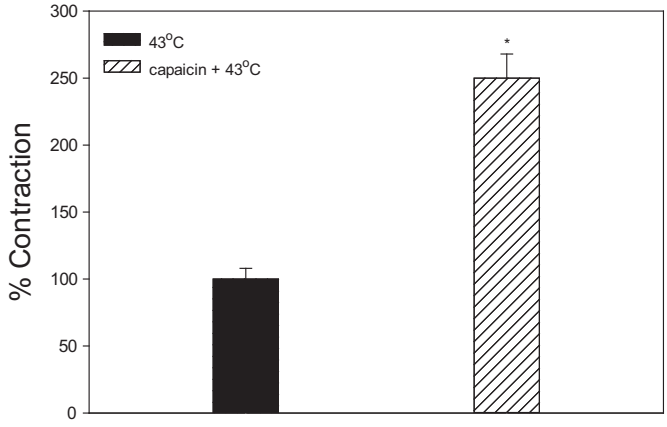

Fig. 3. Effect of capsaicin (100 μM) on 43 °C-induced vasoconstriction of isolated carotid artery segments. Values are means ± SE of 4 experiments. *$p < 0.05$.
(Notice the potentiation effect of capsaicin on heating-induced vasoconstriction).

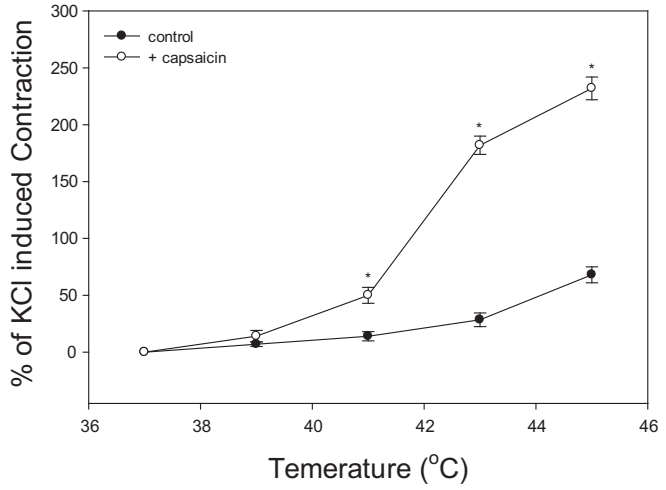

Fig. 4. Effect of capsaicin (100 μM) on heating-induced vasoconstriction of isolated carotid artery segments. Values are means ± SE of 4 experiments. *$p < 0.05$. (Notice the potentiation effect of capsaicin on heating-induced vasoconstriction).

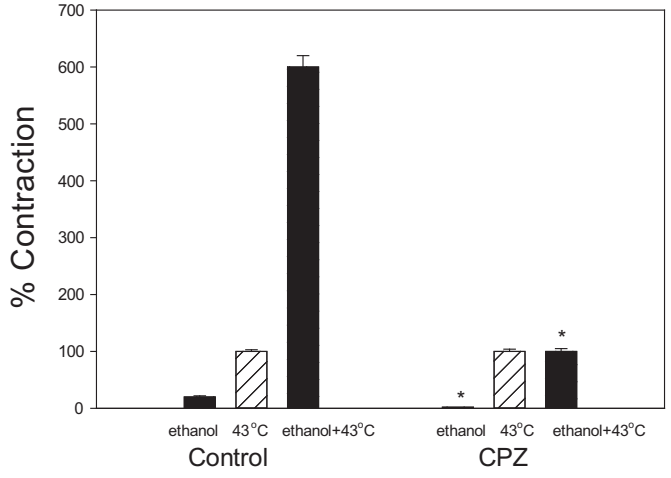

Fig. 6. Effect of ethanol (100 mM) at 37 °C; heating temperature 43 °C and ethanol (100 mM) at 43 °C before and in the presence of capsazepine (30 μM) of isolated carotid artery segments. Capsazepine abolished the effect of ethanol and its effect of potentiation heat-induced vasoconstriction but did not affect heating response. Values are means ± SE of 4 experiments. *$p < 0.05$.

3.2. Ethanol responses

Ethanol (1–1000 mM) was tested on ring segments of carotid artery at 37 °C. A concentration-dependent increase in tension was only observed at 100 mM ethanol concentration. Concentration-response curves for ethanol were repeated at elevated temperatures 39, 41 and 43 °C. The vasoconstrictor responses were increased in magnitude proportional to the heating temperature. The increase in tension started from10 mM at 39 °C, and at 1 mM at higher temperatures, 41 and 43 °C as shown in Fig. 1. Fig. 2 represents the vasoconstriction responses of different concentrations of ethanol at 37 °C and 43 °C. It demonstrates that the vasoconstriction effect of ethanol started at100 mM while it was apparent at concentrations of 1, 50 mM at 43 °C. The ethanol vasoconstriction responses were potentiated during heating.

3.3. Effect of capsaicin

Capsaicin binds to vanilloid receptor subtype 1 (TRPV1)·It had no significant effect on basal tone of the carotid artery preparation. Capsaicin (100 μM) potentiated heating (43 °C)-induced vasoconstriction as shown in Fig. 3.

Capsaicin potentiated the heating-induced vasoconstriction at all the elevated temperatures used in these experiments. Fig. 4 showed the potentiation effect of capsaicin on heat-induced vasoconstrictions.

3.4. Effect of capsazepine

Capsazepine (CPZ) is a synthetic analog of capsaicin and it is a capsaicin antagonist. It had no effect on basal tone of the carotid artery preparation but it abolished the contractile effect of ethanol (100 mM). Capsazepine did not antagonize the effect of heat-induced vasoconstriction. However it abolished the potentiation of ethanol on heat-induced vasoconstriction. This indicates that ethanol is acting through capsaicin receptor (vanilloid receptor) as shown in Figs. 5 and 6.

4. Discussion

Our previous studies [10,17–19] showed that heating induced vasoconstriction of the carotid artery, and it was proportional to temperature. Mustafa et al., 2007b [8] showed that ethanol induced vasoconstriction at 37 °C only at high concentrations (> 100 mM). However, at higher temperatures, its vasoconstrictor effect can be observed at lower concentrations. The increase in tension was in a concentration-dependent manner to ethanol and it was proportional to heating temperature. Ethanol potentiated the effect of heating-induced vasoconstriction [8]. The synergistic effect of ethanol and heating can result in heat stroke and brain damage [8–10]. Therefore, we extended our studies by examining the mechanism of action of ethanol and its potentiation effect.

Capsaicin, a member of the vanilloid family, binds to vanilloid receptor subtype 1. TRPV1 is an ion channel-type receptor and a member of the superfamily of TRP ion channels. There are different TRP ion channels which have been proven to be sensitive to wide ranges of temperatures and are responsible for our range of temperature sensation. TRPV1 can be stimulated with heat. When capsaicin binds to the TRPV1 receptor, it produces the same burning sensations of excessive heat. Our present study shows that capsaicin potentiated the effect of heating-induced vasoconstriction of carotid artery. Capsaicin is acting like ethanol regarding heating effect.

Capsazepine, capsaicin antagonist, did not affect heating-induced

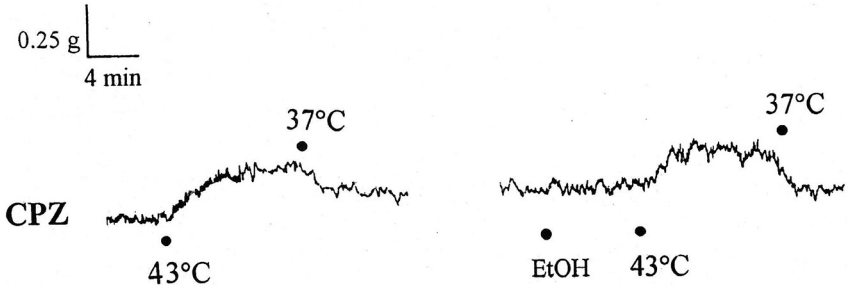

Fig. 5. An original tracing of one segment of isolated carotid artery representing the effect of capsazepine (30 μM) on heating (43 °C)-induced contraction in absence and presence of ethanol (100 mM). Shown are no vasoconstriction for ethanol and no potentiation effect of ethanol on heating-induced vasoconstriction.

vasoconstriction. Capsazepine can block TRPV1 channels stimulated by other chemicals, but not by heat [20]. In the present study, capsazepine abolished the ethanol-induced contraction effect. It also abolished the potentiation effect of ethanol to heating-induced constriction. In the presence of capsazepine, there is no effect due to ethanol either alone or in the presence of heating indicating that it antagonizes ethanol. It has been shown that during inflammation the density of TRPV1 expression is enhanced [21]. It is also known that inflamed tissue is hyperthermic when compared to normal tissue. This explains the sensitivity of inflamed tissue to ethanol with no effect on normal tissue.

In conclusion, ethanol-induced vasoconstriction and its effect on potentiating heat-induced vasoconstriction of the carotid artery is mediated by activation of TRPV1.

Ethanol elicits and potentiates the carotid artery's response to heat via TRPV1. Therefore, the synergistic effect of both ethanol and hyperthermia which may lead to heat stroke and brain damage can be abolished by a TRPV1 antagonist. New specific safe antagonist to ethanol and TRPV1 should be the next target to the pharmaceutical companies to help in improving man's life.

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Declaration of interest

I am declare that there is no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence, this work.

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