Synergistic effects of ethanol and hyperthermia on carotid artery vasoconstriction

S. Mustafa¹, O. Thulesius², A. H. Elgazzar² and H. N. Ismael²

¹College of Health Sciences, The Public Authority for Applied Education and Training, and ²Faculty of Medicine, Kuwait University

Summary

Background: Heatstroke is a serious condition and clinical studies indicate that vascular stroke increases with excessive consumption of alcohol (ethanol). It was our objective to test the influence of ethanol on cerebral perfusion at normal and higher temperatures.

Methods: Recording of isometric tension in rabbit carotid artery strips in organ baths with different concentrations of ethanol at 37°C and during hyperthermia (39–43°C) and scintigraphic cerebral imaging of a radioactive isotope in the control situation and during hyperthermia.

Results: Stepwise heating induced reproducible reversible graded contraction, proportional to temperature. At high concentrations (toxic levels), ethanol induced an increase in tension and heating potentiated these responses. Extracellular Mg²⁺ potentiated both heat-induced contraction and ethanol-induced contraction while extracellular Ca²⁺ had no effect on these responses. During hyperthermia and ethanol scintigraphic isotope uptake was reduced in cortical and cerebellar regions.

Conclusions: Carotid artery vasomotor tone is temperature dependent and heating induces vasoconstriction. Alcohol (ethanol) at 37°C elicited carotid artery contraction at high concentrations (toxic levels) but at any concentration during elevated temperature (39–43°C). Ethanol potentiated the effect of hyperthermia-induced vasoconstriction and reduced cerebral perfusion as shown by radionuclide imaging. The synergistic effect of ethanol and hyperthermia may induce heat stroke and brain damage.

Introduction

Abuse of alcohol is a major cause of morbidity and mortality. Studies show that more than one-third of patients diagnosed with head injury are intoxicated with ethanol and the incidence of vascular stroke and sudden death is increased with the excessive consumption of alcohol (ethanol) or binge-drinking (Hillbom & Numminen, 1987). Serum alcohol levels >100 mg dl⁻¹ at the time of admission after traumatic brain injury is associated with a decrease in cerebral blood flow (CBF) (Alexander et al., 2004). There are also studies suggesting that low levels of alcohol consumption may have some protective effect causing cerebral vasodilatation with an increased blood flow (Blaha et al., 2003), whereas heavy consumption predisposes to stroke (Gill et al., 1991).

Whole body hyperthermia is a distinctive pathological condition with significant impact on tissue metabolism and organ functions (Xi et al., 2001). Hyperthermia can cause brain dysfunction. Toxic substances from infections that affect the temperature-regulating centres and environmental heat exposure may terminate in heatstroke. Fever adversely affects outcome in stroke and also in subarachnoid haemorrhage and is associated with vasospasm and poor outcome (Oliveira-Filho et al., 2001). Heat-related deaths are not uncommon at high environmental temperature, in amphetamine-related hyperpyrexia can occur in elderly incapacitated individuals during heat waves. But so far, the clinical literature does not suggest that a combination of fever with alcohol provokes strokes. We have previously shown that hyperthermia induced a reversible graded vasoconstriction of the carotid artery. In vivo this reaction may lead to a decrease in CBF with brain damage as in heatstroke (Mustafa et al., 2004).

The influence of alcohol (ethanol) has until now only been studied at normal body temperature but not at elevated levels.
Therefore, our aim was to investigate the effect of different concentrations of ethanol at 37°C and during hyperthermia on carotid arterial smooth muscle tone and by brain scintigraphy to explore possible locations and degree of cerebral ischaemia.

Materials and methods

Experimental animals

Twenty adult, male New Zealand White rabbits of the same age (10 weeks) weighing 3.5–4 kg were anaesthetized with sodium pentobarbital (120 mg kg\(^{-1}\) IP). Half the number of these rabbits were used for in vivo imaging and the rest for in vitro experiments. Experiments were performed in accordance with guidelines approved by the Institutional Animal Care and Use Committee of Kuwait University.

In vitro experiments

The carotid arteries were immediately removed and placed in Krebs’ solution with the following composition (in mM): NaCl 118, KCl 5–9, MgSO\(_4\) 1·2, CaCl\(_2\) 2·2, KH\(_2\)PO\(_4\) 1·2, NaHCO\(_3\) 26 and glucose 11·1, at pH 7·4. Connective tissue and fat were carefully removed. The arteries were cut into rings segments 5 mm in length which were mounted on triangular wire supports and suspended in 10-ml organ baths containing Krebs’ solution, maintained at 37°C and gassed with 95% O\(_2\) and 5% CO\(_2\). Tension was continuously recorded using a computerized, automated isometric transducer system (Schuler organ bath 809; Hugo Sachs Electronik, March-Hugstetten, Germany) connected to a Gould recorder (Gould Instrument Inc., Cleveland, OH, USA). The segments were initially loaded to a tension of 2 g, which had been previously determined as an optimal load, and allowed equilibrating for 60 min, during which time they were washed twice. Care was taken not to injure the endothelium during the preparation. The presence of intact endothelium was verified by adding acetylcholine (1 \(\mu\)mol l\(^{-1}\)), which resulted in the relaxation of noradrenaline (1 \(\mu\)mol l\(^{-1}\)) precontracted ring segment. However, in some experiments, the intima of the vessels was gently rubbed with a cotton bud to remove endothelial cells. At the end of each experiment, the muscle was weighed and responses calculated as mg/mg tissue weight. The organ bath temperature was increased using a circulator bath (Haake F3; Fisons, Germany), which had been set to the appropriate temperature. It took 2–3 min to reach the desired temperature in steps from 37°C up to 43°C by 2°C increment. Each heating period was maintained until a peak response had levelled off before further temperature elevation.

Radionuclide imaging

Hyperthermia (43°C) was induced by exposure of the rabbits \((n = 6)\) to heat in a temperature-controlled chamber maintained at 45°C, 40% relative humidity and 0·2 m s\(^{-1}\) air velocity. Rectal temperature was measured using thermistor probes during the experiment. The temperature of the control rabbits was ranging from 37·5 to 38°C. Cerebral blood flow was assessed using technetium-99m-hexamethyl-propyleneamineoxime (99mTc-HMPAO). Each rabbit was injected intravenously with 130 MBq (3·5 mCi). This lipophilic compound can cross the blood brain barrier and is then converted to another compound that cannot escape from the brain for a specific time. This conversion process gives the ability of 99mTc-HMPAO to be retained within the brain. 99mTc-HMPAO is completely cleared from the brain in 72 h. The anaesthetized rabbits were taped to the imaging table in supine position. Scintigraphic images for the brain were acquired using a gamma camera equipped with a low-energy parallel hole and high resolution collimator (Meridian System, T55B-1473, Marconi Medical System Inc., Cleveland, OH, USA) in posterior position. Immediately after tracer administration, a blood pool static image was acquired for 10 min in a 256 × 256 matrix. Seventy-two hours later the same procedure was repeated after ethanol injection. A blood sample was taken to quantify the blood ethanol concentration which was 50 mM. Again after 72 h, an identical protocol was repeated with the same rabbit after elevating his body temperature to 43°C then injected with the same amount of ethanol intravenously in the ear vein. The count per pixel was determined for control (at 37·5–38°C) and during hyperthermia (43°C). Rectal temperature was monitored during the experiment for all the rabbits.

Drugs

Noradrenaline hydrochloride, acetylcholine chloride, verapamil hydrochloride, nifedipine, ethylene glycol bis (\(\beta\)-aminoethyl-ether) O-N,N,N,N-tetraacetic acid (EGTA), were obtained from Sigma Chemicals (St Louis, MO, USA). All drugs were dissolved in distilled water except EGTA, which was dissolved in 0·1 NaOH.

Calculations

Data are presented as mean (SEM) of \((n)\) experiments. Where necessary, differences between two mean values were compared using Student’s-\(t\) test paired or unpaired as appropriate. Where multiple comparisons were necessary one-way analysis of variance (ANOVA) was used followed by Student–Newman–Keuls test. The difference was assumed to be significant at \(p<0.05\).

Results

Carotid artery heating

Before heating all preparations maintained a stable baseline. Stepwise elevation of the bath temperature induced a rapid and reproducible increase in tone, proportional to the heating temperature (Fig. 1). When temperature was reset to 37°C, the tone rapidly returned to basal level. The heating-induced
Contractions could be repeated and were not tachyphylactic in any of the segments tested. To determine whether the endothelium contributes to the heating- or ethanol-induced contractions, experiments were repeated in preparations in which the endothelium had been gently removed. Removal of the endothelial cell layer had no effect on the contractions due to heating or ethanol. These findings indicate that both types of contractions are not generated or altered by mediators released from endothelial cells.

**Ethanol responses**

Ethanol (10–1000 mM) was tested on ring segments of carotid artery at 37°C. Figure 2 shows a typical original trace representing the effect of ethanol on a carotid artery ring segment. Ethanol did not produce any effect at lower concentration than 100 mM. However, it resulted in concentration-dependent contraction development at higher ethanol concentrations.

Contractile responses of carotid artery preparations for ethanol where tested at three concentrations: 10, 50 and 100 mM representing the conventional threshold (minimal), the illegal (severe) and toxic concentrations. These levels were examined at normal body temperature of 37°C and at 39, 41, 43°C representing fever levels. At 37°C, the threshold concentration and the illegal concentration of ethanol did not induce a response. A concentration-dependent increase in tension was only obtained above the highest ethanol concentrations of 100 mM and heating significantly potentiated this response. However, a temperature elevation to 39–41°C at the beginning of the experiment, induced vasoconstriction also with 50 mM ethanol (Fig. 3). The vasoconstrictor responses at illegal and toxic concentrations were increased in magnitude at 41°C and this amounted to three times that obtained at 37°C. This obviously means a potentiation which was proportional to the heating temperature. Figure 4 is an original tracing which shows a six-fold increase in the heating-induced contraction by 100 mM ethanol at 41°C.

**Effect of extracellular Ca²⁺ or Mg²⁺**

Ca²⁺ ions are necessary for excitation and contraction-coupling events while Mg²⁺ ions modulate regulation of Ca²⁺ metabo-
lism and contractile responsiveness in vascular smooth muscle cells. Contraction of the carotid artery was produced by addition of a single dose of ethanol (50 μmol l\(^{-1}\)) to ring segments of carotid artery incubated for 45 min in Ca\(^{2+}\)-free (1 mM EGTA) or Mg\(^{2+}\)-free Krebs’ solution. Removal of extracellular Ca\(^{2+}\) neither changed tone of the carotid smooth muscle nor inhibited the contraction to heat or ethanol. Removal of extracellular Mg\(^{2+}\) did not change basal tone, but potentiated both heat-induced contraction and ethanol-induced contraction. As shown in Fig. 5. Calcium channel antagonists, verapamil (10 μmol l\(^{-1}\)) or nifedipine (1 μmol l\(^{-1}\)) had no inhibitory effect on heat- or ethanol-induced contraction, indicating that contraction to both heat and ethanol is mainly depending on intracellular calcium.

**Radionuclide imaging**

Figure 6 is an anatomical presentation of the rabbit brain next to the image with the normal distribution of the radioactive tracer. This control image covers the forebrain of the large olfactory region, the cerebral hemispheres and the cerebellar region. Figure 7 represents a scan after ethanol injection at normal body temperature of 37°C and at 43°C. The concentration of the injected ethanol was 50 mM. This image clearly displays a reduced activity in the anterior and posterior regions of the brain and thereby clearly supports the concept of a pronounced high-dose ethanol-induced vasoconstriction of the supply arteries to the region of the forebrain, the hemispheres and the cerebellum. Temperature elevation to 43°C clearly displays a more pronounced reaction.

**Discussion**

The results of the present study of in vitro and in vivo experiments clearly support the role of hyperthermia and ethanol as aetiological factors in the development of cerebral ischaemia and brain-damage (stroke). Moreover, it introduces the concept of potentiation for the combined action of ethanol and hyperthermia as they act in synergy to elicit vasoconstriction. This extends our own studies showing that cooling dilates and heating constricts carotid arteries (Mustafa & Thulesius, 2002; Mustafa et al., 2004) and confirms the findings of high-dose ethanol as a vasoconstrictor (Altura et al., 1983).

Judged on a molar basis, the consumption of ethanol far exceeds that of any other drugs. In humans, the concentration of alcohol in the blood is the first concern. By the end of an hour after one drink of alcohol, the blood alcohol level will be 20 mg 100 ml\(^{-1}\) (4 mM). Some degree of euphoria and light-headedness will take place with concentrations of ethanol (10–20 mM). Therefore, we can consider this concentration as the mild pharmacological concentration. Blood alcohol concentration exceeding 80 mg 100 ml\(^{-1}\) (17.4 mM) (the legal limit for driving in Britain) is highly suggestive of alcohol misuse, and values above 150 mg 100 ml\(^{-1}\) (32 mM) are diagnostic. It is generally accepted that a blood level of alcohol of 32 mM may be taken as an evidence that the person is drunk (Faraci & Heistad, 1990). It is known that above 80 mM will severely depress respiratory function and above 100 mM is toxic and fatal. The exact lethal dose in any individual will vary with age, sex, general physical health and the degree of prior tolerance to alcohol (Kinney, 2000). Therefore, we chose 10-, 50- and 100-mM concentration of ethanol in this study as mild, moderate and toxic concentrations.
Our study also indicates that the underlying process of thermal and ethanol-induced vasoconstriction is dependent on intracellular calcium and not blocked by pharmacological antagonists, a finding consistent with previous results using basilar and middle cerebral arteries from dogs, sheep and piglets (Zhang et al., 1993). Mg\(^{2+}\) is thought to act as a naturally occurring Ca\(^{2+}\) antagonist in vascular smooth muscle (Altura et al., 1987). Lowering of extracellular Mg\(^{2+}\) has been demonstrated to increase Ca\(^{2+}\) influx across vascular muscle cell membranes and facilitate intracellular Ca\(^{2+}\) release as well as elevate intracellular Ca\(^{2+}\) in vascular muscle cells (Altura & Altura, 1984; Zhang et al., 1992). As Mg\(^{2+}\) deficiency is associated with ethanol intoxication (Brautbar & Altura, 1987), a supplemental treatment may be considered (Flink, 1986). Low serum concentration of magnesium ions have recently been reported in patients with coronary disease, stroke as well as in patients with cerebral haemorrhage (Li et al., 2001).

A basic question about the validity of our study is the issue of the possible heterogeneity of vasomotor responses within vessels of the cerebral circulation. Are our experiments with the carotid artery representative of the cerebral circulation? Documentation already exists which shows similar vasomotor responses to vasopressin in canine cerebral arteries at the base of the brain (circle of Willis) compared with other intra- and extra-cranial vessels such as the carotid arteries (Suzuki et al., 1993). Another study in cats showed few regional differences to physiological vasoconstrictor agents such as noradrenaline and dopamine, whereas the variability to vasodilation was more pronounced (Hamel et al., 1988). These findings basically support the assumption that no major regional diversity exists to vasoconstrictor responses. Furthermore, our imaging technique, which reflects the level of cerebral blood flow, clearly supports the results of the in vitro vasomotor experiments.

The present investigation clearly shows that hyperthermia induces vasoconstriction of the carotid artery of the rabbit. The degree of heating-induced contraction was proportional to temperatures. The vasoconstrictor effect of alcohol appeared only at high concentration, which is around 100 mM (toxic concentration) at normal body temperature 37°C. During fever (39–41°C) ethanol-induced contractions in the carotid artery with any concentration (1–100 mM). Therefore, the assumption that low levels of alcohol consumption may have some beneficial effects in organ perfusion should emphasize that this...
only applies at 37°C and should be considered dangerous for stroke during fever and other hyperthermic conditions.

At high body temperature, any concentration of ethanol will induce vasoconstriction. Temperature elevation increases the ethanol-induced response and has to be added to the inherent heat-induced degree of vasoconstriction. Therefore, this pattern should result in elimination of any ‘safe’ limits of blood alcohol. Finally, we know about strokes and fever, but we do not know anything of strokes precipitated by a combined effect of fever and alcoholic intoxication in the clinical literature. The demonstration of a synergetic effect between ethanol and hyperthermia is the first study to announce that ethanol can cause cerebral vasoconstriction irrespective of the concentration at elevated temperature. Such interaction may play an important role in stroke associated with alcohol consumption.

References


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