

Hyperthermia-induced vasoconstriction of the carotid artery, a possible causative factor of heatstroke

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Submitted 13 October 2003; accepted in final form 2 December 2003

Mustafa, Seham, O. Thulesius, and H. N. Ismael. Hyperthermia-induced vasoconstriction of the carotid artery, a possible causative factor of heatstroke. *J Appl Physiol* 96: 1875–1878, 2004; 10.1152/japplphysiol.01106.2003.—Clinical and experimental studies indicate that hyperthermia can cause heatstroke with cerebral ischemia and brain damage. However, no study has examined the direct effects of heating carotid artery smooth muscle and tested the hypothesis that hyperthermia induces arterial vasoconstriction and, thereby, decreases cerebral blood flow. We recorded isometric tension of rabbit carotid artery strips in organ baths during stepwise temperature elevation. The heating responses were tested at basal tone, in norepinephrine- and KCl-precontracted vessels, and after electrical field stimulation. Stepwise heating from 37°C to 47°C induced reproducible graded contraction proportional to temperature. The responses could be elicited at basal tone and in precontracted vessels. Heating decreased the contractile responses to norepinephrine and electrical field stimulation but increased contraction to KCl. These responses were not eliminated by pretreatment with the neuronal blocker tetrodotoxin. Our results demonstrate that heating carotid artery preparations above 37°C (normothermia) induced a reversible graded vasoconstriction proportional to temperature. In vivo this reaction may lead to a decrease in cerebral blood flow and cerebral ischemia with brain damage as in heatstroke. The heating-induced contraction is not mediated by a neurogenic process but is due to altered transcellular Ca^{2+} transport. Cooling, in particular of the neck area, therefore, should be used in the treatment of heatstroke.

heating-induced contraction; cerebral ischemia; electric field stimulation; rabbit carotid artery

WHOLE BODY HYPERTHERMIA, such as in heatstroke, is a distinctive pathological condition with significant impact on tissue metabolism and organ functions (18). Heat stroke is associated with cerebral ischemia, and its onset is arbitrarily defined as the beginning of the decline of cerebral blood flow from its peak level (19).

It is well known that there are casualties related to high environmental temperature, amphetamine-related hyperpyrexia, and malignant hyperpyrexia and deaths of elderly incapacitated individuals during heat waves (18). In an epidemiological study during heat waves in urban areas in the United States, the incidence of heatstroke varied from 17.6 to 26.5 cases per 100,000 population, whereas in Saudi Arabia the incidence varies seasonally, from 22 to 250 cases per 100,000 population, and the mortality rate is 50% (1). Hyperthermia and central nervous system dysfunction must be present for a diagnosis of heatstroke. The core temperature may range from 40°C to 47°C (1).

The pathological findings in a person who dies of hyperthermia usually are local hemorrhages and parenchymatous degeneration throughout the body but especially with irreversible cell damage in the brain (1, 6).

Changes of heat balance frequently occur in cardiac surgery, and virtually all such patients are uniquely vulnerable (2). Many are at risk for cerebral ischemia because of vascular disease and embolization. The situation deserves considerable attention, because it has wide consequences. Hyperthermia must be avoided, because there is a relation between postoperative hyperthermia and cognitive dysfunction after cardiac surgery (7).

The threat of heatstroke is increasing. Global warming is already causing heat waves in temperate climates (4, 15–17). The recognition of thermoregulatory failure makes research in this direction a priority.

On a comparative scale, large supply arteries to the brain play a surprisingly large role in regulation of cerebral blood flow, a moderate role in the coronary circulation, and a small role in the mesenteric and skeletal circulation (5). Therefore, constriction and dilatation of large cerebral supply arteries contribute to the regulation of cerebral vascular resistance and, perhaps most importantly, cerebral microvascular pressure and perfusion.

Recently, we showed that cooling the carotid artery is a powerful vasodilator (12). In the present study, we investigate the direct effect of temperature elevation on carotid artery smooth muscle preparations to determine whether heating has the opposite effect and might trigger vasoconstriction and, therefore, be a causative factor in heatstroke.

Hyperthermia is a condition with clinical implications of cerebral damage and offers insights into a key question of thermal biology. However, no study has examined the direct effects of heating on the carotid artery, which provides the main arterial blood supply to the brain. Therefore, the present study was undertaken to investigate the direct effect of heating on carotid artery smooth muscle preparations.

MATERIALS AND METHODS

Experiments were performed in accordance with guidelines approved by the Institutional Animal Care and Use Committee. Adult male New Zealand White rabbits weighing 2–2.5 kg were anesthetized with pentobarbital sodium (120 mg/kg ip). The carotid arteries were immediately removed and placed in Krebs solution with the following composition (in mM): 118 NaCl, 5.9 KCl, 1.2 MgSO_4 , 2.2 CaCl_2 , 1.2 KH_2PO_4 , 26 NaHCO_3 , and 11.1 glucose, pH 7.4. Connective tissue and fat were carefully removed. The arteries were cut into 5-mm-long ring segments. These segments were mounted on triangu-

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lar wire supports and suspended in 10-ml organ baths containing Krebs solution, maintained at 37°C, and gassed with 95% O₂-5% CO₂. Tension was continuously recorded using a computerized, automated isometric transducer system (Schuler organ bath 809, Hugo Sachs Elektronik) connected to a Gould recorder. The segments were initially loaded to the optimum tension of 2 g, which previously had been determined to allow a wide range of contractile and relaxant responses. The specimens were allowed to equilibrate 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 addition of 1 μM acetylcholine, which resulted in (≥75%) relaxation of rings precontracted with 1 μM norepinephrine. At the end of each experiment, the muscle was dried with a filter paper and weighed. Contractile responses were calculated as milligrams per milligram tissue weight.

Heating protocol. The organ bath temperature was increased by an attached thermostated supply bath (Haake F3, Fisons); 1–2 min were required to reach the desired temperature from 37°C to 47°C in 2°C increments (i.e., 37, 39, 41, 43, 45, and 47°C). Each heating period was maintained until a peak response had leveled off before further temperature elevation.

Electrical field stimulation. Tissues were suspended between two platinum plate electrodes. The electrodes were connected to a Grass S8800 stimulator, which delivered square-wave pulses. Optimum electrical stimulation parameters, previously determined (70 V, 0.5 ms), were used for 15 s at frequencies of 2.5–50 Hz.

Drugs. Norepinephrine hydrochloride (NE), acetylcholine hydrochloride (ACh), phentolamine hydrochloride, EGTA, and tetrodotoxin (TTX) were obtained from Sigma Chemical (St. Louis, MO). NE, ACh, phentolamine hydrochloride, and TTX were dissolved in distilled water; EGTA was dissolved in 0.1 NaOH.

Calculations. Values are means ± SE 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 was followed by Student-Newman-Keuls test. The difference was assumed to be significant at *P* < 0.05.

RESULTS

Heating-induced contraction. Before heating, all preparations maintained a stable baseline. Elevation of the organ bath temperature from 37°C to 39, 41, 43, 45, or 47°C induced a rapid and reproducible stepwise increase in tension that was proportional to the bath temperature. A typical original trace represents heating-induced stepwise contraction of a rabbit carotid artery specimen from basal tone. When temperature was reset to 37°C, the tone rapidly returned to basal level (Fig. 1). A temperature-response curve clearly demonstrates that the maximum tension was achieved at 47°C (Fig. 2).

Fig. 1. Original recording of stepwise graded heating and recooling at 37°C of an isolated carotid artery smooth muscle strip from the rabbit at basal tone.

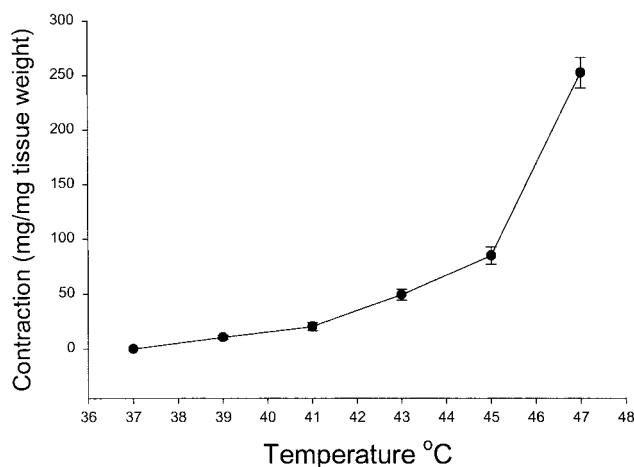
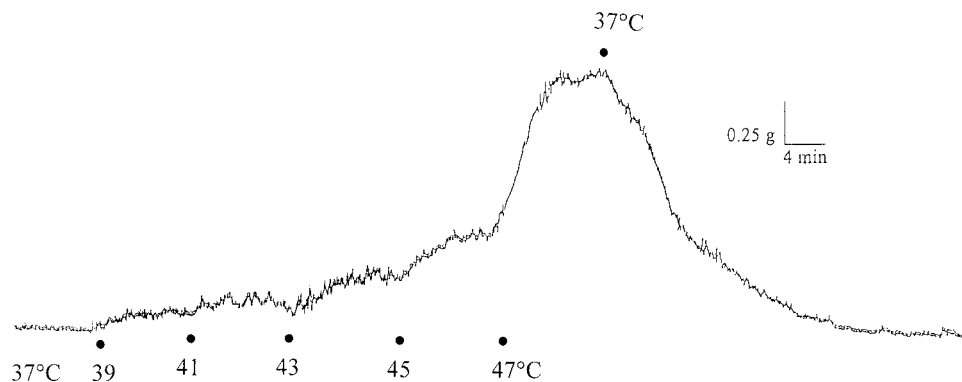


Fig. 2. Effect of heating from basal tone in isolated carotid artery smooth muscle segments of the rabbit. Values are means ± SE of 6 experiments.

Heating and vasoconstrictor drugs. NE (1 nM–100 μM) and KCl (10–50 mM) induced concentration-dependent contractions. Dose-response curves for NE and KCl were obtained at 37, 41, and 43°C. Heating to 43°C significantly inhibited NE-induced contractions but enhanced KCl-induced contractions (Fig. 3). Incubation of preparations in Ca²⁺-free, 2 mM EGTA-containing Krebs solution for 30 min did not change basal tension but almost abolished the contractile responses to KCl and slightly decreased NE-induced contractions.

Nervous control. Electrical field stimulation (EFS) at 37°C (70 V, 2.5–50 Hz) evoked frequency-dependent contractions that were rapid in onset. The contractions were neurogenic and α-adrenergic in origin, because they were abolished by TTX and phentolamine. Incubation of the carotid artery in a Ca²⁺-free, 2 mM EGTA-containing Krebs solution for 30 min did not change basal tension but abolished the responses to EFS, clarifying that these contractions are dependent on extracellular Ca²⁺. The contractile responses to heating were not inhibited by pretreatment with the neuronal blocker TTX (1 μM) and, therefore, were not elicited by a nervous mechanism.

Heating and EFS. Elevating the bath temperature resulted in a distinctly elevated level of contraction. While the temperature of the organ bath was maintained, EFS induced frequency-dependent contractions, but these responses were smaller in amplitude (Fig. 4). Therefore, hyperthermia reduces the effect of vasoconstriction by sympathetic activation, and the reduction was proportional to the temperature.

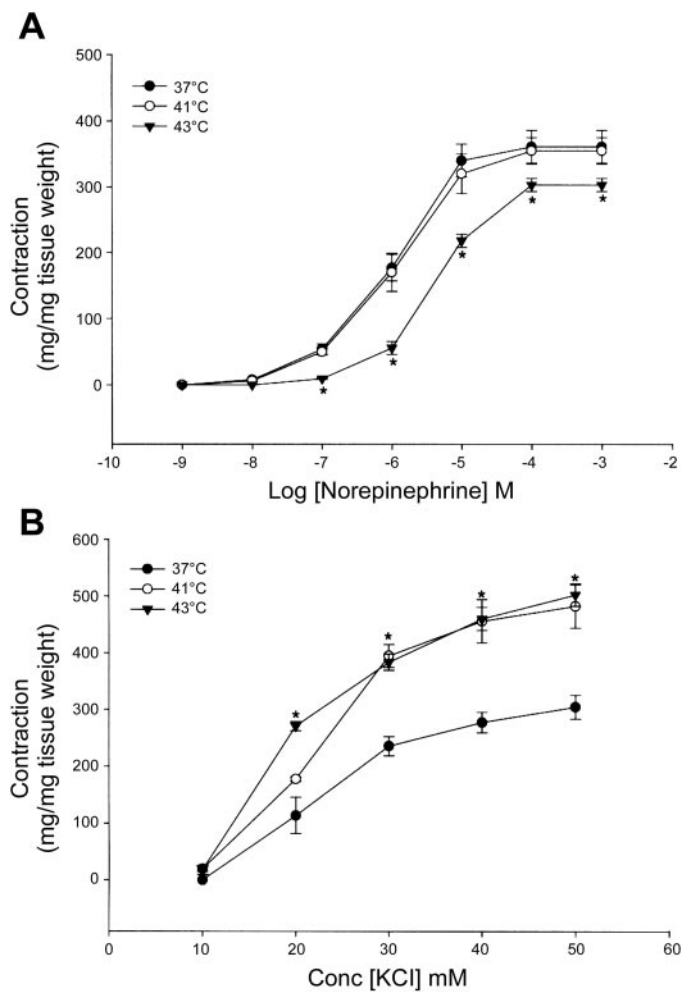


Fig. 3. Influence of heating on norepinephrine (A) and KCl (B) in isolated carotid artery smooth muscle segments. Values are means \pm SE of 5 experiments. Conc, concentration. * $P < 0.05$.

DISCUSSION

The present investigation clearly shows that hyperthermia induces vasoconstriction of the carotid artery of the rabbit by smooth muscle contraction. The degree of heating-induced contraction was proportional to temperature.

These results are clearly opposite to the responses obtained by cooling, which resulted in vasodilatation of the carotid, aortic, and pulmonary arteries as shown by us previously (12, 13). As shown in Fig. 5 quantitatively, on a milligram-per-milligram tissue basis, heating elicits a much more pronounced smooth muscle response than cooling. A heating-induced change of smooth muscle tone at a temperature change of 10°C is nearly five times as powerful as a cooling-induced change.

To the recent extensive review from Saudi Arabia about the importance of a multitude of humoral factors in heatstroke (1), we can add heating-induced arterial vasoconstriction as a component to induce cerebral ischemia. Therefore, neck cooling with a cooling collar should be considered a promising therapeutic strategy for heatstroke patients, in that it eliminates vasoconstriction and offers an additional vasodilatation by cooling cerebral supply vessels (12).

To clarify some aspects of the cellular mechanism of heating-induced contraction, we investigated the effect of the

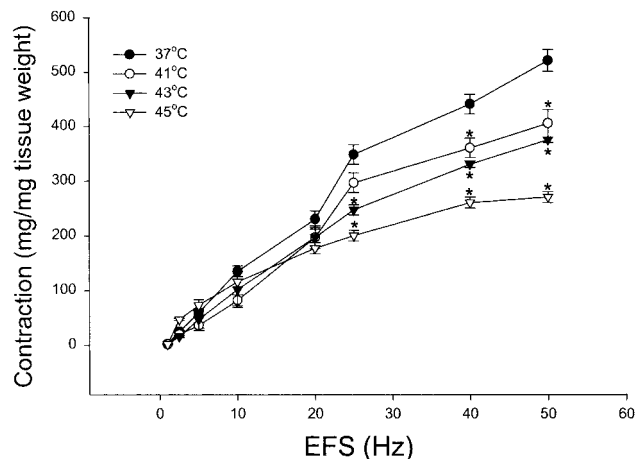


Fig. 4. Influence of heating on frequency-contraction responses to electrical field stimulation (EFS; at 70 V, 0.5 ms, 15 s) of isolated carotid artery smooth muscle segments at 37, 41, 43, and 45°C. Values are means \pm SE of 4 experiments. * $P < 0.05$.

vasoconstrictor agents NE and KCl. Heating potentiated the response to KCl with increasing temperatures; it had the opposite effect on NE-induced contraction and EFS. The reason for this obviously is that NE and KCl have different cellular mechanisms of action: NE acts through a temperature-sensitive mechanism involving phospholipase C, catalyzing the formation of inositol trisphosphate, which leads to increased intracellular Ca^{2+} ; KCl simply depolarizes the muscle cell, permitting extracellular Ca^{2+} influx through voltage-dependent Ca^{2+} channels. Therefore, the action of NE depends mainly on release of intracellular Ca^{2+} and that of KCl on influx of extracellular Ca^{2+} , one being reduced and the other enhanced (3, 14).

Previously we were able to show that carotid artery smooth muscle is innervated by α -adrenoceptors, which can be blocked by phentolamine (12). In the present study, we also were able to clarify the effect of temperature elevation on sympathetic vasoconstrictor tone induced by EFS, mediated by adrenergic nerves releasing NE, the transmitter on α -adrenoceptors. The contractile responses to EFS and NE were progressively reduced with increasing temperature.

Seemingly conflicting evidence was the finding that hyperthermia increased discharge of lumbar, renal, and splanchnic sympathetic nerves (9). We do not know how hyperthermia influences cervical sympathetic outflow, but we can conclude

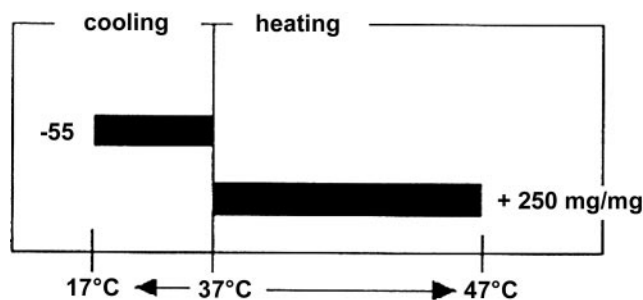


Fig. 5. Schematic representation of the effect of heating and cooling on carotid artery smooth muscle tone. Change of smooth muscle tone is shown in milligrams tension per milligram tissue in response to change of bath temperature from 37°C to 17°C (cooling) and from 37°C to 47°C (heating).

that heating seems to disrupt adrenoceptor function in the carotid artery but does not alter the intrinsic ability of vascular smooth muscle to contract, because the response to KCl was increased by heating, a finding corroborated by Massett et al. (10, 11), who demonstrated that a temperature elevation to 43°C (severe hyperthermia) produced graded contractions in vascular ring segments from rat mesenteric arteries and thoracic aortae. In untreated rings, these contractions were relatively small, whereas hyperthermia elicited near-maximal increases in tension when rings were constricted with phenylephrine or KCl before they were heated (10, 11).

It is well known that activation of the sympathetic nervous system, with liberation of epinephrine and NE, increases the metabolic rate at which the tissues produce a large amount of heat. Therefore, in our study showing that heating inhibited the release, the effect of sympathetic stimulation may be considered a protective feedback mechanism (8).

The membrane potential of arterial smooth muscle cells is controlled by K⁺ channels, a temperature-dependent process and an important regulator of smooth muscle tone and, hence, arterial diameter. We have evidence that the mechanism of heating-induced contraction is mediated by the inhibition of K⁺ channels.

The present investigation is the first to show that heating carotid artery preparations induced a reversible graded vasoconstriction proportional to the temperature, which, during hyperthermia in vivo, may lead to a decrease in cerebral blood flow, leading to cerebral ischemia with damage of brain tissue in heatstroke patients.

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