Cooling-Induced Carotid Artery Dilatation
An Experimental Study in Isolated Vessels

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Background and Purpose—Clinical and experimental studies seem to indicate that hypothermia may improve outcome in stroke victims and reduce experimental brain injury. The current interpretation is that cooling has a neuroprotective effect by reducing brain metabolism. The objective of our study was to test the hypothesis that hypothermia induces arterial vasodilatation and thereby increases cerebral blood flow.

Methods—We recorded isometric tension in rabbit carotid artery strips in organ baths during stepwise cooling. The cooling responses were tested at basal tone, in noradrenaline-precontracted vessels, and after electric field stimulation.

Results—Stepwise cooling from 37°C to 4°C induced reproducible graded relaxation, inversely proportional to temperature. The responses could be elicited at basal tone and in precontracted vessels. Cooling decreased the contractile responses to norepinephrine and potassium chloride. Cooling at 20°C decreased the contractile responses to electric field stimulation, while at 10°C these were totally abolished. Cooling-induced vasodilatation is not dependent on an endothelial mechanism.

Conclusions—Cooling of carotid artery preparations induced a reversible graded vasodilatation and decreased or abolished the effect of vasocontractile neurotransmitters. The effect of local hypothermia could increase cerebral blood flow and may constitute a positive therapeutic modality in stroke patients. (Stroke. 2002;33:256-260.)

Key Words: carotid arteries ■ hypothermia ■ ischemia ■ neuroprotection ■ stroke

We previously studied thermal responses of smooth muscle in various species and organs of the body and found that the degree of active tension is very temperature dependent. Moreover, we detected marked regional differences in the cooling-response pattern in superficial and deep blood vessels, trachea, bronchi, urinary bladder detrusor muscle, and gut. Cutaneous veins contract, whereas deep extremity vessels dilate.1 The aorta and pulmonary artery clearly show a cooling-induced relaxation,2 whereas airways,3 urinary bladder,4 and gut contract.5

As a treatment, hypothermia has been advocated as a potent modality in protecting neurons against lethal ischemic stress. In animal experiments, intraschismic hypothermia has been shown to be neuroprotective in global6 and focal7–9 cerebral ischemia and even when hypothermia is initiated after temporary cerebral ischemia.10 Kuluz et al11 found that selective brain cooling increases cortical cerebral blood flow.

It has been claimed that mild hypothermia is possibly the single most effective method of cerebroprotection developed to date.12 Krieger et al13 recently reported that hypothermia is effective in patients with acute brain infarction, demonstrating that hypothermia appears feasible and safe in cases of acute ischemic stroke even after thrombolysis. However, many questions regarding hypothermia remain to be addressed, and recently some clinical studies have questioned the effectiveness of hypothermia for traumatic brain injury.14 In view of these considerations and the positive reports about hypothermia treatment in stroke victims,15 we decided to extend our studies to include the carotid artery to test the hypothesis of a cooling-induced vasodilatation of brain supply vessels.

Materials and Methods

All procedures followed were within institutional guidelines. Adult male New Zealand White rabbits weighing 2.5 to 3.5 kg were anesthetized with pentobarbital (120 mg/kg IP). The carotid arteries were removed and dissected free of extraneous fat and connective tissue and placed in Krebs’ solution of the following composition (mmol/L): NaCl 118, KCl 5.9, MgSO4 1.2, CaCl2 2.2, KH2PO4 1.2, NaHCO3 26, glucose 11.1, pH 7.4. The arteries were cut into 5-mm ring segments that were mounted on triangular wire supports and suspended in 10-mL organ baths containing Krebs’ solution, maintained at 37°C, and gassed with 95% O2 and 5% CO2. Tension was continuously recorded with the use of a computerized, automated isometric transducer system (Schuler organ bath 809; Hugo Sachs Electronik) connected to a Gould recorder. The segments were initially loaded to a tension of 2 g and allowed to equilibrate for 60 minutes, 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 acetycholine (1 μmol/L), which resulted in relaxation of norepinephrine (1 μmol/L)–precontracted rings. At the end of each experiment the muscle was weighed.

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and responses were calculated as milligram per milligram tissue weight or percent change from norepinephrine-induced condition.

**Cooling Protocol**

The organ bath temperature was reduced with the use of a cooling circulator bath (Haake F3, Fisons), which had been set to the appropriate temperature. It took 2 to 3 minutes to reach the desired temperature in steps from 37°C to 4°C (37°C, 30°C, 25°C, 20°C, 15°C, 10°C, 4°C). Each cooling period was maintained until a peak response had leveled off before the temperature was reduced further.

**Electric Field Stimulation**

For electric field stimulation (EFS), tissues were suspended between 2 platinum plate electrodes. The electrodes were connected to a Grass S8800 stimulator, delivering square wave pulses. Previously determined optimum electric stimulation parameters (70 V, 0.5 ms for 15 seconds with a frequency ranging from 2.5 to 40 Hz) were used.

**Drugs**

Norepinephrine hydrochloride, acetylcholine hydrochloride, sodium nitroprusside, phentolamine hydrochloride, cocaine, propranolol hydrochloride, EGTA, and tetrodotoxin were obtained from Sigma Chemical Co. All drugs were dissolved in distilled water except EGTA, which was dissolved in 0.1 NaOH.

**Statistical Analysis**

Data are presented as mean (SEM). When necessary, differences between 2 mean values were compared by Student’s t test (paired or unpaired as appropriate). When multiple comparisons were necessary, 1-way ANOVA was used, followed by Student-Newman-Keuls test. The difference was assumed to be significant at \( P < 0.05 \).

**Results**

**Cooling-Induced Relaxation**

A typical original tracing representing cooling-induced relaxation of rabbit carotid artery specimens from basal tone and after induction of precontraction with 1 \( \mu \text{mol/L} \) norepinephrine is shown in Figure 1. Before cooling, all preparations maintained a stable baseline. Lowering of the bath temperature induced a rapid and reproducible stepwise decrease in tone from either the basal level or norepinephrine-induced tone, inversely proportional to the temperature. Maximum relaxation was achieved at a temperature of 4°C (Figure 2). When the temperature was reset to 37°C, the tone rapidly returned to the basal level.

Cooling to 15°C relaxed the norepinephrine-induced contraction to the basal level as sodium nitroprusside and acetylcholine in the presence of an intact endothelium, but cooling also relaxed carotid artery strips without endothelium.

**Cooling and Vasoconstrictor Drugs**

Norepinephrine (1 nmol/L to 100 \( \mu \text{mol/L} \)) and KCl (3 to 24 mmol/L) induced concentration-dependent contractions. Dose-response curves for norepinephrine and KCl were obtained at 37°C, 20°C, and 10°C. Cooling to 20°C significantly inhibited norepinephrine- and KCl-induced contractions, and a further reduction of temperature to 10°C abolished contractile responses (Figure 3). Cooling also lengthened the time to the peak tension and doubled the time required to obtain a dose-response curve for both norepinephrine and KCl.

The incubation of carotid artery in calcium-free, EGTA (0.2 mM)-containing Krebs’ solution for 30 minutes did not change the basal tension, did not change the level of basal tone, and did not affect the responses to norepinephrine, but it abolished the contractile response to KCl.
EFS at 37°C

EFS (2.5 to 40 Hz) evoked frequency-dependent contractions that were rapid in onset (Figure 4). The contractions were reproducible, and there was no evidence of tachyphylaxis in any of the preparations tested. Tetrodotoxin (1 μmol/L; n=8; data not shown) abolished these contractions, indicating that they were neurogenic. The contractions were also abolished by phentolamine (1 μmol/L) in all cases, indicating that the excitatory innervation of carotid artery is adrenergic in origin. Propranolol (1 μmol/L) did not increase the responses to EFS, indicating the absence of β-adrenergic receptors in the rabbit carotid artery.

Cocaine (3 μmol/L) inhibited the responses to EFS and also lengthened the time required to return to basal tone after stimulation ceased. Contraction started after 10 seconds of stimulation. This response to cocaine indicates that the reuptake-1 is important and that the artery is highly innervated.

Incubation of the carotid artery in a calcium-free, EGTA (0.2 mM)-containing Krebs’ solution for 30 minutes did not change the basal tension but decreased the responses to EFS. The contraction started after 5 seconds of stimulation while using calcium-free, EGTA (2 mM)-containing Krebs’ solution abolished the responses to EFS.

Cooling and EFS

Reducing the bath temperature from 37°C to 20°C resulted in relaxation. When the temperature was maintained at 20°C, EFS induced frequency-dependent contractions of the carotid artery preparations. However, these responses were slower in onset and smaller in amplitude. The contraction started after 10 seconds of stimulation. Further cooling to 10°C induced more pronounced relaxation of basal tone, and EFS no longer elicited any contractile response. Thus, hypothermia reduced the effect of vasoconstriction by sympathetic activation.

Discussion

The present investigation clearly shows that hypothermia induces vasodilatation of the carotid artery of rabbits by smooth muscle relaxation. The degree of cooling-induced relaxation of isolated rabbit carotid artery preparations is inversely proportional to temperature. These results confirm our earlier findings showing cooling-induced relaxation obtained in deep extremity arteries of sheep1 and the aorta and pulmonary artery of rats.2

To establish the cellular mechanism of cooling-induced relaxation, we studied the effect of the vasoconstrictor agents norepinephrine and KCl. Both of these agents induced contraction that was extensively reduced with cooling. Norepinephrine and KCl have a different cellular mechanism of action: norepinephrine acts through phospholipase C, releasing inositol 1,4,5-triphosphate (IP₃), which leads to increased intracellular Ca²⁺, while KCl depolarizes the muscle cell, permitting extracellular Ca²⁺ influx through voltage-dependent Ca²⁺ channels. Therefore, the action of norepinephrine depends on intracellular Ca²⁺, and that of KCl depends...
on entry of extracellular Ca$^{2+}$; therefore, the use of a Ca-free solution did not affect the contraction to norepinephrine. In a previous study, we showed that hypothermia decreased the influx of Ca$^{2+}$ into the smooth muscle cell$^{12}$; therefore, cooling had a more pronounced effect on KCl than norepinephrine and EFS.

We also studied the neurogenic consequences of hypothermia by monitoring the effect of sympathetic vasoconstrictor tone induced by EFS of the carotid artery strips. The contractile response to EFS was progressively reduced with decreasing temperature and was totally abolished at 10°C. This means that the release of norepinephrine is temperature dependent and is reduced by hypothermia. In addition to the action of hypothermia on neurogenic adrenergic vasoconstrictor tone, cooling slows all energy-requiring metabolic processes and therefore the process of contraction itself at any level of activation.

While cooling lowers pH of the physiological salt solution, the change in pH of Krebs’ solution over the temperature range of 37°C to 4°C was approximately 0.15 U. It is therefore highly unlikely that this change in pH had any effect on the experimental results. This finding is similar to other reports, indicating that the temperature-induced change in pH cannot account for either the relaxation or contraction responses to cooling.$^{2,18}$

Both intracellular alkalization- and acidosis-induced increases in intracellular Ca$^{2+}$ were mediated by both extracellular Ca$^{2+}$ influx and release of stored intracellular Ca$^{2+}$, leading to contraction in isolated canine and ferret pulmonary arterial smooth muscle$^{19,20}$ and rat isolated thoracic aorta.$^{21}$ Our previous study$^2$ showed that neither Ca$^{2+}$-free, EGTA (2 mmol/L)—containing Krebs’ solution nor nifedipine and thapsigargin affected cooling-induced relaxation, indicating that cooling-induced relaxation is not affected by the change in intracellular pH.

In contrast to our present findings of cooling-induced carotid artery relaxation, in previous studies we reported totally divergent responses in other smooth muscle–lined organs. In these organs hypothermia induced a clear-cut increase in basal tone and in some organs induced an increase in the frequency of rhythmic contractions. Ovine airways,$^3$ rat bladder detrusor muscle,$^4$ and rat gastrointestinal smooth muscle$^5$ all contracted when exposed to cooling, contrary to the observed effect in the carotid artery. In these organs, however, pattern of innervation, type of transmitters, release process, and inactivation are different.

Therefore, the basic mechanisms underlying hypothemic reactions of smooth muscle in blood vessels and other conduits of the body are regionally different and adjusted to serve the functional requirements of the organism when exposed to hypothermia. From the functional point of view, it is reasonable to assume that survival during hypothermia requires adjustments in which the priority of organs must be considered. The circulation of blood should be directed toward the central nervous system and the heart, whereas other areas with less demand may be temporarily shut off. A balance of vasoconstriction to prevent heat loss and vasodilatation to secure supply to vital organs can be expected.

From the clinical point of view, our experiments support the view that moderate hypothermia may be useful for maintaining and, if necessary, increasing blood supply to ischemic regions compromised by reduced arterial perfusion. Despite recent reports on the ineffectiveness of generalized hypothermia in the treatment of acute brain injury,$^{17}$ our study may point to an alternative solution: instead of generalized hypothermia, local cooling of the arterial supply vessels to the brain (in the neck) may be considered a new possible therapeutic alternative.

The feasibility of induction of carotid artery cooling (of the arterial wall) and clinical use of cooling-induced relaxation as a therapeutic modality needs to be carefully investigated in further controlled studies. This can be done by monitoring carotid artery flow velocity and diameter with ultrasound as well as measuring tympanic temperature. Administration of graded cooling can be achieved by the external application of an ice-water–perfused neck collar. We are presently performing this phase of research.

Our study is the first to suggest carotid artery cooling as a therapeutic procedure. Previous attempts have used venous cooling of facial veins and dural sinuses,$^{22}$ but our method seems to be more straightforward and is aimed at inducing carotid artery vasodilatation as well as cooling of blood directed to the brain.

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References


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