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Postischemic Attenuation of Cerebral Artery Reactivity Is Increased in the Presence of Tissue Plasminogen Activator

Marilyn J. Cipolla, PhD; Nikola Lessov, MD, PhD; Wayne M. Clark, MD

Background and Purpose—We investigated the combined effect of tissue plasminogen activator and ischemia on middle cerebral artery (MCA) reactivity to determine whether abnormal MCA function after 2 hours of ischemia was worse in arteries perfused with recombinant tissue plasminogen activator (rtPA).

Methods—The intraluminal suture model of focal cerebral ischemia was used to induce 2 hours of ischemia in rats, after which occluded MCAs were removed and studied in vitro with an arteriograph system that allowed control of transmural pressure (TMP) and measurement of lumen diameter. Arteries were either nonischemic (control; n=8), nonischemic and perfused with 400 μg/mL rtPA (rtPA; n=5), ischemic (ISC; n=6), or ischemic and perfused with 400 μg/mL rtPA (ISC-rtPA; n=6). After a 1-hour equilibration at 75 mm Hg, TMP was increased to 125 mm Hg and lumen diameter was recorded at each pressure. Reactivity to acetylcholine (ACh, 0.1 to 10.0 μmol/L) and serotonin (0.01 to 10 μmol/L) was then determined.

Results—Control arteries responded myogenically to pressure and increased the amount of tone from 18.5±3.8% at 75 mm Hg to 24.8±3.0% at 125 mm Hg (P<0.05), which decreased diameter from 241±7 to 232±6 μm. In contrast, all other groups decreased tone at 125 mm Hg, which demonstrated a loss of myogenicity. The percent tone in each group at 75 versus 125 mm Hg was rtPA, 16.0±4.5% versus 11.8±3.8%; ISC, 23.5±4.5% versus 13.5±3.1%; and ISC-rtPA, 23.5±4.2% versus 12.3±3.2% (P<0.05 for all). The percent increase in lumen diameter at each concentration of ACh was diminished in all groups compared with control; ISC-rtPA arteries responded the least, which suggests an additive effect of rtPA in ischemic arteries. The percent increase in lumen diameter at 10⁻³mol/L ACh was 23±4% for control versus 15±2% for rtPA; 17±3% for ISC arteries (P<0.05), and 8±2% for ISC-rtPA arteries (P<0.01). Sensitivity to serotonin was equally diminished in all groups compared with control: EC₅₀ (μmol/L) was 0.06±0.01 for control, 0.17±0.02 for rtPA, 0.22±0.07 for ISC, and 0.16±0.04 for ISC-rtPA (P<0.05).

Conclusions—These results demonstrate that both ischemia and rtPA perfusion diminish cerebral artery reactivity and that the combination may produce an additive effect. This impaired reactivity may contribute to reperfusion-induced injury during or after thrombolysis by altering upstream cerebrovascular resistance. (Stroke. 2000;31:940-945.)

Key Words: arterial wall • ischemia • middle cerebral artery • tissue plasminogen activator

Thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) is a promising treatment for acute ischemic stroke. However, complications associated with intravenous rtPA exposure include symptomatic intracerebral hemorrhage and edema formation after recanalization.1–6 Plasminogen activators have direct effects on vascular tissues, which include altering platelet plug framework, vascular permeability, and vascular integrity at sites of injury that may accelerate disruption of the blood-brain barrier (BBB) and dissolution of the vascular basal laminae.3,7 Therefore, hemorrhagic complications associated with rtPA may be due to a direct effect of this compound on the structure or function of the cerebral circulation.

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Although the complications associated with thrombolysis as a treatment for ischemic stroke are not completely understood, more is known about the effects of thrombolysis on the cerebral microvessels than on other vascular components. The microcirculation is the downstream target of large-artery occlusion and is thought to be central to ischemic brain injury through changes in microvascular permeability and integrity.8,9 However, the cerebral circulation is a unique vascular bed in that the large extracranial vessels and intracranial pial vessels account for approximately half of total cerebrovascular resistance (CVR),10 which serves to protect downstream microvessels as perfusion pressure is increased.11,12 Ineffec-
tive diameter regulation (eg, myogenic reactivity) of these large arteries under pathological conditions such as ischemia or in the presence of a thrombolytic agent could promote hemorrhage and edema as a result of the loss of upstream vascular resistance and increased pressure on the microcirculation.\textsuperscript{13,14} In addition, uncontrolled postischemic perfusion in the presence of a thrombolytic agent could be detrimental under these anti-thrombotic conditions.

The middle cerebral artery (MCA) is the most commonly affected artery in clinical ischemic stroke, the occlusion of which produces a large infarction.\textsuperscript{15,16} Due to its involvement in stroke, this artery is frequently targeted for thrombolytic therapy with rtPA. In previous studies, we found that under normal, nonischemic conditions the MCA possesses considerable tone and responds myogenically to changes in intravascular pressure.\textsuperscript{17} However, this myogenic response was diminished after 2 hours of ischemia and 24 hours of reperfusion.\textsuperscript{17} In the present study, we hypothesized that the effects of ischemia on MCA reactivity worsen in the presence of rtPA. To test this hypothesis, the intraluminal suture model of focal cerebral ischemia was used to induce 2 hours of ischemia, after which the occluded MCA was isolated and studied using in vitro arteriograph methodology that allowed for perfusion of rtPA, control of intravascular pressure, and continuous measurement of lumen diameter.

Subjects and Methods

Rat MCA Occlusion Model

All procedures were approved by the Institutional Animal Care Committee at Oregon Health Sciences University. The focal cerebral ischemia model that we used was a modification of the MCA occlusion model described by Zea Longa et al\textsuperscript{18} and described in detail elsewhere.\textsuperscript{17,19} Male Wistar rats were anesthetized by spontaneous inhalation through a mask of 1.5% halothane in a 70% nitrous oxide/30% oxygen mixture. The right common carotid artery was isolated and coagulated. A 4-0 nylon suture coated with silicon was inserted into the internal carotid artery through the external carotid artery stump and advanced to the anterior cerebral artery, thus occluding the segment most proximal to the circle of Willis contained 6 to 7 collaterals followed distally by a branch-free segment. This branch-free segment of the MCA was consistently used for experiments because vessels with collaterals will leak when pressurized and this

Preparation of Arterial Segments

The MCA from the occluded (right) side of the brain (or from control animals) was carefully dissected, cleared of extraneous connective tissue, and placed in the arteriography chamber. In preliminary studies, we found MCA architecture to be consistent among the rats; the segment most proximal to the circle of Willis contained 6 to 7 collaterals followed distally by a branch-free segment. This branch-free segment of the MCA was consistently used for experiments because vessels with collaterals will leak when pressurized and this identification provided a consistent segment for study. Dissected arteries were mounted on 2 glass microcannulas suspended above an optical window within the chamber, perfused with PSS, and secured with 2 strands of nylon thread (diameter, 10 μm) on both the proximal and distal cannulas. For these experiments, the distal cannula was closed off so that there was no flow through the vessels.

Pressurized Arteriograph System

The arteriograph system (Living Systems Instrumentation) consisted of a 20-mL chamber with inlet and outlet ports for suffusion of PSS and drugs from a 50-mL reservoir. PSS was continually recirculated and pumped through a heat exchanger to warm it to 37°C before it entered the arteriograph chamber and was aerated with a gas mixture of 5% CO:10% O:85% N; to maintain a constant pH of 7.4±0.05. Transmural pressure (TMP) was measured and controlled through a servo-system that consisted of an in-line pressure transducer, miniature peristaltic pump, and controller connected to the proximal cannula. The arteriograph chamber that contained the mounted arteries was placed on an inverted microscope with an attached videocamera and monitor to allow viewing and electronic measurement of lumen diameter. Lumen diameter was measured by the video scan line, which detects the optical contrast of the vessel walls on the video monitor and generates a voltage ramp within the video dimension analyzer which is proportional to diameter.\textsuperscript{20} The output of the video dimension analyzer and pressure controller was recorded on an IBM-compatible computer by means of a serial data-acquisition system (DATAQ) for visualization of dynamic responses of diameter and TMP, in a manner similar to a chart recorder.

Experimental Protocol

Arteries studied were either nonischemic control (CTL, n=8), nonischemic and perfused with 400 μg/mL rtPA (rtPA, n=5), ischemic for 2 hours (ISC, n=6), or ischemic for 2 hours and perfused with 400 μg/mL rtPA (ISC-rtPA, n=6); vessels from 5 animals could not be used for experimentation because of either collateral leaks or equipment difficulties. This concentration of rtPA was chosen because it approximates the dose given for intra-arterial thrombolysis. All arteries were subjected to the following protocol: after a 1-hour equilibration at 75 mm Hg, TMP was increased to 125 mm Hg and lumen diameter recorded. Pressure was returned to 75 mm Hg for the rest of the experiment. Reactivity to acetylcholine (ACh) was determined by commutative addition of ACh (0.1 to 10.0 μmol/L) to the arteriograph bath and measurement of lumen diameter at each concentration once the concentration was stable, after approximately 5 minutes. ACh was washed out of the bath, and serotonin (5-hydroxytryptamine [5HT]) was commutatively added (0.01 to 10 μmol/L). Diameter was recorded at each concentration of 5HT once the concentration was stable, after approximately 5 minutes. At the end of each experiment, a single concentration of papaverine (0.1 mmol/L) was added and a fully relaxed diameter recorded.

Drugs and Solutions

The perfusate and superfusate for all experiments consisted of a bicarbonate-based phosphate buffer (Ringer’s PSS), of the following ionic composition (in mmol/L): NaCl 119.0, NaHCO\textsubscript{3} 24.0, KCl 4.7, KH\textsubscript{2}PO\textsubscript{4} 1.18, MgSO\textsubscript{4} · 7H\textsubscript{2}O 1.17, CaCl\textsubscript{2} 1.6, EDTA 0.026, and glucose 5.5. PSS was made each week and stored without glucose at 4°C. Glucose was added to the PSS before each experiment. 5-HT, ACh, and papaverine were purchased from Sigma Chemical Co and made fresh daily as stock solutions of 10\textsuperscript{-3} and 10\textsuperscript{-4} mol/L. Papaverine was also purchased from Sigma and made fresh each week as a stock solution of 10\textsuperscript{-2} mol/L and stored at 4°C. Reombinant tPA (Activase) was a generous gift from Genentech, Inc, and was mixed fresh in PSS in the appropriate concentration and perfused through the MCA once the MCA was mounted in the arteriograph chamber. The rtPA was left in the perfusate for the entire experiment.

Data Calculations and Statistical Analysis

Spontaneous arterial tone was calculated as a percent decrease in diameter from the fully relaxed diameter in papaverine at each TMP by the following equation:

\[
\text{Spontaneous arterial tone} = \left(\frac{\text{Diameter at each concentration} - \text{Diameter in papaverine}}{\text{Diameter in papaverine}}\right) \times 100
\]
where \(d_{\text{tone}}\) indicates diameter of vessels with tone and \(d_{\text{pass}}\) diameter in papaverine. To assess the behavior of arteries in response to an increase in pressure from 75 to 125 mm Hg, the slope of the pressure-diameter curve was calculated. For 5-HT, the amount of agonist necessary to contract the arteries at 50% of maximum (EC50), was calculated for each artery by first plotting the concentration-response curves on a logarithmic scale and then extrapolating the value from a best-fit line between 20% and 80% contraction. Responses to ACh were assessed by calculating the percent change in diameter from baseline.

All results are expressed as ±SE. Differences in the slope of the pressure-diameter curve, amount of tone at each pressure, and reactivity to ACh and 5-HT were determined by use of 1-way ANOVA in 4 treatment groups. A post hoc Dunnett test was used to determine within-group variability, with the nonischemic CTL arteries as the control. Differences in the amount of tone at 75 versus 125 mm Hg within each group was determined by use of repeated-measures ANOVA. All differences were considered significant if \(P<0.05\).

### Results

#### Diameter Regulation in Response to Increased Pressure

All arteries developed spontaneous tone during equilibration at 75 mm Hg. When pressure was increased to 125 mm Hg, CTL arteries contracted to the increased pressure and decreased diameter. The mean slope of the pressure-diameter curve for CTL arteries was negative (−0.19±0.07), which demonstrated a myogenic response. In contrast, all other groups of arteries increased diameter in response to the increased pressure, which demonstrated a lack of myogenicity. Mean slope of the pressure-diameter curve for all other groups was positive: rtPA, 0.42±0.07; ISC, 0.60±0.14; and ISC-rtPA, 0.86±0.27 (\(P<0.05\) versus CTL for all). Diameters of arteries at 75 and 125 mm Hg are shown in the graph in Figure 1 and summarized in the Table. Diameters of arteries fully relaxed in papaverine were not different between any of the groups at either pressure, as shown in the Table.

#### Myogenic Tone at 75 and 125 mm Hg

The average amount of tone that arteries developed during equilibration at 75 mm Hg was not significantly different between groups, as shown in Figure 2 and summarized in the Table. Although both groups of ischemic arteries tended to have an increased average amount of tone over the nonischemic CTL arteries, these values were not statistically significant. When pressure was increased to 125 mm Hg, only CTL arteries contracted to the increased pressure and significantly increased tone (\(P<0.05\) versus at 75 mm Hg); all other groups had diminished tone at 125 mm Hg (\(P<0.05\) versus at 75 mm Hg).
75 mm Hg). The average amount of tone that the 3 experimental groups possessed at 125 mm Hg was not statistically significant from each other.

**Reactivity to ACh**

Addition of ACh to the arteriograph bath caused vasodilation of all arterial groups that was greatest in CTL arteries, as shown in Figure 3. CTL arteries dilated 23.5 ± 4% with the highest concentration of ACh (10^{-5} mol/L), compared with 15.2 ± 2% for rtPA arteries, and 17.4 ± 3% for ISC arteries (P < 0.05). ISC-rtPA arteries dilated the least with ACh compared with CTL vessels, only 8.7 ± 2% (P < 0.01), which suggests a possible negative synergistic effect of rtPA in ischemic arteries.

**Reactivity to 5HT**

Addition of 5HT to the arteriograph bath caused vasoconstriction in all arterial groups, as shown in Figure 4. All groups were similarly less sensitive to 5HT compared with CTL arteries. EC_{50} for CTL arteries was 0.06 ± 0.01 μmol/L versus 0.17 ± 0.02 μmol/L for rtPA, 0.22 ± 0.07 μmol/L for ISC, and 0.16 ± 0.04 μmol/L for ISC-rtPA arteries (P < 0.05 versus CTL for all).

**Discussion**

The considerable tone and myogenic reactivity of the MCA and other large cerebral arteries serves to protect downstream microvessels by providing appropriate vascular resistance and control of cerebral blood flow (CBF) during changes in perfusion pressures.\textsuperscript{11,12} This important function is demonstrated in the graph in Figure 1, which shows nonischemic CTL arteries contracted to an increase in pressure from 75 to 125 mm Hg, which produced a negative slope on the graph. The lack of a myogenic response produced significantly decreased tone at the higher pressure in all experimental groups. Because these myogenic mechanisms are the primary contributors to autoregulation of CBF and CVR,\textsuperscript{11,12} these results suggest that ischemia and rtPA treatment are associated with impaired autoregulatory function and diminished CVR.

Although myogenic tone in all groups was not significantly different at 75 mm Hg, a significant decrease in tone occurred in all experimental groups at the higher pressure compared with CTL arteries. This effect of ischemia and rtPA could be considered a beneficial means of increasing CBF to an ischemic region. However, uncontrolled perfusion, especially in the presence of a thrombolytic agent, may be detrimental and promote BBB disruption, edema formation, and possibly hemorrhage.\textsuperscript{1–3,13,14} Plasminogen activation by rtPA may increase hemorrhage and edema by affecting existing platelet plug framework and by altering vascular permeability and basal laminae integrity of the microcirculation.\textsuperscript{3,7} A loss of autoregulatory mechanisms (eg, myogenic tone and reactivity) under these conditions may further promote hemorrhage and edema formation by diminishing crucial CVR and protection of the microcirculation during changes in perfusion pressures.\textsuperscript{10–14}

Ischemia and rtPA perfusion have both been associated with fatal edema formation after reperfusion.\textsuperscript{1,21} Several studies have demonstrated that postischemic hyperemia is associated with vasodilatation and exacerbates edema formation.\textsuperscript{22,23} Kuroiwa et al\textsuperscript{22} showed that reactive hyperemia was associated with BBB opening and that suppression of postischemic hyperemia significantly reduced edema formation and the degree of BBB opening. In the present study, we found that both ischemia and rtPA perfusion of MCA diminished the myogenic behavior of these vessels, a result that appears consistent with vasodilatation in postischemic hyperemia.

The mechanism by which either ischemia or rtPA perfusion affects the myogenic properties of these arteries is not clear from the present study. However, in previous studies, we demonstrated that myogenic activity of cerebral arteries depends on the polymerization state of actin in vascular smooth muscle (VSM).\textsuperscript{24,25} It is possible that ischemia and...
rtPA act to diminish myogenic reactivity through a similar mechanism for several reasons. First, ischemia alone is known to have significant effects on the polymerization state of the actin cytoskeleton in many cell types, including endothelial, myocardial, and renal cells. In addition, ischemia has been shown to affect actin-binding proteins that control the state of polymerization. Second, preliminary studies showed that posterior cerebral arteries perfused with rtPA had diminished tone and decreased the pressure at which forced dilatation occurred. This result was remarkably similar to that of arteries in the presence of cytochalasin B, a compound known to inhibit actin polymerization. Because rtPA has been shown to directly bind actin, decreased myogenic activity in the presence of rtPA could be due to an effect of this compound on the dynamics of the actin cytoskeleton in VSM. Third, the effect of rtPA on myogenic reactivity appears to be greater in ischemic MCA, as demonstrated by the steepest slope of the pressure-diameter curve (Figure 1). This possible additive effect may be due to both ischemia and rtPA causing a certain amount of cytoskeletal damage that together is additive. Although this idea is speculative, we are currently investigating the possibility that ischemia and rtPA affect the dynamics of the actin cytoskeleton of VSM, a consequence that may underlie diminished myogenic activity.

Alternatively, the possible additive effect of rtPA perfusion on the diminished myogenic activity of ischemic arteries may be due to ischemic damage that exposes other rtPA binding sites that increase the proteolytic activity of rtPA, such as collagen or other extracellular matrix proteins. Our previous studies with transmission electron microscopy have shown significant structural damage to the arterial wall of MCAs that were ischemic, including areas of endothelial denudation and disruption of the internal elastic laminae. This ischemic damage could be an initial event that in the presence of rtPA is augmented as a result of its proteolytic activity. Although the half-life of rtPA is moderately short, approximately 3 to 5 minutes, it is probably long enough to do significant proteolytic damage to already ischemic arteries.

Ischemia and reperfusion have previously been shown to diminish ACh-induced vasodilatation of MCAs. In the present study, MCAs that were nonischemic and perfused with rtPA or that were ischemic without rtPA had similarly diminished reactivity to ACh compared with CTL arteries, as shown in the graph in Figure 3. However, the combination of ischemia and rtPA perfusion caused a possible decreased response over either ischemia or rtPA alone. Although these results suggest a possible negative synergistic effect, further studies with adequate power to allow for direct pairwise comparison with either exposure alone would be necessary to confirm this finding. In any case, this noted effect may be due to ischemic vessels that express different cell surface receptors such as leukocyte adhesion molecules to which rtPA can bind. Ischemia is known to upregulate endothelial adhesion molecules such as integrins, which rtPA may then bind to: this can cause an increase or alteration in binding and activity of rtPA. Although rtPA has stringent substrate specificity for plasminogen, a study by Ding et al demonstrated that small peptides can mimic determinants that mediate specific proteolysis of rtPA. Therefore, the effect of rtPA may be due to proteolytic damage to the ischemic endothelium, which could alter production and release of endothelium-dependent vasoreactive substances.

The present study also demonstrated a decreased contractile response to 5HT that was similar in all experimental groups. A diminished response to 5HT has been noted previously in MCAs that were ischemic and reperfused. In the present study, diminished 5HT reactivity was also demonstrated in nonischemic arteries perfused with rtPA. This may be due to an overall effect of rtPA on MCA contraction, because these arteries were less responsive to pressure as well (ie, diminished myogenic reactivity). Along these lines, high-affinity binding sites for rtPA have been found on VSM. Binding of rtPA to VSM receptors is thought to increase the functional activity of the protease activity of rtPA as well as induce proliferation. This suggests that rtPA binding may affect intracellular signal transduction pathways of VSM that also may cause a diminished contractile response.

In conclusion, we have demonstrated that both ischemia and rtPA have significant effects on MCA reactivity, which include diminished myogenic reactivity and response to both ACh and 5HT. Although the mechanism of these abnormalities is not clear, these results may underlie some of the detrimental effects of rtPA treatment by diminishing CVR and impairing autoregulation of CBF during reperfusion.

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References

The pathophysiological mechanisms underlying cerebral hemorrhage complicating acute ischemic stroke have interested clinicians and investigators alike for decades. With the introduction of thrombolytic therapy for the treatment of acute ischemic stroke, the interest has intensified, because one of the major complications of thrombolytic therapy is symptomatic intracranial hemorrhage. Efforts to identify potentially treatable or modifiable mechanisms are therefore justified in the hope that such research may lead to improvement in the risk-to-benefit ratio for treatment.

It seems likely that the causes for cerebral hemorrhage complicating thrombolytic therapy for stroke will be multiple. Most often, hemorrhage occurs in the volume of brain undergoing acute ischemia and may occur with or without recanalization of the occluded vessel. In about 1% of cases, hemorrhage occurs in regions of brain distant from the zone of ischemia. In the accompanying article, Cipolla and colleagues show that both ischemia and rtPA exposure alter cerebral artery reactivity and suggest that this may impair the cerebral circulation’s ability to respond to changes in perfusion pressure or other stimuli and thereby potentially lead to complications of either brain hemorrhage or cerebral edema. The data suggest, but do not prove, that the combination of rtPA and ischemia may be a more potent inhibitor than either complication alone. Whether this effect is necessarily detrimental in the setting of acute ischemia or reperfusion remains to be shown. Elucidation of the mechanisms whereby these effects are mediated, and whether they may be blocked pharmacologically, await further research.

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