Attenuation of Thrombin-Induced Brain Edema by Cerebral Thrombin Preconditioning

Guohua Xi, MD; Richard F. Keep, PhD; Ya Hua, MD; Jianming Xiang, MD; Julian T. Hoff, MD

Background and Purpose—Edema formation after intracerebral hemorrhage has been linked to thrombin toxicity induced by the clot. However, thrombin at low concentrations actually protects neurons and astrocytes in culture from hypoglycemic and ischemic cell death. It is also known that a brief episode of brain ischemia increases neuronal tolerance to a subsequent severe ischemic episode. The objective of this study was to investigate whether pretreatment of the brain with low-dose thrombin induces tolerance to a subsequent large dose of thrombin injected into brain parenchyma.

Methods—The rat brain was preconditioned with 1 U thrombin by direct infusion into the right caudate nucleus. After thrombin pretreatment, the effects of a large dose (5 U) of thrombin on brain edema formation were studied at different intervals. We examined whether heat-shock protein (HSP) 27, HSP32, and HSP70 were induced by Western blot analysis, immunocytochemistry, and immunofluorescent double staining.

Results—Thrombin pretreatment significantly attenuated the brain edema that normally follows the infusion of a large dose of thrombin (79.2 ± 0.4 versus 84.0 ± 0.3; P < 0.01). This effect was abolished by the thrombin inhibitor hirudin. Time course studies showed that the maximal effect of thrombin preconditioning (TPC) on brain edema formation was 7 days after pretreatment. This time course corresponded to marked upregulation of HSP27 in the ipsilateral brain. TPC also induced HSP32, but this effect occurred earlier than the effect on edema formation. TPC had no effect on HSP70. Immunocytochemistry and immunofluorescent double labeling showed that HSP27 and HSP32 were expressed in astrocytes after TPC.

Conclusions—The phenomenon of thrombin-induced tolerance of the brain to edema formation may be related to HSP27 induction. ([Stroke. 1999;30:1247-1255.])

Key Words: brain edema ■ cerebral hemorrhage ■ heat-shock proteins ■ thrombin

Brain edema formation after intracerebral hemorrhage (ICH) exacerbates brain injury. An important aspect of ICH management is prevention of edema formation since brain swelling may be more accountable for the poor neurological outcome after ICH than the hematoma mass itself. The coagulation cascade, especially thrombin formation, plays an important role in brain edema formation after ICH.1–4 Direct infusion of thrombin into the caudate nucleus causes inflammation, edema, reactive gliosis, and scar formation in the brain.1–3,5 However, recent studies indicate that low concentrations of thrombin may actually protect neurons and astrocytes from cell death induced by hypoglycemia and ischemia.6,7

The protective effect of ischemic preconditioning, whereby a brief ischemic episode can increase tolerance to subsequent severe ischemia, was first found in the myocardium.8 It is now clear that this ischemic preconditioning (or induced tolerance) phenomenon also occurs in the brain.9–12 The mechanisms of induced ischemic tolerance are not well understood, however. Some investigators have already dem-

See Editorial Comment, page 1255 onstrated that induction of heat-shock proteins (HSPs), including HSP27, HSP32, and HSP70, might be related to cellular protective mechanisms against ischemic and/or oxidative damage.13–16

In this study we examined whether the tolerance phenomenon occurs in the brain after ICH. We tested the effects of large-dose thrombin on brain edema formation after pretreating the brain with a single small dose of thrombin. The expression of HSPs related to thrombin preconditioning (TPC) was also investigated. A preliminary report of these results has been presented in abstract form.17

Materials and Methods
Animal Preparation and Intracerebral Infusion
The protocols for these animal studies were approved by the University of Michigan Committee on the Use and Care of Animals. A total of 91 male Sprague-Dawley rats (weight,
300 to 400 g; Charles River Laboratories) were used in the present study. The animals were anesthetized with pentobarbital (40 mg/kg IP). The right femoral artery was catheterized for continuous blood pressure monitoring and blood sampling. Blood was obtained from the catheter for analysis of blood pH, \( \text{Pao}_2 \), \( \text{Paco}_2 \), hematocrit, and blood glucose. Core temperature was maintained at 37.5°C with the use of a feedback-controlled heating pad. The rats were positioned in a stereotaxic frame (Kopf Instrument), and a cranial burr hole (1 mm) was drilled on the right coronal suture 4.0 mm lateral to the midline. Thrombin or saline (50 \( \mu \)L) was infused into the right caudate nucleus at a rate of 10 \( \mu \)L/min through a 26-gauge needle (coordinates: 0.2 mm anterior, 5.5 mm ventral, and 4.0 mm lateral to the bregma) with the use of a microinfusion pump (Harvard Apparatus Inc). The needle was removed, and the skin incision was closed with suture after infusion.

**Experimental Groups**

The study was divided into 5 parts. The first part tested the effect of intracerebral infusion of a small dose of thrombin on brain water content. In the second part, the effect of such a small dose of thrombin on subsequent edema formation from a large dose of thrombin was evaluated (TPC). The time course of TPC was examined in the third part. For these first 3 parts, the brain samples were used for water and ion contents measurement. In the fourth part, HSPs (HSP27, HSP32, HSP70) were quantified by Western blotting analysis. In this study, the rabbit anti-murine HSP25 polyclonal antibody (StressGen), which specifically recognizes rat HSP27, was used. Immunocytochemistry was performed in the fifth part for HSP27, HSP32, and HSP70, with immunofluorescent double labeling also used to identify the cell types that expressed HSP27 and HSP32. Although the brain water contents were measured by the same individual who performed the intracerebral infusion, Western blot analysis, immunocytochemistry, and immunofluorescent double labeling were performed by a “blinded” observer.

**Part 1**

Three groups of 5 rats each were examined. Rats of the first group received 50 \( \mu \)L saline infusion. Rats of the second and third groups received 1 \( \mu \)L rat thrombin (Sigma) in 50 \( \mu \)L saline. The animals were killed at 24 hours for the first and second groups and at 7 days for the third group to determine brain water and ion contents.

**Part 2**

Three groups of 5 rats each were used in this part. The first group had 50 \( \mu \)L saline infusion, the second group received 1 \( \mu \)L thrombin in 50 \( \mu \)L saline, and the third group received 1 \( \mu \)L thrombin plus 1 \( \mu \)L hirudin (Sigma) in 50 \( \mu \)L saline. Seven days after the first infusion, all rats in these 3 groups received a second infusion (5 \( \mu \)L thrombin in 50 \( \mu \)L saline). All rats in this part were decapitated 24 hours after the second infusion to determine brain water and ion contents.

**Part 3**

Five groups of 5 or 6 rats each were tested in this part. Rats in the first group had 5 \( \mu \)L thrombin only. It was reported that a 50-\( \mu \)L blood clot can produce \( \approx 8 \) to 10 \( \mu \)L thrombin.\(^2\) Rats in the second to fifth groups received 1 \( \mu \)L thrombin first, then received a second infusion (5 \( \mu \)L thrombin in 50 \( \mu \)L saline) at either 3, 7, 14, or 21 days after the first infusion. All rats were killed at 24 hours after the large dose (5 \( \mu \)) of thrombin to determine brain water and ion contents.

**Part 4**

Seven groups of 3 rats each were investigated in this part. The first group had no infusion, while the second group received 50 \( \mu \)L saline. Animals of both groups were decapitated 7 days later. The other groups received 1 \( \mu \)L thrombin, then were killed at either 1, 3, 7, 14, or 21 days. All groups were used for Western blot analysis.

**Part 5**

Eight rats were used in this part. Five rats had 1 \( \mu \)L thrombin infusion, and 3 rats had 50 \( \mu \)L saline infusion, then were killed at 7 days for immunocytochemistry and immunofluorescent double staining.

**Brain Water, Sodium, and Potassium Contents**

Animals were decapitated under pentobarbital anesthesia (60 mg/kg). The brains were removed, and a coronal brain slice (\( \approx 3 \) mm thick) 4 mm from the frontal pole was cut with a blade. The brain slice was divided into 2 hemispheres along the midline; each hemisphere was dissected into cortex and basal ganglia. The cerebellum was also detached to serve as control. Thus, a total of 5 samples from each brain were obtained: ipsilateral and contralateral cortex, ipsilateral and contralateral basal ganglia, and cerebellum. Brain samples were immediately weighed on an electronic analytical balance (model AE 100, Mettler Instrument Co) to obtain the wet weight. Brain samples were then dried in a gravity oven (Blue M. Electric Co) at 100°C for 24 hours to obtain the dry weight. Water contents were expressed as a percentage of wet weight. The formula for calculation was (Wet Weight−Dry Weight)/Wet Weight. The dehydrated samples were digested in 1 mL of 1 mol/L nitric acid for 1 week. The sodium and potassium contents of this solution were measured with the automatic flame photometer (model IL943, Instrumentation Laboratory Inc). Ion content was expressed in milliequivalents per kilogram of dehydrated brain tissue (mEq/kg dry wt).

**Western Blotting Analysis**

Animals were anesthetized and decapitated at different time points. Brain was perfused with saline, and brain tissues were sampled as described in the preceding paragraph. The brain tissues were immersed in 0.5 mL Western sample buffer (62.5 mmol/L Tris-HCl, pH 6.8, 2.3% sodium dodecyl sulfate, 10% glycerol, and 5% \( \beta \)-mercaptoethanol) and then were sonicated for 10 seconds. Twenty microliters of the sample solution was taken for protein assay (Bio-Rad), while the rest was frozen at \(-20°C\) for Western blot. Western blot analysis was performed as described previously from our laboratory.\(^1\) Briefly, 50 \( \mu \)g protein was run on 15% polyacrylamide gels with a 4% stacking gel (SDS-PAGE) after 5 minutes of boiling at 95°C. The protein was transferred to hybond-C pure nitrocellulose membrane (Amersham). The
membranes were blocked in 5% Carnation nonfat dry milk in TBST (150 mmol/L NaCl, 100 mmol/L Tris base, 0.1% Tween 20, pH 7.6) buffer for 1 hour at 37°C. After they were washed in TBST buffer 3 times, membranes were probed with 1:2500 dilution of the primary antibody (HSP25, HSP32, or HSP70, StressGen) for 1.5 hours at room temperature. After the membranes were washed with TBST buffer 3 times, the membranes were immunoprobed again with 1:800 dilution of the primary antibody (rabbit anti-mouse HSP25, rabbit anti-HSP32 antibody; (5) and (6) mouse anti–neuron-specific enolase monoclonal antibody (Chemicon) and rabbit anti-HSP25 or anti-HSP32 antibody; (3) and (4) mouse anti–neuron-specific enolase monoclonal antibody (Chemicon) and rabbit anti-HSP25 or anti-HSP32 antibody, Each primary antibody (1:100 dilution) was incubated overnight at 4°C. Fluorescein isothiocyanate (FITC)–labeled horse anti-mouse (1:100) and rhodamine-conjugated goat anti-rabbit (1:100) second antibodies were incubated with sections for 2 hours at room temperature. The double labeling was analyzed by a fluorescence microscope (Nikon Microphoto-SA) with the use of a rhodamine filter and a FITC filter.

**Statistical Analysis**

All data in this study are presented as mean±SD. Data were analyzed with ANOVA with a Scheffé F test. Statistical significance was accepted at \( P<0.05 \).

**Results**

There were no significant differences in mean arterial blood pressure, blood gases, blood pH, blood glucose, and hematocrit among the groups during intracerebral infusion. The combined mean physiological variables are shown in Table 1.

**Thrombin-Induced Brain Edema and TPC**

Intracerebral infusion of 1 U thrombin did not significantly affect brain water content at either 1 day or 7 days (Table 2). In contrast, large-dose thrombin (5 U) infusion caused a marked increase of water content. Intracerebral infusion of 1 U thrombin infusion (TPC) 7 days before infusion of 5 U thrombin significantly reduced edema formation compared with a group in which saline was infused 7 days before the infusion of 5 U thrombin (Figure 1A). There was no protective effect when 1 U thrombin was coinfused with 1 U

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**TABLE 1. Physiological Parameters During Intracerebral Infusion**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>108±9</td>
</tr>
<tr>
<td>pH</td>
<td>7.43±0.03</td>
</tr>
<tr>
<td>( Pao_2 ), mm Hg</td>
<td>81.2±3.6</td>
</tr>
<tr>
<td>( Paco_2 ), mm Hg</td>
<td>45.7±3.7</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>41.2±1.3</td>
</tr>
<tr>
<td>Glucose, mg %</td>
<td>121±15</td>
</tr>
</tbody>
</table>

Values are mean±SD. These are combined data since there are no significant differences among study groups.

**TABLE 2. Brain Water Contents After Saline or Thrombin (50 \( \mu L \)) Infusion**

<table>
<thead>
<tr>
<th>Region</th>
<th>Saline 50 ( \mu L ), 1 d</th>
<th>Thrombin 1 U, 1 d</th>
<th>Thrombin 1 U, 7 d</th>
<th>Thrombin 5 U, 1 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral cortex</td>
<td>79.4±0.3</td>
<td>79.4±0.3</td>
<td>79.6±0.3</td>
<td>79.5±0.4</td>
</tr>
<tr>
<td>Ipsilateral cortex</td>
<td>79.8±0.3</td>
<td>80.1±0.4</td>
<td>80.0±0.4</td>
<td>82.7±0.3*</td>
</tr>
<tr>
<td>Contralateral basal ganglia</td>
<td>77.9±0.2</td>
<td>77.9±0.1</td>
<td>78.3±0.2</td>
<td>78.3±0.6</td>
</tr>
<tr>
<td>Ipsilateral basal ganglia</td>
<td>78.6±0.3</td>
<td>79.8±1.3</td>
<td>78.7±0.4</td>
<td>84.0±0.3*</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>76.2±0.1</td>
<td>77.8±0.2</td>
<td>77.9±0.2</td>
<td>78.2±0.3</td>
</tr>
</tbody>
</table>

Values are mean±SD.

* \( P<0.01 \) vs the other groups.
hirudin, a thrombin inhibitor (Figure 1A). Increases in brain sodium ion concentrations in the 3 groups corresponded to the changes in brain water content (Figure 1B).

We determined the optimal interval between thrombin pretreatment and the second thrombin infusion (5 U thrombin) for reducing the formation of brain edema. Water and ion contents were measured 24 hours after the second infusion following the first thrombin pretreatment and the second thrombin infusion intervals of 3, 7, 14, or 21 days. All these time intervals showed a protective effect on brain edema; the maximal protective interval was 7 days (Figure 2A). Reductions in brain edema formation with TPC were also associated with reduced sodium accumulation (Figure 2B) and reduced potassium loss (Figure 2C).

**TPC and Heat-Shock Proteins**

According to our water content measurements, the most effective interval for TPC was 7 days. The concentrations (density of protein band) of HSP27 and HSP32 were therefore quantified by Western blot 7 days after intracerebral infusion of 1 U thrombin (Figure 3). The concentration of HSP27 in the ipsilateral basal ganglia was \(10\)-fold higher than that of the control group (Figure 3A) and 3-fold higher than that of the contralateral side (Figure 3B). The concentration of HSP32 in the basal ganglia ipsilateral to the thrombin infusion was also 3-fold higher than that of the control group (Figure 3A) and 4-fold higher than that of the contralateral basal ganglia (Figure 3B).

**Figure 1.** Brain water (A) and sodium ion (B) contents at 24 hours after intracerebral infusion of 5 U thrombin. The brains had been infused with either saline, 1 U thrombin, or 1 U thrombin+1 U hirudin 7 days before the large dose of thrombin. Values are expressed as mean±SD; n=5. *P<0.05 vs saline; #P<0.01 vs saline or thrombin+hirudin.

**Figure 2.** Basal ganglia water (A), sodium (B), and potassium (C) contents 24 hours after 5 U thrombin infusion. The rats had received either no TPC or the low dose of thrombin (1 U) either 3, 7, 14, or 21 days before the large dose. Values are expressed as mean±SD; n=5. *P<0.05 vs No TPC; #P<0.01 vs No TPC.

Western blot analysis showed that HSP27 protein concentration increased gradually after infusion of 1 U thrombin, reached a peak between 7 and 14 days (5-fold increase versus the first day; Figure 4) and started to decline by 21 days (5-fold increase versus the first day; data not shown). In contrast to HSP27, the concentration of HSP32 protein reached a peak on the first day and then declined progressively (Figure 4). HSP70 was undetectable by Western blot analysis at any point after infusion of 1 U thrombin.
Western blot analysis.

Figure 4. Time course of HSP27 and HSP32 protein levels in ipsilateral basal ganglia with TPC. Lanes 1 and 2 were 1 day after 1 U thrombin infusion; lanes 3 and 4, 3 days after 1 U thrombin infusion; lanes 5 and 6, 7 days after thrombin pretreatment; and lanes 7 and 8, 14 days after TPC. Equal amounts (50 μg) of protein were analyzed by Western blot.

Immunocytochemistry showed expression of HSP27 and HSP32 after TPC. Seven days after 1 U thrombin infusion, HSP27 immunoreactivity was observed in all ipsilateral basal ganglia but was only observed in part of the ipsilateral cortex (Figure 5A). HSP27 immunoreactivity was not observed in the contralateral hemisphere (Figure 5B). HSP32 immunoreactivity was also detected ipsilaterally (Figure 5C), but not in the contralateral hemisphere (Figure 5D). HSP70 immunoreactivity was only observed around needle track (Figure 6). In the ipsilateral hemisphere, GFAP immunoreactivity was significantly increased, and the shape of astrocytes was changed (Figure 5E). GFAP immunoreactivity was detected in the contralateral hemisphere as well (Figure 5F). The morphological appearance of HSP27- and HSP32-positive cells was similar to that of astrocytes. In saline injection control rats, the immunoreactivities of HSP27, HSP32, and HSP70 were only detected around needle track.

Discussion

The present study demonstrates that the intracerebral infusion of a low dose of thrombin, which fails to produce marked brain edema by itself, significantly attenuates formation of brain edema induced by a large dose of thrombin infused 7 days later. This protective effect of TPC can be blocked by hirudin, a thrombin inhibitor, indicating that the effects of TPC are due to thrombin. A low dose of thrombin increased levels of HSP27 and HSP32 in the brain, suggesting that the former, in particular, may be involved in the protective process induced by low-dose thrombin.

Thrombin is a serine protease produced immediately in the brain after ICH, brain trauma, or blood-brain barrier breakdown following many kinds of brain injury. Direct infusion of large doses of thrombin into brain causes inflammatory cell infiltration, mesenchymal cell proliferation, brain edema formation, and an increase in reactive astrocytes.1–3,5 However, a recent in vitro study has demonstrated that a low concentration of thrombin (100 pmol/L to 100 nmol/L) protects rat neurons and astrocytes from cell death caused by hypoglycemia, growth supplement deprivation, or ischemia.6,7 The protective effects of thrombin shown by Vaughan et al6 were blocked by a thrombin inhibitor, protease nexin-1. The mechanism of thrombin protection remains unknown. Recent reports indicate that thrombin may regulate a variety of activities in the brain. Thrombin enhances the synthesis and secretion of nerve growth factor in glial cells,19 modulates neurite outgrowth,20 reverses process-bearing stellate astrocytes to epithelial-like astrocytes,21 and stimulates astrocyte proliferation.21–23 Our present study indicates that thrombin also influences the expression of HSP27 and HSP32.

Our present TPC-edema time course shows that the most effective tolerance against thrombin-induced edema appeared 7 days after thrombin pretreatment. This time delay for tolerance induction suggests that new proteins may be synthesized. The HSP27 Western blot time course demonstrated that the expression of HSP27 (also called HSP25 in the mouse) reaches a peak at 7 to 14 days after thrombin pretreatment. The close relationship between HSP27 expression and induced tolerance suggests that there may be a causal relationship. It may also suggest that induction of HSP27 by other methods might cause the same protection.

HSP27 immunoreactivity is not detected in adult rat cerebrum and cerebellum.24 However, HSP27 does accumulate to high levels in cells after stresses, such as hyperthermia, and contributes to temporary heat-shock resistance.25 It has been suggested that HSP27 protects against heat shock.26 and oxidative stress27 through stabilization of actin microfilaments. Stabilization of actin microfilaments is related to activation of a stress-sensitive mitogen-
activated protein (MAP) kinase signal transduction pathway that induces resistance to stress-induced actin fragmentation. Activation of MAP kinase activates mitogen-activated protein kinase-activated protein kinase 2 (MAPKAP kinase-2), which in turn causes HSP27 phosphorylation. HSP27 then modulates the cytoskeleton by altering actin dynamics. We hypothesize that HSP27 accumulation and possibly activation of the MAP kinase signal transduction pathway after TPC may induce reorganization of the actin cytoskeleton and thus alter cell swelling after a subsequent injection of large-dose thrombin.

Our hypothesis is supported by the study of Lavoie et al., which found that HSP27 overexpression modifies cellular actin distribution, including more cortical F-actin and less cytoplasmic stress fiber. Interestingly, their study also found a 2-fold increase in actin polymerization activity and accumulation of F-actin after stimulation by thrombin in serum-starved CCL39-neo cells. In the present study, HSP27 upregulation after thrombin injection was associated with a change in astrocyte shape (Figure 5E), demonstrated earlier in vitro by Cavanaugh et al.

Although we hypothesize that HSP27 may exert its protective effects through changes in the cytoskeleton, HSP27...
may have other protective effects, such as acting as a molecular chaperone and increasing glutathione levels, which protect against oxidative injury.

Through immunocytochemistry we found that HSP27 immunoreactivity at 7 days after thrombin pretreatment was related to increased GFAP immunoreactivity in the ipsilateral basal ganglia. Immunofluorescent double staining demonstrated that HSP27-positive cells were also GFAP positive.

HSP27 expression in glial cells has been correlated with development of ischemic tolerance. Recently, Plumier et al found that cortical application of potassium chloride triggered HSP27 in astrocytes, suggesting that expression of HSP27 increased resistance to ischemic injury. Whether the effects of TPC on thrombin-induced edema formation are limited to modulating astrocyte swelling has yet to be determined.

Figure 6. HSP70 immunoreactivity after 1 U thrombin pretreatment. Shown are HSP70 immunoreactivity in contralateral parietal cortex (A) and in ipsilateral parietal cortex around needle track (B). The asterisk indicates needle track. Examples of HSP70 immunoreactive cells are shown by arrows. Scale bar = 50 μm.

Figure 7. Double immunofluorescent labeling for GFAP and HSP27 or HSP32 7 days after 1 U thrombin infusion in the right rat caudate nucleus. A, C, FITC-labeled GFAP; B, rhodamine-labeled HSP27; D, rhodamine-labeled HSP32. Examples of double-labeled cells are shown by arrows. Scale bar = 20 μm.
Intracerebral infusion of a low dose of thrombin also induced an increase in HSP32 immunoreactivity. Thus, at 7 days, HSP32 immunoreactivity was significantly higher than that of control or in the contralateral basal ganglia (∼3-fold or 4-fold increase, respectively). HSP32, also called heme oxygenase 1 (HO-1), is a stress protein and the rate-limiting enzyme in the heme degradative pathway. HO-1 cleaves heme to release carbon monoxide, iron, and bilirubin. Carbon monoxide has been suggested as a second messenger in the central nervous system. Bilirubin itself has antioxidant properties. Recent studies suggest that HO-1 may play an important role in cytoprotection against oxidative injury as well as heme- and hemoglobin-induced toxicity. Induction of HO-1 by pretreating rats with hemoglobin, a potent HO-1 inducer, provided protection against lethal endotoxemia in rats. This hemoglobin-induced protective effect is blocked by tin protoporphyrin, an inhibitor of HO-1. This might suggest that HSP32 could also be involved in TPC. In contrast, to HSP27, the time course for the upregulation of HSP32 is faster than our observed effects of TPC on edema formation, indicating that this may not be the case.

HSP70 expression may be responsible for ischemic tolerance through the preconditioning process. Our Western blot analysis data, however, demonstrate that HSP70 induction did not occur at any time points in TPC. With immunocytochemistry, some HSP70 immunoreactive cells were found, but these were only localized around needle track. Our finding of HSP 70 immunoreactivity along the needle track confirms the findings of Matz et al. while Brown et al. demonstrated localized induction of HSP70 mRNA along the wall of a small surgical cut in the rat cerebral cortex.

In conclusion, our data demonstrate that pretreatment with a low dose of thrombin can attenuate edema induced by a large dose 7 days later. This phenomenon of induced tolerance is shown for the first time in brain and may involve the induction of HSPs, particularly HSP27. Although this study is not strictly randomized, our findings may stimulate further investigation of the mechanism of edema formation after ICH.

Acknowledgment

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Editorial Comment

The authors showed in previous work that thrombin injection into the brain causes edema. Because there is thrombin present after intracerebral hemorrhage, this process may underlie some of the edema formation that occurs after intracerebral hemorrhage. In this communication, they show that injection of a low dose of thrombin into the rat caudate nucleus decreases edema formation in response to a larger dose of thrombin administered 7 days later. The levels of the heat-shock proteins (HSPs) 27, 32, and 70 were measured and the preconditioning effect of thrombin correlated with the time course of expression of HSP27.

It is known that a brain preconditioned by one or more episodes of brief, mild ischemia is protected against a subsequent, more severe ischemic insult. The mechanism of this effect is under investigation and has been suggested to involve immediate early genes, HSPs, and a variety of cytokines.1 The work of Xi et al shows that the preconditioning response to thrombin is at least associated with increases in HSP27. The heat-shock response has been the subject of recent review to which I refer the interested reader.2 One potentially important implication of the findings is that if the mechanism of the preconditioning effect were known, then presumably some treatment aimed at inducing this before the insult or immediately thereafter might alleviate some of the detrimental effects of the insult.

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References


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