Intracerebral infusion of thrombin as a cause of brain edema

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PURIFIED thrombin from an exogenous source is a hemostatic agent commonly used in neurosurgical procedures. The toxicity of thrombin in the brain, however, has not been examined. This study was performed to assess the effect of thrombin on brain parenchyma, using the formation of brain edema as an indicator of injury.

Ten μl of test solution was infused stereotactically into the right basal ganglia of rats. The animals were sacrificed 24 hours later, and the extent of brain edema and ion content were measured. Concentrations of human thrombin as low as 1 U/μl resulted in a significant increase in brain water content. Rats receiving 10 U/μl had a mortality rate of 33% compared to no mortality in the groups receiving smaller doses. Thrombin-induced brain edema was inhibited by a specific and potent thrombin inhibitor, hirudin. A medical grade of bovine thrombin commonly used in surgery also caused brain edema when injected at a concentration of 2 U/μl. Edema formation was prevented by another highly specific thrombin inhibitor, Nα-(2-Naphthalenesulfonylglycyl)-4-amidino-DL-phenylalaninepiperidide (α-NAPAP). Thrombin-induced brain edema was accompanied by increases in brain sodium and chloride contents and a decrease in brain potassium content. Changes in brain ions were inhibited by both hirudin and α-NAPAP, corresponding to the inhibition of brain water accumulation. This study shows that thrombin causes brain edema when infused into the brain at concentrations as low as 1 U/μl, an amount within the range of concentrations used for topical hemostasis in neurosurgery.

KEY WORDS • thrombin • brain edema • hirudin • Nα-(2-Naphthalenesulfonylglycyl)-4-amidino-DL-phenylalaninepiperidide (α-NAPAP) • rat

THROMBIN applied topically to the brain is an effective means of achieving hemostasis in neurosurgery. A recent clinical report on the use of thrombin to control intracranial bleeding during stereotactic procedures has suggested that this hemostatic agent may cause cerebral infarction. However, there have been no studies of thrombin’s toxicity in the brain.

Extensive research on thrombin has revealed several mechanisms of action throughout the body. The protease activity of thrombin in blood is responsible for its procoagulant effects including the conversion of fibrinogen to fibrin. Receptor-mediated effects have also been described including modulation of capillary permeability, regulation of vascular tone, and promotion of inflammation. The interaction of thrombin with nervous system tissue is nevertheless not well understood. The current study was undertaken to determine the effect of thrombin on brain tissue, with edema formation used as an indicator of injury.

Materials and Methods

Animal Preparation

A total of 71 adult male Sprague Dawley rats, weighing between 250 and 350 g, were anesthetized in a closed chamber with 5% isoflurane. A 16-gauge catheter was inserted into the trachea of each animal using an oral intubation technique. The rats were ventilated with a Harvard rodent ventilator using a mixture of oxygen (21%), nitrogen (76%–77%), and isoflurane (2%–3%). A PE-50 catheter was inserted into the femoral artery and arterial blood pressure was monitored continuously. The blood pressure was titrated with isoflurane to maintain a mean pressure of 100 mm Hg. Arterial blood was obtained from the femoral artery catheter for blood gas analysis. The ventilator rate, tidal volume, and inspired gas concentrations were adjusted to maintain normal blood oxygen and carbon dioxide tensions. Body temperature was maintained at 37.5°C using a rectal thermometer and a feedback-controlled heating pad.

Intracerebral Injection

The rats were positioned in a stereotactic frame and the scalp of each animal was incised along the sagittal midline. An operating...
microscope was used for the remainder of the procedure, and hemostasis was maintained with bipolar electrocautery. A 1-mm burr hole was placed in the skull near the right coronal suture 3 mm lateral to the bregma. A 26-gauge needle was inserted into the right basal ganglia using stereotactic guidance.

Solutions were infused into the brain using a Harvard pump. Ten microliters of test solution was infused over 3 minutes. A purified form of human thrombin containing 3000 U of activity per milligram of protein was dissolved in normal saline. Different thrombin concentrations were infused (0.1 U/μl in six animals; 1 U/μl in seven animals; and 10 U/μl in five animals) and the treated animals were compared to sham animals injected with saline (six animals). The range of thrombin concentrations was chosen to reflect both the amounts used in surgery and the quantity released from a hematoma. In an additional group of rats (five animals), 1 U/μl of hirudin, a naturally occurring thrombin inhibitor, was injected along with 1 U/μl of human thrombin.

A commonly used operating room preparation of bovine thrombin containing approximately 40 U of activity per milligram of protein was injected into groups of rats at various concentrations (0.1 U/μl in six animals; 1 U/μl in seven animals; 10 U/μl in five animals) and the treated animals were compared to a sham group (six animals) injected with control vehicle containing 90 mM NaCl, 14 mM CaCl2, 1.2% glycine (weight/volume), and 5% bovine serum albumin (weight/volume). A separate group of rats (six animals) received intracerebral injections of the synthetic thrombin inhibitor Nex-2-Naphthalenesulfonylgluceryl-4-amidino-DL-phenylalaninepiperidide (α-NAPAP) (B). The values shown are expressed as the mean ± standard error of the mean. The Neuman–Keuls test was used for comparison.

**p < 0.01. The Dunnett t-test was used for comparison.**

**FIG. 1.** Bar graph depicting the water content (percent of wet weight) in the cortex and basal ganglia contralateral (CONTRA) and ipsilateral (IPSI) to the site of intracerebral injection of 10-μl solutions of 0, 0.1, 1, and 10 U/μl of thrombin. The values shown are expressed as the mean ± standard error of the mean. The Neuman–Keuls test was used for comparison.

**FIG. 2.** Bar graphs showing the water content (percent of wet weight) in the basal ganglia ipsilateral to the site of injection of human thrombin with and without the inhibitor hirudin (A) and of bovine thrombin with and without the inhibitor Nex-2-Naphthalenesulfonylgluceryl-4-amidino-DL-phenylalaninepiperidide (α-NAPAP) (B). The values shown are expressed as the mean ± standard error of the mean. The Neuman–Keuls test was used for comparison.

**Magnetic Resonance Imaging**

Rats were injected with either 10 μl of 1 to 2 U/μl thrombin (three animals) or 10 μl of saline (three animals) using the methods described above. Twenty-four hours after intracerebral injection, the animals were anesthetized with ketamine and xylazine. Standard T2-weighted magnetic resonance (MR) imaging was performed using a 2-tesla/31-cm Omega CSI imaging spectrometer. The T2-weighted, spin-echo acquisition parameters were: repetition time 2.8 sec, echo time 80 msec, field of view 30 mm, 128 × 128 matrix, slice thickness 0.8 mm, and number of signal averages 2, 16 slices.

**Statistical Analysis**

Multiple groups of animals were compared using analysis of variance. The differences between groups of rats in water and ion contents were evaluated using the Neuman–Keuls and Dunnett t-tests of significance where indicated. A two-tailed probability value of less than 0.05 was used to indicate a significant difference.

**Sources of Supplies and Equipment**

Human thrombin and the thrombin inhibitors hirudin and α-NAPAP were obtained from Sigma Chemical Co., St. Louis, MO; and bovine thrombin was purchased from Johnson and Johnson Medical Inc., Arlington, TX.

The gravity oven used to dry the tissue samples was obtained from Blue M Electric Co., Blue Island, IL. Measurements of sodium and potassium contents were made using the IL-943 Automatic Flame Photometer, Instrumentation Laboratory, Inc., Lexington, MA; measurement of chloride content was made using the model 442-5000 digital chloridometer, Haake Buchler Instruments, Inc., Saddlebrook, NJ.

Magnetic resonance imaging was performed using an Omega CSI imaging spectrometer obtained from Bruker Instruments (formerly GE NMR, Fremont, CA).

**Results**

**Physiological Parameters**

Table 1 shows the mean values for the physiological contents were evaluated using the Neuman–Keuls and Dunnett t-tests of significance where indicated. A two-tailed probability value of less than 0.05 was used to indicate a significant difference. **p < 0.01. The Dunnett t-test was used for comparison.**

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**Results**

**Physiological Parameters**

Table 1 shows the mean values for the physiological
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parameters recorded in the groups of rats used in this study. The mean values for blood pressure, blood gases, and hematocrit were within normal range for each group of rats.

Brain Water Content

Intracerebral infusion of 10-μl solutions of 1 and 10 U/μl of human thrombin produced a significant increase in brain water content in the ipsilateral basal ganglia compared to that measured in rats that received control vehicle (Fig. 1). No edema was produced when 0.1 U/μl was injected into the brain.

Water content in the ipsilateral basal ganglia was 4% greater in animals receiving an intracerebral infusion of 1 U/μl of human thrombin compared to animals receiving control saline (Fig. 1). An increase of 5.8% over sham values was found in the group receiving 10 U/μl of human thrombin. Animals receiving 1 U/μl also had brain edema in the ipsilateral cortex but not in the contralateral hemisphere. At 10 U/μl the ipsilateral cortex, contralateral cortex, and contralateral basal ganglia also had increased water content. The animals receiving this higher dose of thrombin had a 33% mortality rate. No mortality occurred in any of the other groups. In all groups the cerebellum showed no evidence of edema formation.

An increase in brain water content similar to that caused by human thrombin was demonstrated using bovine thrombin (Table 2). Increases in brain water were prevented by the thrombin inhibitors hirudin and α-NAPAP (Fig. 2).

Brain Ion Content

Brain sodium and chloride contents increased in rats receiving thrombin at doses of at least 1 U/μl (Table 3); however, the brain potassium content decreased. These brain ion changes were significantly lessened by the presence of the thrombin inhibitors hirudin and α-NAPAP. In general, brain sodium and chloride changes corresponded directly to the degree of edema fluid produced. The brain potassium levels had an inverse relationship to brain water accumulation.

Magnetic Resonance Imaging

Figure 3 shows representative T2-weighted MR images...
TABLE 2

Water content in rat brains following 10-µl intracerebral injections of solutions containing thrombin alone or with inhibitor *

<table>
<thead>
<tr>
<th>Water (g/g DW)</th>
<th>Cortex</th>
<th>Basal Ganglia</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contralateral</td>
<td>Ipsilateral</td>
<td>Contralateral</td>
</tr>
<tr>
<td>human thrombin (U/µl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>3.76 ± 0.04</td>
<td>3.88 ± 0.07</td>
<td>3.51 ± 0.05</td>
</tr>
<tr>
<td>0.1</td>
<td>3.78 ± 0.01</td>
<td>3.95 ± 0.05</td>
<td>3.55 ± 0.04</td>
</tr>
<tr>
<td>1.0</td>
<td>3.86 ± 0.02</td>
<td>4.45 ± 0.06†</td>
<td>3.66 ± 0.05</td>
</tr>
<tr>
<td>10.0</td>
<td>3.96 ± 0.05†</td>
<td>4.77 ± 0.11†</td>
<td>3.88 ± 0.09†</td>
</tr>
<tr>
<td>human thrombin (1.0 U/µl) + hirudin (1.0 U/µl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>3.76 ± 0.02‡</td>
<td>4.17 ± 0.05‡§</td>
<td>3.52 ± 0.04‡</td>
</tr>
<tr>
<td>bovine thrombin (U/µl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>3.80 ± 0.04</td>
<td>3.93 ± 0.04</td>
<td>3.71 ± 0.05</td>
</tr>
<tr>
<td>0.1</td>
<td>3.87 ± 0.03</td>
<td>4.15 ± 0.08</td>
<td>3.66 ± 0.03</td>
</tr>
<tr>
<td>1.0</td>
<td>3.79 ± 0.00</td>
<td>4.00 ± 0.08</td>
<td>3.61 ± 0.03</td>
</tr>
<tr>
<td>2.0</td>
<td>3.87 ± 0.07</td>
<td>4.39 ± 0.15**</td>
<td>3.68 ± 0.05</td>
</tr>
<tr>
<td>10.0</td>
<td>4.06 ± 0.05†</td>
<td>4.73 ± 0.14†</td>
<td>4.09 ± 0.09†</td>
</tr>
<tr>
<td>bovine thrombin (2.0 U/µl) + α-NAPAP (0.2 mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>3.85 ± 0.03</td>
<td>4.04 ± 0.07‡</td>
<td>3.77 ± 0.04</td>
</tr>
</tbody>
</table>

* Values expressed are the mean ± standard error of the mean. The terms “ipsilateral” and “contralateral” are used in reference to the side of the infusion of solutions. α-NAPAP = Nα-(2-Naphthalenesulfonylglycyl)-4-amidino-DL-phenylalaninepiperide; DW = dry weight.
† p < 0.05 compared to sham-treated animals using the Neuman–Keuls test.
‡ p < 0.01 compared to sham-treated animals using the Neuman–Keuls test.
§ p < 0.05 compared to animals receiving thrombin using the Neuman–Keuls test.
|| p < 0.05 compared to sham-treated animals using the Neuman–Keuls test.
** p < 0.05 compared to sham-treated animals using ANOVA with Dunnett’s t-test.

The difference, after 24 hours, in brain water content between a rat that received an intracerebral infusion of saline (Fig. 3A) and a rat injected with 10 µl of thrombin at 1 to 2 U/µl (Fig. 3B). Images obtained in six animals were examined. The edema was prevalent around the point of injection in the basal ganglia and along white matter tracts, with evidence of extension into the contralateral hemisphere. The cortical edema was at least partially due to the egress of thrombin along the needle tract as seen in the MR imaging slice through the path of the needle.

Discussion

In this study, both human and bovine thrombin were shown to cause brain water and ion changes consistent with brain edema. Edema, produced by thrombin at a dose commonly used in neurosurgical procedures, involved the cortex as well as the basal ganglia in the hemisphere on the side of injection, 24 hours later. At higher concentrations of thrombin, edema also involved the opposite hemisphere. The distribution of edema was due either to diffusion of thrombin into these areas, producing a direct toxic effect, or to bulk flow of edema fluid to distant regions. To confirm that the edema response was caused by thrombin, additional tests were performed using the inhibitors of thrombin, hirudin and α-NAPAP. Both inhibitors reduced the brain water and ion changes induced by thrombin.

Hirudin, a biological substance found in leeches, is the most potent and specific naturally-occurring thrombin inhibitor.6,16,18 This protein is currently produced by recombinant deoxyribonucleic acid technology and has been used medically as an anticoagulant.20,27 The synthetically produced thrombin inhibitor, α-NAPAP, is also highly specific and potent for thrombin.16,19,26

Thrombin is a serine protease produced from its inactive zymogen, prothrombin, which has procoagulant effects in blood that affect several different steps in the coagulation cascade.7 Thrombin is responsible for the conversion of fibrinogen to fibrin. In addition to the enzymatic activity in blood, thrombin has cellular effects mediated through thrombin receptors located on platelets, endothelial cells, fibroblasts, neurons, and glia.10,22,25 Thrombin induces mitosis and the subsequent proliferation of cells including glia. In addition, this molecule is important in wound healing and inflammation. It stimulates chemotaxis of leukocytes, expression of adhesion molecules on endothelial cells, and release of cytokines.4 A receptor-mediated effect on endothelial cells causes the release of platelet-activating factor. Platelet activation by thrombin leads to aggregation, secretion, and production of thromboxane A2.23 Thrombin has vasoactive properties that cause vasodilation or vasoconstriction, depending on the artery.1,11,13 The molecule stimulates endothelial cells to secrete endothelin.3

In the brain, thrombin causes inflammation, scar formation, and reactive gliosis.22 The brain and spinal cord have a large number of thrombin binding sites.21 The molecule binds to neuroblastoma cells, which have characteristics similar to normal neurons, and it stimulates the production of cyclic guanosine monophosphate.20 During central nervous system development and regeneration, thrombin and its inhibitors are thought to be involved in neurite outgrowth, thereby controlling the growth and morphology...
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TABLE 3
Brain ion contents in the ipsilateral basal ganglia of rats following 10-μl intracerebral injections of solutions containing thrombin alone or with inhibitor*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sodium (μEq/g DW)</th>
<th>Potassium (μEq/g DW)</th>
<th>Chloride (μEq/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>193 ± 2</td>
<td>437 ± 4</td>
<td>142 ± 6</td>
</tr>
<tr>
<td>human thrombin (1 μl)</td>
<td>413 ± 6†</td>
<td>363 ± 23†</td>
<td>330 ± 39†</td>
</tr>
<tr>
<td>human thrombin (1 μl) + hirudin (1 μl)</td>
<td>303 ± 6</td>
<td>392 ± 6‡</td>
<td>249 ± 78</td>
</tr>
<tr>
<td>vehicle</td>
<td>224 ± 14</td>
<td>462 ± 10</td>
<td>182 ± 17</td>
</tr>
<tr>
<td>bovine thrombin (2 μl)</td>
<td>407 ± 91**</td>
<td>374 ± 31†</td>
<td>298 ± 54**</td>
</tr>
<tr>
<td>bovine thrombin (2 μl) + α-NAPAP (0.2 mM)</td>
<td>220 ± 9</td>
<td>436 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

* Values expressed are the mean ± the standard error of the mean. The term “ipsilateral” is used in reference to the side of the infusion of solutions. α-NAPAP = N-(2-Naphthalenesulfonylglycyl)-4-amidino-2-phenylalaninepiperidide; DW = dry weight.
† p < 0.01 compared to sham-treated animals using analysis of variance (ANOVA) with Dunnett’s t-test.31
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§ p < 0.01 compared to sham-treated animals using the Neuman–Keuls test.31
∥ p < 0.05 compared to animals receiving thrombin using the Neuman–Keuls test.31
** p < 0.05 compared to sham-treated animals using ANOVA with Dunnett’s t-test.

of neurons.15 Reports suggest that thrombin contributes to cell damage through its ability to cause retraction of axons, dendrites, and other cell processes of neurons and glia.28

The brain may be more susceptible to the effects of thrombin than other organs. Thrombomodulin, a potent inhibitor found in vessels throughout the body, is absent from endothelial cells in the brain.14 The extracellular matrix contains a novel thrombin inhibitor, protease nexin-1.28

Thrombin may affect the permeability of the blood-brain barrier. Studies of endothelial cell monolayers suggest that thrombin increases permeability in a dose-related manner.12,17 Microvessel endothelial cells from various organs show differential responsiveness to thrombin. Lysin sinusoidal endothelial cells are relatively unresponsive, whereas brain endothelial cells exhibit significant morphological changes in response to nanomolar concentrations of thrombin.5

Thrombin could play a role in edema formation that is caused by intracerebral hemorrhage. Studies of intracerebral hemorrhage have shown water and ion changes after exposure of the brain to blood that are similar to thrombin-mediated changes.20 There is also evidence that this molecule is involved in vasospasm caused by subarachnoid hemorrhage. The naturally occurring inhibitor, antithrombin III, reverses the delayed arterial narrowing caused by subarachnoid hemorrhage in rabbits and dilates precontracted basilar arteries in vitro.29 Glycosaminoglycans, such as heparin, accelerate the reaction of thrombin with antithrombin III, which may explain clinical reports that suggest that heparin is useful in the prevention of delayed ischemic events caused by vasospasm.20,24

This study demonstrates that exogenous thrombin (bovine or human) applied to brain parenchyma causes brain edema in rats. Thrombin should, therefore, be used judiciously in surgery of the neuraxis. A study of the effect of thrombin-soaked gelfoam on brain edema would further clarify this issue. Identification of the precise mechanism by which thrombin causes the formation of brain edema will require further study.

References
21. McKinney M, Snider RM, Richelson E: Thrombin binding to
human brain and spinal cord. 


