The kallikrein-kinin system as mediator in vasogenic brain edema

Part 1: Cerebral exposure to bradykinin and plasma

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Plasma and bradykinin were perfused into the ventricular system of mongrel dogs to investigate whether either or both induce brain edema. Formation of cerebral edema was determined by measurement of cerebral water and electrolytes in periventricular white matter, cerebral cortex, and caudate nucleus. The response of cerebral tissue to exposure to bradykinin or to plasma, as a carrier of kininogens, was analyzed by assessment of the perfusate composition after ventricle passage. The authors report that cerebral administration of bradykinin induces cerebral edema. Ventricular perfusion with plasma also led to an increase of cerebral water content which was restricted to the white matter, but involved all brain tissue areas, if bradykinin was used. Ventricular perfusion with plasma was associated with consumption of the kinin precursor (kininogens) indicative of formation of kinins. Significant consumption of the precursor was found in five out of nine animals subjected to plasma perfusion of the ventricular system. In these animals a close correlation between the increase of white matter water content and kininogen-consumption as a measure of kinin-formation was obtained. Marked kinin-degrading activity was observed during ventricular perfusion with bradykinin as concluded from a considerable decrease of bradykinin concentration in the cisternal effluent compared to the inflowing perfusate concentration. Ventricular perfusion with plasma was associated with a decrease of K⁺ clearance capacity with continued duration, and in two animals with a release of glutamate into the plasma perfusate, suggesting an involvement of cytotoxic mechanisms.

These findings provide support for the hypothesis of a mediator function of the kallikrein-kinin (KK) system in vasogenic brain edema. The next question that needs to be answered to complete the picture — does spontaneous activation of the KK system occur in conditions leading to vasogenic edema? — is studied in a subsequent report.

KEY WORDS □ vasogenic brain edema □ kallikrein-kinin system □ bradykinin □ ventriculocisternal perfusion

Vasogenic brain edema occurs in association with many cerebral disorders, including brain tumors, focal cerebral ischemia, and severe head trauma. It is caused by a defect of the blood-brain barrier in areas of focal necrosis, allowing for penetration of a protein-rich plasma-like fluid from the intravascular space into the cerebral parenchyma.²⁵,³⁹

Although our knowledge of the origin and composition of vasogenic edema fluid has progressed considerably, questions remain unanswered on the mechanisms of uptake, spread, and persistence of vasogenic edema. Moreover, it is not clear whether vasogenic edema fluid, which has a composition different from extracellular fluid of the brain, induces functional or structural damage to nerve and glial cells.²⁷ Such damage includes the development of secondary cytotoxic swelling of nerve and glial cells, and the splitting of myelin lamellae.⁵,¹⁹,²⁰,²⁵,³⁷

It has been postulated that formation of primary tissue necrosis together with the opening of the blood-brain barrier by a primary lesion provides the basis for production and release of substances mediating and enhancing secondary brain damage, such as vasogenic edema.²,⁴ The mediator compounds may be derived from irreversibly damaged cells of a cerebral lesion, or taken up from the intravascular space through the broken barrier into focal and perifocal tissue.⁴ Mediator compounds are considered to induce a variety of patho-
physiological mechanisms, such as enhancement of the barrier defect, disturbances of the microcirculation in the focus or the perifocal brain, or formation of cytotoxic swelling of nerve and glial cells. The concept of factors mediating secondary brain damage may provide a basis for improvement of therapy.\(^3\) Up to now, no compound has been identified as a mediator, although many candidates have been subjected to rigorous experimental and clinical testing.\(^4\)

The kallikrein-kinin (KK) system is a promising candidate as mediator of secondary brain damage due to the powerful pharmacological actions of kinin peptides. The basis of this concept is that the components of the plasma-KK system penetrate into brain parenchyma as a result of traumatic or ischemic damage of the blood-brain barrier, and are activated in focal and perifocal tissue. Formation of kinins in primarily damaged brain tissue may enhance disturbances of the microcirculation, blood-brain barrier dysfunction, and formation of secondary cell swelling.\(^4\)'\(^4\)'\(^7\)

Physiological or pathological phenomena associated exclusively with an activation of the KK system have not been found, although an involvement in blood clotting, nociception, alterations of peripheral macro- as well as microcirculation and of capillary permeability have been reported.\(^1\)'\(^7\)'\(^2\)'\(^3\)'\(^3\) Relationship of the KK system to blood clotting, peripheral vasodilation, and increase of vascular permeability is particularly pertinent for a role in secondary brain damage. Sicuteri, et al.,\(^4\)'\(^3\) have postulated activation of the KK system in subarachnoid hemorrhage. However, there is not yet direct evidence that kinins induce cerebral damage. Therefore, we have investigated whether cerebral administration of bradykinin or of plasma, as a carrier of the intravascular KK system, causes brain edema. For this purpose, homologous plasma or bradykinin were administered to the cerebrum by ventriculocisternal perfusion in order to bypass the blood-brain barrier.\(^4\)'\(^6\) Preliminary findings were reported elsewhere.\(^4\)

**Materials and Methods**

Ventriculocisternal perfusion was performed in 31 mongrel dogs of 11 to 13 kg body weight under pentobarbital (30 mg/kg) anesthesia, relaxation with succinylcholine, and mechanical ventilation. Ventilation was continuously controlled by monitoring of tidal CO\(_2\) and intermittent blood gas analysis. Both lateral ventricles were punctured by free-hand technique with plastic cannulas at a point 10 mm lateral to the median plane and 4 mm posterior to the coronal suture. The cannulas were fixed to the skull by a rapidly polymerizing dental cement. The atlanto-occipital membrane was exposed, and an incision was made for introduction of a plastic catheter into the cerebellomedullary cistern to collect the perfusate after passage through the ventricles. The technical details are shown in Fig. 1.

In control as well as in experimental animals, test perfusion of the ventricles commenced with artificial cerebrospinal fluid (CSF) under continuous perfusion pressure control.\(^*\) The perfusion rate was 0.5 ml/min/ventricle. Perfusion pressure was maintained at 10 mm Hg by adjustment of the cisternal outflow tube. After 30 minutes of control perfusion with artificial CSF, ventriculocisternal perfusion was continued for 3 hours. In Group 1 (eight dogs), artificial CSF was used throughout (controls). In Group 2 (13 dogs), the perfusion fluid was changed to homologous plasma as carrier of kininogens. In Group 3 (10 dogs), the perfusate was bradykinin (1200 to 4100 ng/ml) dissolved in isotonic and buffered artificial CSF. The perfusate concentrations of bradykinin were selected in this amount as they are equivalent to the amount of kinins maximally releasable from kininogens normally present in dogs' plasma (see below). The purity of the commercially obtained bradykinin preparation was 96% as determined by reversed phase high-pressure liquid chromatography.\(^\dagger\)

Artificial CSF was prepared as described by Merlis.\(^3\)\(^2\) Osmolarity and pH of the perfusates were adjusted prior to perfusion to physiological levels. For ventricular perfusion with plasma, mongrel donor dogs (each weighing 15 to 18 kg) were bled from the femoral artery under pentobarbital anesthesia. Coagulation of the freshly drawn blood was prevented by addition of 500 IU heparin/100 ml. Centrifugation ensued to obtain the plasma perfusion fluid. Prior to the experiment the plasma perfusate was kept in siliconized beakers at

\(*\) Gould pressure transducer, Model PD23 ID, manufactured by Gould, Inc., 3631 Perkins Avenue, Cleveland, Ohio.\n
\(\dagger\) Liquid chromatography was conducted by Serva Co., Heidelberg, West Germany.
TABLE 1
Cerebral water and electrolyte content in brain areas of control and experimental animals*

<table>
<thead>
<tr>
<th>Parameter &amp; Group</th>
<th>Parameter</th>
<th>No. of Dogs</th>
<th>Cortex</th>
<th>No. of Dogs</th>
<th>Caudate Nucleus</th>
<th>No. of Dogs</th>
<th>White Matter</th>
<th>No. of Dogs</th>
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<tbody>
<tr>
<td>water content (ml/100 gm fw)</td>
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<td></td>
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<tr>
<td>control group</td>
<td>80.94 ± 0.58</td>
<td>7</td>
<td>80.29 ± 0.84</td>
<td>6</td>
<td>66.17 ± 1.04</td>
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<tr>
<td>plasma group</td>
<td>80.95 ± 0.75</td>
<td>12</td>
<td>80.26 ± 0.70</td>
<td>12</td>
<td>68.51 ± 1.51§</td>
<td>9</td>
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<tr>
<td>bradykinin group</td>
<td>81.63 ± 0.35†</td>
<td>8</td>
<td>81.89 ± 0.40‡</td>
<td>8</td>
<td>68.01 ± 1.70‖</td>
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<td>Na⁺ content (mM/kg dw)</td>
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<tr>
<td>control group</td>
<td>279.9 ± 25.3</td>
<td>6</td>
<td>262.1 ± 40.5</td>
<td>6</td>
<td>155.5 ± 22.5</td>
<td>6</td>
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<tr>
<td>plasma group</td>
<td>299.4 ± 38.5</td>
<td>13</td>
<td>249.7 ± 23.6</td>
<td>13</td>
<td>176.2 ± 19.6</td>
<td>13</td>
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<tr>
<td>bradykinin group</td>
<td>287.5 ± 25.9</td>
<td>8</td>
<td>308.7 ± 14.4‡</td>
<td>8</td>
<td>162.5 ± 9.4</td>
<td>8</td>
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<tr>
<td>K⁺ content (mM/kg dw)</td>
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<td></td>
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<tr>
<td>control group</td>
<td>548.4 ± 27.8</td>
<td>6</td>
<td>503.4 ± 46.1</td>
<td>6</td>
<td>272.6 ± 21.8</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>plasma group</td>
<td>540.6 ± 43.7</td>
<td>13</td>
<td>506.1 ± 19.8</td>
<td>13</td>
<td>273.5 ± 30.6</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bradykinin group</td>
<td>524.7 ± 27.7</td>
<td>8</td>
<td>494.3 ± 35.8</td>
<td>8</td>
<td>248.3 ± 24.0</td>
<td>8</td>
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* Significance compared to control group levels: † = p < 0.02; ‡ = p < 0.002; § = p < 0.01; ‖ = p < 0.05. fw = fresh tissue weight; dw = dry weight.

37°C. Siliconized tubes were used for perfusion and collection of the cisternal effluent to prevent spontaneous activation of the KK system. The pH of the plasma perfusate was controlled and adjusted to 7.35 if necessary by addition of phosphate buffer. Carbon-14 (¹⁴C)-labeled inulin was added to the perfusate as a volume marker. The cisternal effluent was collected at 30-minute intervals.

Osmolarity, pH, glucose, lactic and pyruvic acid, Na⁺, K⁺, glutamate, ¹⁴C-inulin, bradykinin, or kininogens, respectively, were measured in perfusate samples prior to and after ventricular passage. Per fusate infl ow samples were taken at the beginning and at termination of ventricular perfusion. Perfusion pressure, arterial blood pressure, heart rate, body temperature, hematocrit, arterial pO₂, and acid-base status were continuously monitored. Intravenous injection of 1 to 2 ml/kg of 2% Evans’ blue solution was administered to visualize gross damage to the blood-brain barrier. At the end of the experiment, the animals were sacrificed by an intravenous bolus of saturated KCl causing immediate cardiac arrest. This did not markedly affect cerebral K⁺ concentration as shown by comparison with other methods of sacrifice. The cerebral cortex was removed and separated into cortex, caudate nucleus, and periventricular white matter. For each region, water content was determined gravimetrically after drying to constant weight, and Na⁺ and K⁺ contents were evaluated using flame photometry.

Glucose and lactic, pyruvic, and glutamic acid concentrations in the perfusate samples were measured by enzymatic tests after deproteinization and subsequent neutralization with perchloric acid and KOH. Levels of ¹⁴C-inulin were determined by liquid scintillation spectrometry after incubation in 70% perchloric acid (0.2 ml) and 30% hydrogen peroxide (0.2 ml). Bradykinin concentrations were determined by a rat-uterus bioassay as described by Mann, et al., against a bradykinin standard. The uterus of female rats 100 to 200 gm in body weight was sensitized by 0.1 mg diethylstilbestrol 12 to 16 hours prior to sacrifice of the rats. Plasma kininogens were determined after tryptic cleavage as kinins according to the method of Diniz and Carvalho. Acetic acid, 5 ml of 0.1 M, was added to 0.2 ml of perfusate and heated for 30 minutes at 100°C. Then, 0.2 ml of 0.1 M NaOH, 1.5 ml of 0.3 M Tris buffer, and 0.5 ml of 1% trypsin were added. The material was incubated at 37°C for 25 minutes. The proteolytic reaction was terminated by the addition of 0.4 ml (20,000 KIU/ml) aprotinin (Trasylol). The test solutions were stored at −20°C until measurement. The results were statistically evaluated after testing for normal distribution, by the Student t-test, Mann-Whitney U-test, or Wilcoxon test.

Results

Brain Water and Electrolytes

Cerebral water and electrolyte contents of the cortex, caudate nucleus, and white matter in control and experimental animals are given in Table 1. Group 2 animals, perfused with homologous plasma, had a significant increase in water content in the periventricular white matter directly exposed to the perfusate of 2.34% as compared to controls (p < 0.01) corresponding to a swelling of 7.4%. White matter Na⁺ content was increased to more than 20 mM/kg dry weight, but the increase was not significant (Table 1). The cerebral K⁺ content remained virtually unchanged.

Ventricular perfusion with bradykinin led to a marked increase of water content in the white matter, basal ganglia, and cerebral cortex (p < 0.05, < 0.002, and < 0.02, respectively). Formation of edema in the white matter was, however, somewhat less pronounced.

Bradykinin was provided by Sandoz AG, Basel, Switzerland.
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FIG. 2. Clearance of K⁺ and uptake of glucose from perfusates by the brain in eight control dogs and in animals with ventriculocisternal perfusion with plasma (13 dogs) or bradykinin (10 dogs). Values represent the mean ± standard error of the mean. Measurable clearance of K⁺ ions was found only in animals perfused with plasma, probably due to the higher K⁺ concentrations in the plasma perfusate than in normal cerebrospinal fluid (CSF). In the remaining experimental groups, the K⁺ concentration of the perfusate was adjusted to that of CSF. K⁺ clearance from the plasma perfusate became attenuated with continuing ventricular perfusion. Perfusion of the ventricles with the different experimental solutions started at 0 minutes after a preceding control period of 30 minutes using mock CSF. Therefore, K⁺ clearance was negligible at 0 minutes. Appreciable uptake of glucose clearance was also observed only in animals perfused with plasma. A decline in glucose clearance was not observed during ventricular perfusion with plasma. The differences were statistically analyzed by the Student t-test, Mann-Whitney U-test (+), or Wilcoxon test (++).

Cerebrospinal Fluid Formation

The effects of plasma and bradykinin on formation of CSF were analyzed according to the method of Pappenheimer, et al., using ¹⁴C-inulin as a volume marker. Formation of CSF fell continuously in the control group and in animals perfused with homologous plasma. On the other hand, formation of CSF was initially inhibited upon ventricular perfusion with bradykinin, but tended to recover toward the end of the experiment.

Perfusion Fluid after Ventricular Passage

The Na⁺ concentration of the perfusate remained unchanged during exposure to both bradykinin and plasma. Since K⁺ concentration of the plasma perfusate exceeded that of normal CSF, K⁺ ions were cleared from the perfusate in animals subjected to ventricular perfusion with plasma. Adjustment of the K⁺ concentration of the plasma perfusate to that of normal CSF was not attempted prior to ventricular perfusion. With continuing perfusion, cerebral clearance of K⁺ ions from the perfusate became attenuated. The decrease in K⁺ clearance during perfusion with plasma in 13 animals was statistically significant as demonstrated by a
regression analysis ($r = 0.83, p < 0.001$; Fig. 2). Clearance of K$^+$ ions from the perfusate did not occur in the eight control experiments or during perfusion with bradykinin in the 10 dogs in Group 3 where K$^+$ concentration of the perfusate was adjusted to that of normal CSF.

Concentrations of lactic and pyruvic acid in the perfusate remained unchanged during ventricular perfusion with either artificial CSF, plasma, or bradykinin; the osmolarity of the different perfusates was also constant during perfusion. The pH of the plasma perfusate equilibrated at 7.35, and that of artificial CSF, and of the bradykinin perfusate at 7.05. The decrease in pH probably resulted from uptake of CO$_2$ into the perfusates which were buffered only by H$_2$PO$_4^-$/HPO$_4^{2-}$. Again, glucose concentrations of the plasma perfusate were higher than in the perfusates of control animals (artificial CSF) or animals exposed to bradykinin. In the latter groups, glucose levels were adjusted to normal. Thus, in the 13 animals perfused with plasma, glucose was cleared from the perfusate at a rate of 0.5 to 1.0 $\mu$M/min (Fig. 2). This is in agreement with clearance of K$^+$ ions (see above) where perfusate concentrations were above normal. However, glucose concentrations in this group did not decrease to normal.

Glutamate concentration in the plasma perfusate was approximately 30 $\mu$M/liter. Cerebral clearance of glutamate was found in 11 of 13 experiments. A clearance rate of 20 nM/min was observed, resulting in a decrease of glutamate in the perfusate after ventricular passage to 10 $\mu$M/liter. However, release of glutamate from the brain instead of uptake was observed in two animals during ventricular perfusion with plasma. In these experiments, glutamate levels rose to 100 $\mu$M/liter in the cisternal effluent. Interestingly, these animals had the highest increase in periventricular water content. Artificial CSF or bradykinin never induced release of glutamate from cerebral tissue into the perfusate.

In the 10 animals perfused with the peptide, the mean (± standard deviation) bradykinin concentration of the perfusate was 2476 ± 1020 ng/ml (ranging from 1200 to 4100 ng/ml) prior to ventricular perfusion. This was due to a variability of bradykinin concentrations found in batches of the commercially available preparations. Adjustment of bradykinin to obtain equal concentrations in the inflowing perfusates for each experiment was not attempted. During ventricular passage, 60% to 80% of inflowing bradykinin concentration was cleared. This amounts to 1500 to 1900 ng of bradykinin cleared per minute by periventricular brain tissue during passage of the perfusate (Fig. 3). The result demonstrates an enormous capacity of cerebral tissue to clear kinins from the CSF either by uptake or metabolism.

**Consumption of Kininogens by the Brain During Ventricular Passage of Homologous Plasma**

Kininogen concentration in plasma perfusate ranged from 1500 to 4000 ng releasable kinins/ml (mean ± standard deviation: 2686 ± 810 ng/ml). Consumption of the kinin precursor (kininogens), indicative of formation of kinins, was found in five out of nine animals. This was calculated from a decrease of kininogens in the perfusate during ventricular passage to 90% to 50% of the concentration present in the inflowing perfusate (Fig. 4). Potential dilution by endogenous CSF was corrected for by using $^{3}C$-inulin as a volume marker. In another group of four animals, kininogens of the perfusate were unchanged, or somewhat increased after ventricle passage (Fig. 4). Formation of kinins during ventricular perfusion with plasma from 0 to 180 minutes was calculated according to the following equation:

$$
\text{Formation of kinins} = \int_{0}^{180 \text{min}} \left(1 - \frac{K'_{\text{gen,eff}}}{K'_{\text{gen,in}}}\right) \times 100 \cdot dt,
$$

where $K'_{\text{gen,in}}$ = kininogen concentration in the perfusate prior to perfusion (ng/ml), $K'_{\text{gen,eff}}$ = kininogen concentration in cisternal effluent (ng/ml); and $dt$ = differential of $t$ (Fig. 5).

**Discussion**

A mediator of vasogenic brain edema should induce cerebral damage, such as edema, or be released or produced under conditions leading to edema; thus, specific inhibition of its formation or release should limit brain edema secondary to a vasogenic insult. 29 At the moment, no compound has been described that entirely fulfills these requirements. Nevertheless, phys-

**Fig. 3. Upper:** Bradykinin concentrations (mean ± standard error of the mean (SEM) in 10 dogs) in inflowing perfusate (dashed line) and cisternal effluent (dotted line). **Lower:** Cerebral clearance (mean ± SEM) of bradykinin from the perfusate. As seen, 60% to 80% of the inflowing kinin concentration was removed from the perfusate during ventricular passage. The first cisternal effluent sample for assessment of clearance was available 30 minutes after the start of ventricular perfusion with bradykinin. The differences were statistically analyzed by the Student t-test, or Wilcoxon text (+).
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Fig. 4. Time course of kininogen concentrations (mean ± standard error of the mean) in the cisternal effluent in experiments with and without formation of kinins during ventricular perfusion with plasma. Kininogen concentrations of the perfusate prior to ventricle perfusion are given for comparison. The intercepts of regression curves of the kininogen concentrations in in- and outflowing perfusate (upper) differ at p < 0.05, indicative of a significant fall of kininogens during passage of the plasma perfusate through the ventricles. No differences between in- and outflowing concentrations were found in experiments in four animals (lower).

Fig. 5. Regression analysis between the integer of kinin formation and water content in the white matter of animals with consumption of kininogens during ventricular perfusion with plasma. The coordinates of control dogs perfused with artificial cerebrospinal fluid (CSF) are given for comparison. Neither kininogens nor kinins were found in the cisternal effluent of the control animals. FW = fresh tissue weight.
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Perfusion with Plasma

The current studies demonstrate clearly that cerebral administration of plasma causes edema of the periventricular white matter. The accumulation of water was moderate, although statistically significant. This may be due to the relatively short period of ventricular perfusion of only 3 hours before sacrifice, and to restriction of penetration of the perfusate components, for instance, of the plasma proteins into the periventricular tissue. Marmarou, et al., also reported that cerebral exposure to plasma increases the cerebral water content. After infusion of 0.25 ml autologous serum into a cerebral hemisphere of cats, they found that the tissue water content of infused and adjacent areas was elevated for at least 3 days, by which time it had returned to normal if artificial CSF were used instead. The extent of penetration of kininogens from the plasma perfusate into adjacent tissue may have influenced formation of edema. Based on kinetic and ultrastructural studies with labeled albumin or peroxidase, respectively, uptake of the perfusate components into periventricular and subarachnoidal tissue can be assumed. Due to the ubiquitous presence of kininases in the brain were definitely higher in animals perfused with bradykinin than in animals perfused with plasma. Quantitative estimates of bradykinin concentrations in the periventricular tissue are not possible, however, because of the ubiquitous presence of kininases in the brain. Cerebral kininases led to considerable clearance of the peptide during ventricular passage (see Fig. 3), raising the possibility of a pathophysiological role of bradykinin split-products. However, the potency of kinin degradation products on peripheral effects, such as blood pressure or contraction of uterus muscle, were reported as only 0.1% or less, as compared to native kinin. A dose-effect relationship was not observed between the bradykinin concentrations in the perfusate and the increase of water content in cerebral tissue. The bradykinin concentrations used for ventricular perfusion were probably above the dose-dependency range. Hence, concentrations lower than those employed might be sufficient to induce brain edema.

Perfusion with Bradykinin

Formation of edema by cerebral administration of bradykinin has not been observed so far. Kinin concentration in the perfusate entering the brain was about 2500 ng/ml. This concentration corresponds to the amount of kinins maximally releasable from plasma kininogens of the animals used. In this study, the releasable kinin concentration in dogs was 2860 ± 810 ng/ml plasma. However, under physiological conditions, concentrations of free kinins in plasma, as well as in brain tissue, are virtually negligible on account of naturally occurring powerful inhibitors and kininases. The bradykinin concentrations employed in these experiments for ventricular perfusion are therefore clearly in a pathophysiological range. Based on the findings obtained by ventricle perfusion with bradykinin, a marked enhancement of edema formation must be considered if the kininogens present in vasogenic edema fluid were completely converted to kinins.

The bradykinin concentrations fell eventually to approximately 1000 ng/ml during ventricular passage (see Fig. 3). Nevertheless, the actual concentrations of kinins present at the ventricular and subarachnoid surface of the brain were definitely higher in animals perfused with bradykinin than in animals perfused with plasma.
of vasogenic edema, reports abound on tissue damage attributed to the edema fluid. Structural abnormalities from discrete demyelination, or astrocytic swelling in the white matter to widely spread degeneration and formation of cystic necrosis, have been described under experimental and clinical conditions where damage by the primary lesion can be ruled out.

Homologous instead of autologous plasma was used for ventriculocisternal perfusion in order to have sufficient quantities of the perfusate. This raises the possibility of an involvement of immunological mechanisms. Damage by anti-brain antibodies present in the donor plasma might be considered. However, to the best of our knowledge, there is no evidence of the presence of spontaneous anti-brain antibodies in dogs. Cross matches for blood transfusion between donor and recipient dogs initially conducted in the present study were always negative. The formation of edema by activation of immunological pathways appears unlikely.

A few additional points may be discussed concerning the specificity of the KK system in formation of edema during ventricular perfusion with plasma. Certainly, the evidence available at the moment on the specificity of the system as an edematogenic mediator in plasma is as yet incomplete, although many findings are impressive enough to make further efforts worthwhile. The following results strongly support a role of the system in vasogenic edema. Besides an induction of edema by ventricular perfusion with bradykinin, it was recently observed that superfusion of the pia-arachnoid surface of the brain with the peptide increases the permeability of the blood-brain barrier to intravenously injected indicators, such as Na+ fluorescein. Moreover, formation of kinins was found in experimental vasogenic edema in focal and perifocal tissue areas, which was enhanced by additional ischemia.

In this study, of nine animals with ventricular plasma perfusion, five exhibited formation of kinins in relation to the extent of brain edema (Fig. 5). We have found that in vivo inhibition of kallikrein (the enzyme that activates the KK system) by a protease inhibitor (aprotinin) significantly reduces the extent of brain swelling secondary to cold injury. (A. Unterberg, et al., in preparation). The findings taken together fulfill almost completely the requirements necessary for identification of a mediator compound (see above). Additional evidence may be obtained by cerebral exposure to plasma after selective elimination of the components of the KK system. For that purpose, purification procedures of the perfusate were required to remove kininogens exclusively, but no other plasma proteins. However, such a method is not available for dog plasma. Alternatively, depletion of plasma kininogens might be attempted by the addition of proteolytic enzymes, such as kallikrein or trypsin. However, the perfusate would then be contaminated with material which itself damages cerebral tissue.

The fact that brain edema results from the mere contact of cerebral tissue with plasma, as currently demonstrated, is certainly important. It suggests again that vasogenic edema fluid has a potential for damage independent from its other effects, such as intracranial space occupation, or depression of cerebral blood flow.

**Nature of Brain Edema Induced by Kinins**

Only a few pathophysiological observations on kinins pertain to cerebral edema. Injection of plasma proteins into the cisterna magna of rabbits increased blood-brain barrier permeability to intravascular albumin. The simultaneous administration of a kallikrein-inhibitor (aprotinin) inhibited this effect, suggesting involvement of kinins. Our findings agree only partially with these observations. In the current studies, penetration of intravenous Evans blue into cerebral tissue was only seen in areas adjacent to the perfusion cannulas. We have found in recent studies that gross opening of the blood-brain barrier by kinins is unlikely. Superfusion of feline cerebral cortex with bradykinin in mock CSF led to penetration of intravenously injected Na+ fluorescein, but not of dextran, which is indicative of selective rather than complete loss of barrier function caused by kinins. This, however, may suffice to enhance cerebral uptake of electrolytes and water in pathophysiological conditions. Additional vasodilation induced by kinins would increase such an edema mechanism.

Although the evidence so far accumulated is incomplete as to a mediator function of kinins in brain edema, the presently available results provide substantial support for such a role, making the KK system a worthwhile candidate for further investigations.

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**References**

6. Blasberg RG: Clearance of serum albumin from brain
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