Inhibition of Bradykinin- and Kallikrein-Induced Cerebral Arteriolar Dilation by a Specific Bradykinin Antagonist

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We have previously shown that topical brain application of kallikrein, an enzyme which converts kininogen to bradykinin, induces rabbit pial arteriole dilation. The purpose of the present investigation was to utilize a newly developed competitive kinin receptor antagonist to test the hypothesis that kallikrein-induced dilation was due to the conversion of brain kininogen to vasoactive kinins. As in our previous study, we measured rabbit pial arteriole diameter with a microscope using the closed cranial window technique. The kinin antagonist (6 μM) reduced the dose-dependent dilation produced by bradykinin and blocked the dilation induced by kallikrein. In addition, the kinin antagonist was specific since it did not alter the cerebral arteriole dilation produced by adenosine, acetylcholine, or vasoactive intestinal polypeptide. These experiments provide further evidence for a possible role of the endogenous brain kallikrein-kinin system in the modulation of the cerebral circulation and provide the necessary pharmacologic foundation for future use of this antagonist in testing the role of kinins in the normal or altered cerebral circulation. (Stroke 1987;18:792-795)

Materials and Methods

A total of 48 male New Zealand white rabbits weighing 3.3–4.2 kg were studied under anesthesia produced by 25 mg/kg i.v. sodium pentobarbital, 560 mg/kg subcutaneous brethane, and 38 mg/kg subcutaneous α-chloralose as previously reported. Supplemental doses of pentobarbital were given as needed to maintain anesthesia. Under this regimen, surgical anesthesia was quickly induced with minimum respiratory depression, and the need for additional doses of pentobarbital was reduced.

After completion of a tracheotomy, each rabbit was ventilated with room air. The end-expiratory CO₂, of each rabbit was continuously monitored with an Avarad Inc. infrared CO₂ analyzer (Santa Barbara, Calif.) and was maintained at approximately 30 mm Hg throughout each experiment by adjusting the respirato-
ry rate and volume. Arterial blood pressure was measured with a Statham P23Db pressure transducer connected to a cannula inserted into the left femoral artery. Arterial blood samples were periodically analyzed with an Instrumentation Laboratory blood pH-blood gas analyzer to ensure a normal range of $P_{O_2}$, $P_{CO_2}$, and blood pH.

Pial arterioles ranging between 29 and 100 μm in diameter were examined using the previously described cranial window technique. The 10-mm diam. cranial window was implanted on the midline just caudal to the suture connecting the frontal and parietal cranial window was equipped with 3 openings; 2 were used as inlet and outlet for filling the space under the cranial window with test solutions, and the third was connected to a Statham pressure transducer for continuous measurement of intracranial pressure (ICP). The ICP outlet of the window was connected to plastic tubing, the open end of which was placed at a fixed level to give a constant ICP of 5 mm Hg throughout the experiment. The space under the window and the plastic tubing connected to it were filled with artificial cerebrospinal fluid (CSF) equilibrated with gas containing 6.6% oxygen, 5.9% carbon dioxide, and 87.5% nitrogen, which gives gas tensions and a pH in the normal range for CSF. The experiments were conducted using groups of 3–7 rabbits.

Bradykinin triacetate, kallikrein (from porcine pancreas), acetylcholine, adenosine, and indomethacin were purchased from Sigma Chemical Co. (St. Louis, Mo.), and vasoactive intestinal polypeptide (VIP) and Substance P were obtained from Peninsula Laboratories (Belmont, Calif.). All reagents were directly dissolved in distilled water to produce a stock solution of 10 KU/ml that was added to artificial CSF to produce a final concentration of 1, 0.3, or 0.1 KU/ml CSF. All agents were applied topically; 1 ml of artificial CSF containing the agent or agents to be examined was perfused through the inlet and outlet ports over a period of approximately 1 minute. Arteriole diameter was then measured 2 and 5 minutes after completion of drug application.

The data describing the dose response to BK in the presence or absence of the BK antagonist were analyzed by repeated-measures analysis of variance (ANOVA) followed by examination of the group × dose interaction effect. The effect of the BK antagonist on kallikrein-induced dilation was evaluated by ANOVA and Tukey's Studentized range test. A $p$ value $\leq 0.05$ was considered to be statistically significant.

### Figure 1
Comparison of amino acid sequence of bradykinin (BK) and BK antagonist. Replacement of proline residue at Position 7 of BK with a $\alpha$-phenylalanine (DPhc) residue converts BK to BK antagonist. Replacement of phenylalanine residues at Positions 5 and 8 with isosteric $\beta$(2-thienyl)-alanine (Thi) increases potency of the BK antagonist. This antagonist is specific for the kinins, including BK, kallidin (lys-bradykinin) and metlys-bradykinin.

### Figure 2
The effect of topically applied bradykinin antagonist (BK + ANTAG) on bradykinin-induced pial arteriole dilation (mean ± SEM). The bradykinin only (BK) data ($\Delta$) was gathered from 17 arterioles in 5 rabbits. In a separate group of 23 arterioles in 7 rabbits, we tested the effect of 6 μM antagonist alone and 6 μM antagonist in the presence of increasing doses of BK (○). The dose-response curves for group × dose interactions were different at $p < 0.02$, indicating that the antagonist reduced the effect of exogenous BK.

Results and Discussion

The mean arterial blood pressure in all rabbits was $88 ± 1$ mm Hg (mean ± SEM) and therefore was similar to that reported for unanesthetized rabbits. Arterial blood gases and pH were $P_{O_2}, 91 ± 1$ mm Hg; $P_{CO_2}, 30 ± 1$ mm Hg; and pH, $7.49 ± 0.01$. Topical application of the various agents to the pial arterioles had no effect on systemic arterial blood pressure, blood gases, or pH.

Figure 1 compares the amino acid composition of BK and the BK antagonist. Figure 2 shows that 6 μM BK antagonist alone induced a slight but significant
(p<0.05) pial arteriole dilation. The antagonist blocked the effect of 8 x 10^{-7} M BK and reduced the response to 8 x 10^{-6} M BK by 40%. ANOVA was performed followed by examination of the group x dose interaction; the interaction was different at p<0.02. The dose-response curves of BK only and BK plus antagonist were different, indicating that the antagonist significantly reduced the response to BK. Limited availability of antagonist prevented our testing the effects of higher concentrations of antagonist; however, a higher concentration would likely have more completely blocked the effect of 8 x 10^{-6} M BK since the antagonist acts competitively.

Figure 3 shows that increasing doses of kallikrein produced a dose-dependent arteriole dilation. These results, like our previous studies, show that the dilation induced by topical application of 1 KU/ml CSF is approximately equivalent to the dilation produced by 8 x 10^{-6} M exogenous BK (Figure 2). Since previous reports of BK and kininogen levels in whole brain tissue are orders of magnitude lower than 8 x 10^{-6} M,^{11-13} we hypothesize that the kininogen which is acted upon by kallikrein must be in or near the vascular BK receptors, such that kallikrein induces a local kinin concentration equivalent to that produced by 8 x 10^{-6} M exogenous BK. However, if this highly localized concentration were diluted by more distant tissue, as in analysis of whole brain, the BK concentration per gram of whole tissue would be much less.

Figure 3 again shows that the antagonist by itself produces a 6% dilation. ANOVA of the kallikrein and kallikrein plus antagonist responses shown in Figure 3 indicated a significant difference at p<0.0001. Analysis by Tukey’s Studentized range test showed that the response to each dose of kallikrein alone was significantly different from the response to the same dose of kallikrein in the presence of the BK antagonist. Since the antagonist completely blocked the response to 0.1 and 0.3 KU/ml CSF, these results imply that the kallikrein-induced dilation at these concentrations is due solely to the formation of kinin.

Vavrek and Stewart have previously reported that the BK antagonist which we employed has no effect on the rat blood pressure, rat uterus, or guinea pig ileum responses to Angiotensin II or Substance P. However, we wished to determine if the BK antagonist was a specific inhibitor in the cerebral circulation. We therefore tested the effect of the BK antagonist on the response to adenosine, acetylcholine, or VIP, 3 chemically dissimilar agonists known to produce cerebral arteriole dilation. Each agent was employed at approximately its ED_{50}, as determined by our published and unpublished investigations in rabbits, because that concentration allowed us to determine if the antagonist increased or decreased the response to each dilator. Figure 4 shows that the BK antagonist did not affect cerebral arteriole dilation in response to adenosine, acetylcholine, or VIP, providing further proof that the BK antagonist specifically blocks the action of kinins. We attempted to study the effect of the BK antagonist on Substance P activity; however, this was not completed since in 18 arterioles in 6 rabbits we found that 8 μM Substance P induced only 9 ± 1% arteriolar dilation.

The current results are among the first to show that the competitive kinin antagonist developed by Vavrek and Stewart can block kinins generated from endogenous kininogen. These results strengthen our previous suggestion that the brain kallikrein-kinin system may play a role in modulating the cerebral circulation. In addition, our current data provide the pharmacologic foundation for future studies using the BK antagonist to explore the possible role of the kallikrein-kinin system in the brain’s cerebrovascular response to several pathophysiologic phenomena. For example, Wei et al and Kontos et al have shown that concussive brain injury or acute hypertension injure cerebral vessels by a cyclooxygenase-dependent mechanism. We
suggest that the kallikrein-kinin system may be a biochemical link between brain injury or acute hypertension and the stimulation of cyclooxygenase activity. Furthermore, BK is known to cause cerebral edema, and experimental cerebral edema is associated with increased brain levels of BK.16,17 The BK antagonist should be useful in further probing the specific role of the endogenous kallikrein-kinin system and the therapeutic potential of the BK antagonist in these pathophysiologic phenomena.

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References


Key Words: bradykinin antagonist • kinin cerebral blood flow • brain microcirculation • kallikrein
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