Role of Peptidases in Bradykinin-Induced Increase in Vascular Permeability In Vivo


The purpose of this study was to examine whether neutral endopeptidase and angiotensin I–converting enzyme, two membrane-bound metalloenzymes that are widely distributed in the microcirculation, play a role in bradykinin-induced increase in vascular permeability in the hamster cheek pouch. Changes in vascular permeability were quantified by counting the number of leaky sites and by calculating the clearance of fluorescein isothiocyanate (FITC)–dextran (molecular mass, 70,000 d) during suffusion of the cheek pouch with bradykinin. Bradykinin produced a concentration- and time-dependent increase in the number of leaky sites and clearance of FITC-dextran. The selective, active site–directed neutral endopeptidase inhibitors phosphoramidon (1.0 μM) and thiorphan (10.0 μM) and the selective angiotensin I–converting enzyme inhibitor captopril (10.0 μM) each shifted the concentration–response curve to bradykinin significantly to the left. During suffusion with bradykinin (1.0 μM) and phosphoramidon, the number of leaky sites increased significantly from 17±2 to 27±4 sites per 0.11 cm² (mean±SEM, p<0.05), and FITC-dextran clearance increased significantly from 1.0±2 to 2.1±0.3 ml/sec×10⁻⁴. During suffusion with bradykinin (1.0 μM) and captopril, the number of leaky sites increased significantly from 10±2 to 41±3 sites per 0.11 cm², and FITC-dextran clearance increased significantly from 0.8±0.3 to 3.2±0.8 ml/sec×10⁻⁴. During suffusion with bradykinin (1.0 μM) and thiorphan, the number of leaky sites increased significantly from 15±3 to 47±7 sites per 0.11 cm², and FITC-dextran clearance increased significantly from 0.8±0.2 to 4.7±0.6 ml/sec×10⁻⁴. Suffusion of both phosphoramidon and captopril was associated with an additive effect on bradykinin-induced responses. Other proteinase inhibitors had no significant effect on bradykinin-induced increase in vascular permeability. In addition, adenosine (1.0 μM)-induced increase in leaky site formation was not potentiated by phosphoramidon and captopril. We conclude that neutral endopeptidase and angiotensin I–converting enzyme each play an important role in modulating bradykinin-induced increase in vascular permeability in vivo. (Circulation Research 1992;70:952–959)

Key Words • angiotensin I–converting enzyme • neutral endopeptidase • proteinase inhibitors • microcirculation • inflammation • hamsters

One characteristic feature of inflammation is an increase in postcapillary venular permeability to plasma proteins. Although the sequence of events that leads to the increase in vascular permeability during inflammation is still unknown, current concepts suggest that perturbation of endothelial cell integrity in postcapillary venules plays a major role. Vascular endothelial cells are the target of the potent nonapeptide bradykinin, which is generated and released locally at sites of tissue injury and inflammation.

One component of the inflammatory response to bradykinin is an increase in vascular permeability to plasma proteins. This increase promotes the development of localized edema that may disrupt regional hemodynamics and amplify tissue injury.

Little is known about the factors that abrogate the increase in vascular permeability induced by bradykinin in vivo. Bradykinin is hydrolyzed very efficiently by a variety of peptidases in vitro. However, their relative contribution to bradykinin degradation in the microcirculation in vivo remains unclear. Vascular endothelial cells possess surface peptidases, neutral endopeptidase (NEP, EC 3.4.24.11) and angiotensin I–converting enzyme (ACE, EC 3.4.15.1), that hydrolyze bradykinin at the Pro³–Phe⁴ bond to inactive fragments 1-7 and 8-9. The location of NEP and ACE in anatomic proximity to the receptors of bradykinin on postcapillary venular endothelial cells suggests that they may play an important role in modulating the edema-forming effects of the peptide in vivo.

We postulated that NEP and ACE each play an important role in modulating the edema-forming effects of bradykinin in vivo. We reasoned that if endogenous NEP and ACE degrade bradykinin to inactive fragments, then selective pharmacological inhibition of
these peptidases (but not of other proteinases) should potentiate bradykinin-induced increase in vascular permeability. This potentiation may result from a decrease in peptide degradation leading to higher concentrations of bradykinin near or at postcapillary venular endothelial cell membrane receptors. To test our hypothesis, we used the hamster cheek pouch model, because it has been used previously to study bradykinin-induced changes in vascular permeability and because it contains both NEP- and ACE-like activities (I. Rubinstein, I.F. Ueki, and J.A. Nadel; personal communications). We found that selective, active site–directed NEP and ACE inhibitors significantly potentiated bradykinin-induced increase in vascular permeability in the hamster cheek pouch and that these effects were specific.

**Materials and Methods**

**Experimental Techniques**

**Preparation of animals.** Adult hamsters weighing 120–140 g were anesthetized with pentobarbital sodium (6 mg/100 g body wt i.p.). A tracheostomy was performed to facilitate spontaneous breathing. The left femoral vein was cannulated to inject the intravascular tracer fluorescein isothiocyanate (FITC)-dextran (molecular mass, 70,000 d; 40 mg/100 g body wt) and supplemental anesthesia (2–4 mg/100 g body wt/hr). The left femoral artery was cannulated to obtain arterial blood samples and to measure arterial blood pressure during the experiments. To visualize the microcirculation of the hamster cheek pouch, we used a method that has been described by Mayhan and Joyner. Briefly, the left cheek pouch of the hamster was spread gently over a small plastic baseplate, and an incision was made in the outer skin to expose the cheek pouch membrane. The avascular connective tissue layer was carefully cut away so as not to damage the microvasculature of the cheek pouch. An upper plastic chamber was positioned over the baseplate and secured in place by suturing the skin around the upper chamber with a purse-string technique. This chamber was used to contain the suffusion fluid. This arrangement forms a triple-layered complex: the baseplate, the upper chamber, and the cheek pouch membrane exposed between the two plates.

After these initial procedures, the hamster was transferred to a heated microscope stage. The chamber was connected to a reservoir containing bicarbonate buffer, which allows continuous suffusion of the cheek pouch. The bicarbonate buffer was bubbled continuously with 95% nitrogen and 5% carbon dioxide (pH 7.4), and its temperature was maintained constant (36–37°C). The chamber was also connected via a three-way valve to an infusion pump (Sage Instruments, Cambridge, Mass.) that allowed constant administration of drugs into the suffusate.

**Determination of microvascular leaky sites.** The cheek pouch microcirculation was visualized using an Olympus microscope (Jacobs Instruments, Shawnee Mission, Kan.) coupled to a 100-W mercury light source. Fluorescence microscopy was accomplished with the aid of filters that matched the spectral characteristics of FITC-dextran.

Macromolecular leakage was determined by the extravasation of FITC-dextran, which appeared as fluorescent “spots” or leaky sites. The number of leaky sites was determined by counting three random fields every minute for the first 7 minutes and then at 5-minute intervals for 30 minutes after each intervention. The number of leaky sites observed in these three fields was averaged and expressed as the number of leaky sites per 0.11 cm² of tissue, which is the area of one microscopic field.

**Determinations of microvascular clearance of FITC-dextran.** In experiments in which clearance of FITC-dextran was calculated, the suffusion fluid was collected at 5-minute intervals throughout the experiment using a fraction collector (Micro-fractionator, Gilson Medical Electronics, Inc., Middleton, Wis.). Samples were collected in glass test tubes, and the concentration of FITC-dextran was determined. Arterial blood samples were collected in heparinized capillary tubes (70 μl volume, Scientific Products, McGaw Park, Ill.) beginning 5 minutes before and 10, 50, 100, and 160 minutes after injection of FITC-dextran in all successive experimental procedures. All plasma samples were analyzed for FITC-dextran concentration.

To quantitate the concentration of FITC-dextran in the plasma and suffusate, a standard curve for FITC-dextran concentration versus percentage emission was performed on a spectrophotofluorometer (The Perkin-Elmer Corp., Norwalk, Conn.). The standard was FITC-dextran that was prepared on a weight-per-volume basis. Using bicarbonate buffer as background, a standard curve was generated for each experiment, and each curve was subjected to linear regression analysis. The percent emission for unknown samples (plasma and suffusate) was measured on the spectrophotofluorometer, and the concentration of FITC-dextran was calculated from the standard curve. In preliminary experiments, minimal fluorescence signal (<2% above background) was detected when drugs were added to the bicarbonate buffer and when plasma and suffusate samples were examined before the addition of FITC-dextran.

Clearance of FITC-dextran was determined by calculating the ratio of suffusate (in nanograms per milliliter) to plasma (in milligrams per milliliter) concentration of FITC-dextran and multiplying this ratio by the suffusate flow rate (2 ml/min).

**Experimental Protocols**

**Effects of phosphoramidon on bradykinin-induced increase in vascular permeability.** In seven hamsters, the cheek pouch was first suffused with bicarbonate buffer for 30 minutes (equilibration period). FITC-dextran was injected, and the number of leaky sites and the clearance of FITC-dextran were determined for 30 minutes. Then, increasing concentrations of bradykinin (0.1, 0.5, and 1.0 μM; 5 minutes each) were suffused over the cheek pouch microcirculation. The time interval between each subsequent application of bradykinin was at least 30 minutes. It has been shown previously that in the hamster cheek pouch intermittent stimulation with bradykinin produced a reproducible response that is not reduced when applied at 30-minute intervals. This time interval was applied in all subsequent experiments, including those using bradykinin fragments (see below).
The number of leaky sites was determined every minute for 7 minutes and at 5-minute intervals for 30 minutes thereafter. Clearance of FITC-dextran was determined at 5-minute intervals for 30 minutes. After suffusion of the final dose of bradykinin (1.0 μM) and determining the number of leaky sites and clearance of FITC-dextran for 30 minutes, phosphoramidon (1.0 μM) was suffused over the cheek pouch microcirculation for 30 minutes. The number of leaky sites and FITC-dextran clearance were determined during this 30 minutes as described above. Then, bradykinin suffusion was repeated as described above. The concentration of phosphoramidon used in these experiments was based on data in the literature. In preliminary experiments, we determined that repeated exposures of the hamster cheek pouch microcirculation to bradykinin (0.1, 0.5, and 1.0 μM) before and after suffusion with saline (vehicle of peptidase inhibitors) for 30 minutes was associated with reproducible increases in vascular permeability (data not shown).

Effects of thiorphan on bradykinin-induced increase in vascular permeability. In seven hamsters, the cheek pouch was first suffused with bicarbonated buffer alone for 30 minutes. FITC-dextran was injected, and the number of leaky sites was determined for 30 minutes. Then, increasing concentrations of bradykinin (0.1, 0.5, and 1.0 μM; 5 minutes each) were suffused over the cheek pouch microcirculation. The number of leaky sites was determined every minute for 7 minutes and at 5-minute intervals for 30 minutes thereafter. Clearance of FITC-dextran was determined at 5-minute intervals for 30 minutes. After suffusion of the final dose of bradykinin (1.0 μM) and determination of the number of leaky sites and clearance of FITC-dextran for 30 minutes, thiorphan (10.0 μM) was suffused over the cheek pouch microcirculation for 30 minutes. The number of leaky sites and FITC-dextran clearance were again determined during this 30-minute period. Then, bradykinin suffusion was repeated as described above. The concentration of thiorphan used in these experiments was based on data in the literature.

Effects of captopril on bradykinin-induced increase in vascular permeability. In four hamsters, the cheek pouch was first suffused with bicarbonated buffer alone for 30 minutes. FITC-dextran was injected, and the number of leaky sites was determined for 30 minutes. Then, increasing concentrations of bradykinin (0.1, 0.5, and 1.0 μM; 5 minutes each) were suffused over the cheek pouch microcirculation. The number of leaky sites was determined every minute for 7 minutes and at 5-minute intervals for 30 minutes thereafter. Clearance of FITC-dextran was determined at 5-minute intervals for 30 minutes. After suffusion of the final dose of bradykinin (1.0 μM) and determination of the number of leaky sites and clearance of FITC-dextran for 30 minutes, captopril (10.0 μM) was suffused over the cheek pouch microcirculation for 30 minutes. The number of leaky sites and FITC-dextran clearance were again determined during this 30-minute period. Then, bradykinin suffusion was repeated as described above. The concentration of captopril used in these experiments was based on data in the literature.

Effects of phosphoramidon and captopril together on bradykinin-induced increase in vascular permeability. In preliminary experiments, we suffused the hamster cheek pouch with bradykinin (1.0 μM) for 10 minutes and after 10 minutes immediately after suffusing phosphoramidon (1.0 μM) and captopril (10.0 μM) for 30 minutes as described above. Samples of the suffusate (1 ml) were removed at the end of each suffusion period of bradykinin into test tubes containing glacial acetic acid (50 μl). The concentration of bradykinin in each sample was determined by reverse-phase high-performance liquid chromatography (Perkin-Elmer) as previously described. Briefly, 500 μl suffusate was injected onto a 0.46x25-cm Vydac 21TP54 column equilibrated with 0.1% (vol/vol) trifluoroacetic acid at a flow rate of 1.5 ml/min. The concentration of acetonitrile in the eluting solvent was raised to 35% (vol/vol) over 30 minutes using a linear gradient. Bradykinin was eluted from the column with a retention time of 25.9 minutes, and its concentration in the suffusate was estimated from the area of the peak. We found that the concentration of bradykinin in the suffusate containing the ACE and NEP inhibitors was 17.6% higher than that in the control suffusate. These data indicate that NEP and ACE both degrade bradykinin in the hamster cheek pouch.

Next, to determine whether the inhibition of both NEP and ACE together was associated with a positive effect on bradykinin-induced increase in vascular permeability in the hamster cheek pouch, we determined the increase in the number of leaky sites and in the clearance of FITC-dextran in response to bradykinin (0.1 and 0.5 μM) in two groups of animals. In one group, the response was determined before and after suffusing phosphoramidon (1.0 μM, 30 minutes) first and then captopril (10.0 μM, 30 minutes); in the second group, the order of suffusion of the NEP and ACE inhibitors was reversed.

Effects of bradykinin fragments on vascular permeability. In this series of experiments, we examined the effects of the major peptide fragments of bradykinin hydrolysis by NEP and ACE (fragment 1-7 and fragment 8-9) on vascular permeability in the hamster cheek pouch. In three animals, increasing concentrations of fragment 1-7 (0.1, 0.5, and 1.0 μM; 5 minutes each) were suffused over the hamster cheek pouch microcirculation before and after suffusing phosphoramidon (1.0 μM, 30 minutes) or captopril (10.0 μM, 30 minutes). The number of leaky sites and clearance of FITC-dextran were determined after each intervention as described above. Similarly, in three animals, increasing concentrations of fragment 8-9 (0.1, 0.5, and 1.0 μM; 5 minutes each) were suffused before and after suffusing phosphoramidon or captopril as described above.

Effects of adenosine on vascular permeability. To examine the specificity of phosphoramidon and captopril potentiation of bradykinin-induced increase in vascular permeability, we examined the effects of phosphoramidon (1.0 μM) and captopril (10.0 μM) on adenosine-induced increase in vascular permeability in the hamster cheek pouch. Adenosine, as well as bradykinin, induces microvascular leaky sites in the hamster cheek pouch at postcapillary venules through a receptor-mediated mechanism. In three animals, the cheek pouch was first suffused with bicarbonated buffer for a 30-minute equilibration period. FITC-dextran was injected, and the number of leaky sites was determined for 30 minutes. Then, adenosine (1.0 μM) was suffused
over the cheek pouch microcirculation for 5 minutes. The number of leaky sites was determined every minute for 7 minutes and at 5-minute intervals for 30 minutes thereafter. After the number of leaky sites returned to control, phosphoramidon (1.0 μM) was suffused over the cheek pouch microcirculation for 30 minutes. The number of leaky sites was determined at 5-minute intervals during this period. Then, suffusion of adenosine was repeated as described above. In three additional animals, the same experimental protocol was repeated except that captopril (10.0 μM) was substituted for phosphoramidon.

**Effects of other proteinase inhibitors on bradykinin-induced increase in vascular permeability.** To determine whether other proteinase inhibitors modulate bradykinin-induced increase in vascular permeability in the hamster cheek pouch, we examined the effects of a combination of leupeptin (10.0 μM), bestatin (10.0 μM), and DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (MGTA, 10.0 μM) to inhibit aminopeptidases, thiol proteinases, and carboxypeptidase N, respectively, on bradykinin-induced responses. In four animals, the cheek pouch was first suffused with bicarbonate buffer for 30 minutes. FITC-dextran was injected, and the number of leaky sites was determined for 30 minutes. Then, increasing concentrations of bradykinin (0.1, 0.5, and 1.0 μM; 5 minutes each) were suffused over the cheek pouch microcirculation. The number of leaky sites was determined every minute for 7 minutes and at 5-minute intervals for 30 minutes thereafter. After the number of leaky sites returned to control, the combination of the proteinase inhibitors was suffused over the cheek pouch microcirculation for 30 minutes, and the number of leaky sites was determined during this period. Then, suffusion of bradykinin was repeated as described above.

**Drugs**

FITC-dextran, bradykinin, phosphoramidon, DL-thiorphan, and bradykinin fragment 1-7 were purchased from Sigma Chemical Co., St. Louis, Mo. Bradykinin fragment 8-9 was purchased from BACHEM Bioscience Inc., Philadelphia, Pa. Leupeptin and bestatin were purchased from Peninsula Laboratories, Belmont, Calif., MGTA was purchased from Calbiochem Corp., San Diego, Calif. Captopril was a gift from E.R. Squibb & Sons, Inc., Princeton, N.J. The drugs were prepared daily before each experiment and were diluted with saline to the desired concentrations.

**Data Analysis**

Data are expressed as mean±SEM where appropriate. The concentration–response curves were compared by a two-way analysis of variance and Newman–Keuls test for multiple comparisons. An unpaired Student’s t test was used to compare the effects of phosphoramidon, captopril, and thiorphan. A value of p<0.05 was considered significant.

**Results**

**Effects of Bradykinin on Vascular Permeability**

Bradykinin induced a significant time- and concentration-dependent increase in the number of leaky sites and clearance of FITC-dextran in the hamster cheek pouch (Figure 1, n=7). Leaky sites occurred around postcapillary venules ~2 minutes after the start of bradykinin suffusion and reached a maximum at 5–7 minutes. The changes in vascular permeability returned to control values within 30 minutes after suffusion of bradykinin was stopped.

**Effects of Phosphoramidon on Bradykinin-Induced Increase in Vascular Permeability**

Phosphoramidon significantly potentiated bradykinin-induced increases in the number of leaky sites and clearance of FITC-dextran at all bradykinin concentrations tested (Figure 1, p<0.05, n=7). During suffusion with the highest concentration of bradykinin (1.0 μM) in the presence of phosphoramidon (1.0 μM), the number of leaky sites increased significantly from 17±2 sites per 0.11 cm² before suffusion to 27±4 sites per 0.11 cm² after suffusion with phosphoramidon (p<0.05); likewise, clearance of FITC-dextran increased significantly from 1.0±0.2 ml/sec×10⁻⁶ before suffusion to 2.1±0.3 ml/sec×10⁻⁶ after suffusion with phosphoramidon (p<0.05). Suffusion with phosphoramidon alone did not induce any leaky site formation nor an increase...
in clearance of FITC-dextran (0.2±0.1 ml/sec×10^-6 under control conditions and 0.3±0.1 ml/sec×10^-6 during suffusion of phosphoramidon; p=NS).

**Effects of Thiorphan on Bradykinin-Induced Increase in Vascular Permeability**

Thiorphan significantly potentiated bradykinin-induced increases in the number of leaky sites and clearance of FITC-dextran at all bradykinin concentrations tested (Figure 2, p<0.05, n=7). During suffusion with the highest concentration of bradykinin (1.0 µM) in the presence of thiorphan (10.0 µM), the number of leaky sites increased significantly from 14±2 sites per 0.11 cm² before suffusion to 47±7 sites per 0.11 cm² after suffusion with thiorphan (p<0.05); likewise, clearance of FITC-dextran increased significantly from 0.8±0.2 ml/sec×10^-6 before suffusion to 4.7±0.6 ml/sec×10^-6 after suffusion with thiorphan (p<0.05). Suffusion with thiorphan alone did not induce any leaky site formation nor increase in clearance of FITC-dextran (0.3±0.1 ml/sec×10^-6 under control conditions and 0.3±0.1 ml/sec×10^-6 during suffusion of thiorphan; p=NS).

**Effects of Captopril on Bradykinin-Induced Increase in Vascular Permeability**

Captopril significantly potentiated bradykinin-induced increases in the number of leaky sites and clearance of FITC-dextran at all bradykinin concentrations tested (Figure 3, p<0.05, n=4). During suffusion with the highest concentration of bradykinin (1.0 µM) in the presence of captopril (10.0 µM), the number of leaky sites increased significantly from 10±2 sites per 0.11 cm² before suffusion to 40±3 sites per 0.11 cm² after suffusion with captopril (p<0.05); likewise, clearance of FITC-dextran increased significantly from 0.8±0.3 ml/sec×10^-6 before suffusion to 3.2±0.8 ml/sec×10^-6 after suffusion with captopril (p<0.05). Suffusion with captopril alone did not induce leaky site formation or increase in clearance of FITC-dextran (0.3±0.1 ml/sec×10^-6 under control conditions and 0.3±0.1 ml/sec×10^-6 during suffusion of captopril; p=NS).

**Effects of Phosphoramidon and Captopril Together on Bradykinin-Induced Increase in Vascular Permeability**

The potentiation of bradykinin-induced increase in vascular permeability by the combination of phosphoramidon and captopril was greater than that induced by either of these inhibitors alone (Figure 4, p<0.05, n=8). The effects of phosphoramidon and captopril were additive. There was no significant difference in the potentiation of bradykinin-induced responses whether phosphoramidon (Figures 4A and 4C) or captopril (Figures 4B and 4D) was suffused first over cheek pouch

![Figure 2. Bar graphs showing the effects of bradykinin on the formation of microvascular leaky sites (panel A) and clearance of fluorescein isothiocyanate–dextran (panel B) in the absence (open bars) and presence (shaded bars) of thiorphan (10.0 µM). All values are mean±SEM; *p<0.05 versus response in the absence of thiorphan.](image)

![Figure 3. Bar graphs showing the effects of bradykinin on the formation of microvascular leaky sites (panel A) and clearance of fluorescein isothiocyanate–dextran (panel B) in the absence (open bars) and presence (shaded bars) of captopril (10.0 µM). All values are mean±SEM; *p<0.05 versus response in the absence of captopril.](image)
concentration of fragment 8-9 (1.0 μM), clearance of FITC-dextran was similar to that of the control value (0.3±0.1 ml/sec×10⁻⁶ before and 0.2±0.1 ml/sec×10⁻⁶ after suffusion fragment 8-9; p=NS, n=3).

**Effects of Adenosine on Vascular Permeability**

Adenosine (1.0 μM) induced a significant increase in the number of leaky sites in the hamster cheek pouch. However, suffusion with phosphoramidon (1.0 μM, n=3) or captopril (10.0 μM, n=3) had no effect on adenosine-induced increase in the number of leaky sites (14±4 sites per 0.11 cm² before and 14±4 sites per 0.11 cm² after suffusion with phosphoramidon, and 16±4 sites per 0.11 cm² before and 16±4 sites per 0.11 cm² after suffusion with captopril; p=NS).

**Effects of Other Proteinase Inhibitors on Bradykinin-Induced Increase in Vascular Permeability**

Suffusion of the hamster cheek pouch with a combination of the proteinase inhibitors leupeptin, bestatin, and MGTA (10.0 μM each) had no significant effect on bradykinin-induced increase in the number of leaky sites. The number of leaky sites was 4±2 and 12±1 sites per 0.11 cm² during suffusion of 0.5 and 1.0 μM bradykinin alone, respectively, versus 7±3 and 16±2 sites per 0.11 cm² during suffusion of 0.5 and 1.0 μM bradykinin, respectively, with the proteinase inhibitors (n=4).

**Discussion**

Our data show that selective pharmacological inhibition of NEP and ACE potentiated a bradykinin-induced increase in vascular permeability in the hamster cheek pouch, most likely by inhibiting peptide degradation. The similar magnitude and additive effects of NEP and ACE inhibitors in potentiating bradykinin-induced responses suggest that these two enzymes have comparable physiological roles in regulating the edema-forming effects of bradykinin in the peripheral microcirculation. These effects were specific, because NEP and ACE inhibitors did not potentiate the receptor-mediated increase in vascular permeability induced by adenosine.8,9 In addition, the peptide fragments released by NEP and ACE hydrolysis of bradykinin (fragment 1-7 and fragment 8-9) had no effect on vascular permeability. These findings indicate that in the hamster cheek pouch the edema-forming effects of bradykinin are mediated by the C-terminal of the molecule. Our data suggest that any reduction in NEP and ACE activity in the peripheral microcirculation would potentiate the bradykinin-induced increase in vascular permeability that occurs in tissue injury and inflammation.2-4,7,16,18,21

**Consideration of Methods**

We chose the hamster cheek pouch as a model for our studies because it has been used extensively to study the role of inflammatory mediators, including bradykinin, in regulation of vascular permeability.8-11,22 This model allows the measurement of solute efflux from postcapillary venules in two ways, enumeration of leaky sites and clearance of FITC-dextran, thereby providing a quantitative appraisal of macromolecular transport in situ. In addition, NEP- and ACE-like activities are present in the hamster cheek pouch. Finally, we examined the effects of selective, active site-directed NEP
and ACE inhibitors on bradykinin-induced increase in vascular permeability, because they have been shown to inhibit NEP- and ACE-like activities, respectively, in the hamster cheek pouch tissue in vitro (I. Rubinstein, I.F. Ukei, J.A. Nadel; personal communications).

Consideration of Previous Studies

Bradykinin increases vascular permeability in the peripheral microcirculation by forming large intercellular gaps between adjacent endothelial cells predominantly in postcapillary venules.1-2,22-24 In the hamster cheek pouch, these effects are mediated by B2-receptors and the activation of protein kinase C in response to bradykinin.6,9

NEP is a membrane-bound metalloenzyme that is widely distributed in the peripheral microcirculation and hydrolyzes bradykinin primarily at the Pro7-Phe8 bond, releasing the inactive fragments 1-7 and 8-9.2,5,5 Previous studies have suggested that NEP plays an important role in modulating tachykinin-induced plasma extravasation in the airway and skin of rodents.12,18,20,21 Pretreatment with NEP inhibitors was associated with potentiation of tachykinin-induced plasma extravasation in these tissues, whereas administration of recombinant human NEP attenuated these responses.12,20 Although bradykinin is hydrolyzed very efficiently by NEP in vitro, little is known about the role of NEP in modulating the edema-forming effects of the peptide in vivo.

Like NEP, ACE is a membrane-bound metalloenzyme that is widely distributed in the peripheral microcirculation and hydrolyzes bradykinin primarily at the Pro7-Phe8 bond.2-5,13,18 Few studies have examined the role of ACE in regulating the edema-forming effects of bradykinin in vivo, and the results are conflicting. Adamski et al.15 showed that the bradykinin-induced increase in protein efflux and edema formation in the canine forelimb were potentiated by pretreatment with captopril. The results indicated that ACE may play an important role in modulating the vasoactive effects of bradykinin. In contrast, Fantone et al.17 showed that captopril inhibited bradykinin-induced wheal formation in rat skin. The inhibitory effect of captopril was unrelated to inhibition of serum ACE activity or serum prostatlandin E2 levels and was not prevented by pretreatment of the animals with indomethacin. Finally, Horii et al.16 showed that plasma extravasation in kaolin-induced pleurisy in rats was mediated, in part, by kinins and that captopril potentiated these effects. However, these authors did not examine the mechanism(s) by which captopril potentiated plasma extravasation into the pleural space. These data suggest that the role of ACE in modulating bradykinin-induced increase in vascular permeability may be species and tissue specific.

Consideration of the Present Study

We examined the role of NEP and ACE, two membrane-bound metalloenzymes that are widely distributed in the peripheral microcirculation and hydrolyze bradykinin very efficiently in vitro, in modulating the bradykinin-induced increase in vascular permeability in the hamster cheek pouch. We postulated that the anatomic localization of NEP and ACE in proximity to the site of action of bradykinin in postcapillary venules is likely to be an important determinant of bradykinin-induced responses. Our data show that the bradykinin-induced increase in vascular permeability was potentiated by phosphoramidon and thiorphan, selective NEP inhibitors, and by captopril, a selective ACE inhibitor. The similar magnitude and additive effects of NEP and ACE inhibitors in potentiating bradykinin-induced responses suggest that these two enzymes have comparable physiological roles in regulating the edema-forming effects of bradykinin in the peripheral microcirculation. These effects were specific, because NEP and ACE inhibitors did not potentiate the receptor-mediated increase in vascular permeability induced by adenosine.5,9 In addition, the peptide fragments released by NEP and ACE hydrolysis of bradykinin (fragment 1-7 and fragment 8-9) had no effect on vascular permeability. Taken together, these data suggest that degradation of bradykinin by NEP and ACE in vivo abrogates its edema-forming effects in the peripheral microcirculation. Furthermore, if NEP and ACE activity in the peripheral microcirculation is decreased by factors that also induce tissue injury and inflammation, such as oxygen free radicals,2-4,21 then bradykinin concentrations near or at its receptors on postcapillary venular endothelial cells will increase, thereby amplifying plasma extravasation.

The significance of our findings is derived from the following observations: 1) bradykinin is generated and released during tissue injury and inflammation; 2) bradykinin has potent edema-forming effects in vivo; 3) bradykinin is degraded by NEP and ACE in vitro; 4) bradykinin is involved in local generation and release of other proinflammatory and vasoactive mediators in the peripheral microcirculation, such as prostaglandins and platelet activating factor; 5) NEP-like activity is decreased during tissue injury and inflammation in vivo; and 6) exogenous administration of recombinant human NEP attenuates kinin-induced increase in plasma extravasation in the guinea pig skin.1,2,12,13,20-24 The latter observation may have important therapeutic implications in the treatment of inflammatory and allergic disorders in which bradykinin generation and release is increased and/or its catabolism decreased. Finally, our findings support and extend the observations made by Adamski et al.15 by demonstrating a role for both NEP and ACE in modulating the edema-forming effects of bradykinin in vivo.

In summary, the results of the present study suggest the presence of an important mechanism that regulates the edema-forming effects of bradykinin in the peripheral microcirculation, namely enzymatic degradation of the peptide by NEP and ACE in postcapillary venules. We postulate that this mechanism is important in the pathogenesis of tissue injury and inflammation. Our findings may also provide a rationale for the development of alternative therapeutic approaches to abrogate the edema-forming effects of bradykinin in inflammation.

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