Biochemical Basis of Angioedema Associated With Recombinant Tissue Plasminogen Activator Treatment
An In Vitro Experimental Approach

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Background—Angioedema has been reported during recombinant tissue plasminogen activator (rtPA) treatment of acute ischemic stroke, often with concomitant use of angiotensin I–converting enzyme inhibitor treatment. Angioedema has been partly attributed to the nonapeptide bradykinin (BK), although its precise role has been poorly documented until now. The purposes of this report are 2-fold. First, we sought to define and characterize the in vitro kinin-forming capacity of rtPA when incubated with human plasma at a concentration within the therapeutic concentration range of rtPA attained in blood in vivo during fibrinolysis. Second, we sought to define the mechanism by which rtPA liberates BK from purified human single-chain high-molecular-weight kininogen, a key constituent of the contact system of plasma and the precursor of BK.

Summary of Report—When incubated with human plasma, in the presence of an angiotensin I–converting enzyme inhibitor, rtPA generates BK, which is further metabolized to des-Arg\(^9\)-BK. The quantity of kinins generated by rtPA is similar to that observed during the activation of the contact system of plasma with a negatively charged surface, suggesting that it is physiologically relevant. The total amount of des-Arg\(^9\)-BK liberated during the incubation period depends on the aminopeptidase P activity, its main degrading peptidase. Additionally, incubations using purified proteins of the fibrinolytic and the contact system pathways show that the rtPA kinin-forming capacity is mediated by plasmin.

Conclusions—We conclude that rtPA used in vitro at a therapeutic concentration has the capacity to generate significant quantities of kinins from human plasma. This kinin-forming activity depends on the activation of the fibrinolytic pathway. These data suggest that angioedema associated with rtPA treatment of ischemic stroke results directly from plasmin-mediated release of BK.

Key Words: angioneurotic edema | kinins | stroke | tissue plasminogen activator

Serious allergic reactions (anaphylactoid reactions or angioedema) have been observed during thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) (alteplase) used for the treatment of acute ischemic stroke, myocardial infarction, and deep vein thrombosis.\(^1\)\(^-\)\(^8\) A substantial number of these patients were simultaneously treated with an angiotensin I–converting enzyme (ACE) inhibitor. Angioedema, a local acute, potentially life-threatening inflammatory reaction, has been reported in patients with acute ischemic stroke treated with rtPA with a frequency of 1.9%. This frequency is higher than that observed in patients treated with an ACE inhibitor for hypertension or heart failure (0.1% to 0.2%).\(^1\)\(^-\)\(^4\)\(^,\)\(^9\) In these different clinical situations, angioedema has been attributed, at least in part, to bradykinin (BK).

BK is the prototype of a family of powerful vasodilatory peptides, the kinins. BK is released from high-molecular-weight kininogen (HK) when hydrolyzed by plasma kallikrein. This hydrolysis occurs, at least in vitro, when plasma is in contact with a negatively charged surface.\(^10\) In human plasma, we have shown that BK is metabolized mainly by 3 metallopeptidases: ACE, aminopeptidase P (APP), and kininase I. ACE and APP are the first and second main inactivating pathways of BK, respectively. Kininase I represents a minor pathway unless ACE is inhibited, in which case it transforms BK into its active metabolite, des-Arg\(^9\)-BK, which in turn is inactivated by APP and ACE, APP being its major inactivating metallopeptidase.\(^11\) BK and des-Arg\(^9\)-BK exert their pharmacological activity locally, at their site of formation, stimulating B2 and B1 receptors, respectively.\(^12\)\(^,\)\(^13\)

In a previous study we defined the in vitro kinetics of activation of the contact system of human plasma in 116 healthy individuals as a reference for pathophysiological studies for ACE inhibitor–associated side effects in which the activation of the kinins system could be involved.\(^11\) Subsequently, and more recently, we characterized the metabolism of endogenous BK and des-Arg\(^9\)-BK during in vitro activa-
tion of the contact system of plasma in the presence of an ACE inhibitor to address the pathophysiology of angioedema associated with ACE inhibitor therapy in hypertension.\(^1\)\(^4\) When compared with a control group of hypertensive patients and with the reference population values previously defined, the hypertensive patients with angioedema showed decreased degradation of BK but mainly des-Arg\(^9\)-BK, which was strongly correlated with lower activity of APP.\(^1\)\(^4\)

The purposes of this report are (1) to evaluate the capacity of rtPA to generate kinins, similarly to glass beads used in previous experiments, when incubated with human plasma, at a concentration within the therapeutic concentration range of rtPA attained in blood in vivo during fibrinolysis and (2) to characterize the capacity of rtPA to release BK when incubated with purified human single-chain HK, the precursor of BK. These experiments seek to explore the role of BK and its active metabolite des-Arg\(^9\)-BK in the development of rtPA-associated angioedema.

### Materials and Methods

rtPA was obtained from Chromogenix and from Hoffmann-LaRoche Ltd (Activase rt-PA). Both forms of rtPA were used for the different experiments. Enalaprilat (Vasotec I.V.) was from Merck Frosst Canada & Co. Single-chain HK, glu-plasminogen, plasma prekallikrein (pKK), factor XII (Hageman factor), and plasmin were from Enzyme Research Laboratories. All other biochemical reagents were from Fischer Scientific.

### Plasma Activation

Plasma samples were from 2 healthy male volunteers, previously analyzed to define the reference values of the metabolism of endogenous kinins.\(^1\) Plasma 1 exhibits a high APP activity (27 nmol·L\(^{-1}\)·min\(^{-1}\)), and plasma 2 exhibits a low activity (5 nmol·L\(^{-1}\)·min\(^{-1}\)) (reference values: 19±7 nmol·L\(^{-1}\)·min\(^{-1}\)).\(^1\)\(^1\)

One milliliter of each plasma sample was incubated in polypropylene tubes for 20 minutes at 37°C with enalaprilat at a concentration of 130 nmol/L, which totally inhibits plasma ACE activity. The contact system of plasma was activated by adding glass beads (37°C, with agitation), according to a method developed in our laboratory and described earlier.\(^1\)\(^1\)

The kinin-forming capacity of rtPA (10 μg/mL) was evaluated in a similar way by incubation with both plasma samples for a period of 8 hours. The ACE activity was also inhibited to mimic possible results in vivo.

At different time intervals, the incubation was stopped by adding cold ethanol. After evaporation to dryness, the extracts were used for the quantification of BK and des-Arg\(^9\)-BK with highly specific and sensitive immunoassays developed in our laboratory.\(^1\)\(^5\)

### Effect of rtPA on Single-Chain HK

rtPA (1 to 50 μg/mL) or plasmin (100 μg/mL) or rtPA plus glu-plasminogen (100 μg/mL) was incubated with purified HK (100 μg/mL) for different intervals of time ranging from 10 to 180 minutes at 37°C, under agitation. The incubation mixtures were then submitted to a sodium dodecyl sulfate–polyacrylamide gel electrophoresis in a precast Ready gel (4% to 15% Tris; Biorad). The immunoreactive BK was also quantified in the incubation mixtures.\(^1\)\(^5\)

### Results

**Incubation of rtPA With Human Plasma Generates BK and des-Arg\(^9\)-BK**

Figures 1 and 2 represent the kinetics of plasma kinin metabolism after activation of the contact system with glass beads (Figure 1A and Figure 2A) or when plasma was incubated in the presence of 10 μg/mL rtPA (Figure 1B and Figure 2B).

Although the kinetic profile of BK in the presence of rtPA is delayed in time (maximum concentration measured at 180 minutes versus 5 minutes) compared with that observed during the activation of the contact system by glass beads (Figure 1), the total BK released during the activation period is similar in both incubation conditions. In fact, the area under the curve is equal to 6.1 nmol·mL\(^{-1}\)·60 minutes for subject 1 and 5.8 nmol·mL\(^{-1}\)·60 minutes for subject 2, respectively (reference value for activation by glass beads: 7.0±2.3 nmol·mL\(^{-1}\)·60 minutes).\(^1\)\(^1\)

Figure 2 illustrates the kinetic profiles for des-Arg\(^9\)-BK. Similarly to BK, the profiles of des-Arg\(^9\)-BK release with rtPA are delayed in time compared with the kinetic profiles observed with glass beads (maximum values measured at 180 minutes versus 20 minutes). In this case, however, the profile of subject 1 (high APP activity: 27 nmol·mL\(^{-1}\)·60 minutes) is similar to that of the reference population (mean APP activity: 19±7 nmol·mL\(^{-1}\)·min\(^{-1}\)) but differs greatly from that for subject 2 (low APP activity: 5 nmol·mL\(^{-1}\)·min\(^{-1}\)). The areas under the curve for subject 1 are 6.7 nmol·mL\(^{-1}\)·480 minutes and 3.7 nmol·mL\(^{-1}\)·120 minutes for rtPA or glass beads incubation, respectively, but are 18.8 nmol·mL\(^{-1}\)·480 minutes and 20.5 nmol·mL\(^{-1}\)·120 minutes for subject 2 (reference value for men for activation by glass beads: 9.0±6.5 nmol·mL\(^{-1}\)·60 minutes).\(^1\)\(^1\) At a concentration of rtPA of 100 μg/mL, a significantly increased quantity of BK
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demonstrated when different constituents of the fibrinolytic system were incubated in similar conditions with purified HK and factor XII, ie, only plasminogen plus rtPA or plasmin alone exhibited proteolytic activity when incubated with purified pKK or factor XII (data not shown).

In this report we show for the first time that rtPA (pure laboratory reagent or injectable drug) used in vitro at a therapeutic concentration of 10 \( \mu \text{g/mL} \) \((\text{Activase rt-PA, Hoffmann-LaRoche Ltd})\) has the capacity to generate immunoreactive BK when incubated with human plasma. This B2 receptor agonist, in turn, is metabolized to des-Arg\(^{9}\)-BK, a potent B1 receptor agonist. A decreased plasma metabolism of des-Arg\(^{9}\)-BK characterizes hypertensive patients who presented an ACE inhibitor–related angioedema.\(^{14}\)

The metabolic profiles of both kinins, in the presence of rtPA and an ACE inhibitor, are quantitatively similar to those measured during the in vitro activation of the contact system cascade by glass beads, an experimental approach we have applied previously to a healthy reference population and to hypertensive patients who presented an ACE inhibitor–related angioedema.\(^{14}\) We have previously shown in hypertensive angioedema patients that APP activity in the presence of ACE inhibitor plays the major role in des-Arg\(^{9}\)-BK metabolism: the lower the activity of this enzyme, the higher is the accumulation of the B1 receptor agonist during the 120-minute observation period. These data suggest that low APP activity in the presence of an ACE inhibitor may also play a role in rtPA-associated angioedema.

The kinetic profiles obtained in the presence of rtPA are qualitatively different than those measured in the presence of glass beads: the maximum concentration of kinins is delayed in time. This observation suggests the existence of a second control mechanism that regulates the release of BK from HK, in addition to the APP activity. This second mechanism, further defined with the incubation of purified proteins, may be the protease-antiprotease system. Both APP activity and the protease-antiprotease balance could explain the severity and the duration of the angioedema symptoms in stroke patients treated with rtPA.

The incubation of pure constituents of the fibrinolytic system with HK, pKK, and factor XII allowed further study of the mechanism of the kinin-forming capacity of rtPA in plasma. The kinin-forming activity of rtPA depends on its activation of plasminogen into plasmin, which in turn activates the different constituents of the contact system of plasma. In these incubation conditions, the release of BK could be observed at concentrations of rtPA as low as 1 \( \mu \text{g/mL} \) after only a 10-minute incubation period. These results contrast with our observations in human plasma, in which the kinin-forming capacity is delayed in time and becomes evident only for therapeutic concentrations of rtPA, within the range found in blood in vivo during fibrinolysis. These observations suggest that the kinin-generating ability of rtPA in human plasma could be regulated by inhibitors, possibly antiproteases known to control the activation of contact system (C\(_{1}\) esterase inhibitor) and/or of the fibrinolytic cascade (plasminogen activator inhibitor-1 and \( \alpha_{2} \)-antiplasmin).

Our results complete and extend earlier observations showing that purified plasmin liberates bioactive BK when incubated in a presence of purified HK. They show that the kinin-forming capacity of rtPA depends on its triggering activity on the fibrinolytic pathway, which in turn activates

\[ \text{Kinin generation.} \]

**Figure 2.** A, Metabolic profiles of des-Arg\(^{9}\)-BK for subjects 1 and 2, compared with the mean kinetic profile for a reference population, after activation of the contact system of plasma with glass beads, in the presence of enalaprilat. B, Kinetic profiles of des-Arg\(^{9}\)-BK for subjects 1 and 2, while incubated with 10 \( \mu \text{g/mL} \) of rtPA, in the presence of enalaprilat. Profile of control plasma without rtPA is also shown (dotted line) and shows no kinin generation.

(10 times higher) during the incubation period could be measured.

This kinin-forming capacity could not be detected when plasma samples were incubated in the absence of rtPA or in the presence of 1 \( \mu \text{g/mL} \) rtPA.

**Interaction of the Constituents of the Fibrinolytic Pathway With HK**

As shown in Figure 3A, neither rtPA nor plasminogen alone had a proteolytic activity when incubated with HK. The electrophoretic profile of HK obtained after incubation of plasminogen plus rtPA, however, is similar to that observed with plasin. This presence or absence of a proteolytic activity could be measured for incubation periods as short as 10 minutes (results not shown) and for concentrations of rtPA as low as 1 \( \mu \text{g/mL} \). Moreover, the quantity of immunoreactive BK released in the different incubation conditions (Figure 3B) could be related to the electrophoretic pattern (Figure 3A). In fact, and contrarily to what happens with plasin or rtPA plus plasminogen, no significant release of BK could be measured after incubation with rtPA or plasminogen alone with HK for periods varying from 10 to 180 minutes.

Finally, a similar proteolytic pattern could also be measured when different constituents of the fibrinolytic system were incubated in similar conditions with purified pKK and factor XII, ie, only plasminogen plus rtPA or plasmin alone exhibited proteolytic activity when incubated with purified pKK or factor XII (data not shown).

**Discussion**

In this report we show for the first time that rtPA (pure laboratory reagent or injectable drug) used in vitro at a therapeutic concentration of 10 \( \mu \text{g/mL} \) \((\text{Activase rt-PA, Hoffmann-LaRoche Ltd})\) has the capacity to generate immunoreactive BK when incubated with human plasma. This B2 receptor agonist, in turn, is metabolized to des-Arg\(^{9}\)-BK, a potent B1 receptor agonist. A decreased plasma metabolism of des-Arg\(^{9}\)-BK characterizes hypertensive patients who presented an ACE inhibitor–related angioedema.\(^{14}\)

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Our results complete and extend earlier observations showing that purified plasmin liberates bioactive BK when incubated in a presence of purified HK. They show that the kinin-forming capacity of rtPA depends on its triggering activity on the fibrinolytic pathway, which in turn activates
the different components of the contact system in a noncascade way. Moreover, the equivalent kinin-forming capacity of both forms of rtPA constitutes an experimental argument against the fact that angioedema observed in stroke during fibrinolysis could be attributed to the arginine content of the injectable form, as previously suggested.1–8

Our in vitro observations suggest that further characterization of the metabolic pathways (protease-antiprotease balance, metallopeptidases) controlling the release and the inactivation of BK and its active metabolite des-Arg9-BK in stroke patients who presented an rtPA-related angioedema is warranted. In conclusion, our data strongly suggest that, similar to other acute side effects of ACE inhibitor, such as angioedema, rtPA-associated angioedema seen in stroke patients may also result from BK release.

Figure 3. A, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of 5 μg of the following components: molecular weight (MW) markers (well 1), rtPA (well 2), glu-plasminogen (plgn) (well 3), rtPA incubated with glu-plasminogen (well 4), plasmin (plas) (well 5), HK (HMWK) (well 6), rtPA incubated with HK (well 7), rtPA plus glu-plasminogen incubated with HK (well 8), and plasmin incubated with HK (well 9). B, Quantity of immunoreactive BK released from HK (pmol/pmol) after incubation with rtPA (filled column), glu-plasminogen (open column), plasmin (hatched column), and rtPA plus glu-plasminogen (cross-hatched column).

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References

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