**EDITORIAL COMMENT**

Tissue-Type Plasminogen Activator Release

New Frontiers in Endothelial Function*

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Tissue-type plasminogen activator (t-PA) is a pivotal enzyme in the mammalian fibrinolytic system. It exerts its action by converting the zymogen plasminogen into the proteolytically active serine protease plasmin, which, in turn, degrades fibrin clots. Because of its affinity for fibrin, t-PA is the prototypic fibrin-specific plasminogen activator (1). This specificity and its lack of antigenicity have made it a powerful tool in the treatment of acute myocardial infarction (2). The t-PA is inactivated by its suicide substrate, plasminogen activator inhibitor-1 (PAI-1) (3).

**Storage of t-PA in endothelial cells.** The t-PA is synthesized and stored in endothelial cells and vascular neurons (4,5). There has been much controversy in the published data regarding the specific endothelial cell vesicle in which t-PA is stored. It has been suggested that because t-PA is often released concomitantly with von Willebrand factor (vWF), both proteins are stored in Weibel-Palade bodies. Emeis et al. (6) demonstrated that this is not necessarily the case. In studies using rat lung homogenates fractionated by density gradient centrifugation, particles containing a majority of the t-PA antigen and activity were found at similar densities in sucrose and Nycodenz gradients. The Nycodenz gradient co-localized t-PA granules with vWF containing Weibel-Palade bodies. However, the two proteins fractionated at different densities in sucrose. Using double-immunofluorescence staining of human umbilical vein endothelial cells, t-PA and vWF particles did not co-localize (6). Further evidence that t-PA and vWF are stored in separate granules is based on the observation that these proteins are differentially released by specific stimuli. For example, ionomycin-induced vWF release is dose-dependent and correlates with the increase in intracellular calcium concentration. In contrast, t-PA is not released until the calcium concentration exceeds a threshold of 500 nM (7). Thus, it appears that t-PA and vWF vesicles fractionate at different densities, do not co-localize by immunofluoroscopy, and can be induced to be released separately.

**Molecular mechanisms of t-PA release.** In vitro studies of t-PA release have revealed much about the signal transduction pathways involved in its release. Early studies revealed that constitutive release of t-PA from the endothelium can be augmented in a dose- and time-dependent manner by protein kinase C (PKC) activation. In PKC-activated cells, cyclic adenosine monophosphate (cAMP) agonists augment t-PA secretion (8). Furthermore, PKC-augmented t-PA secretion may be dependent on an increase in t-PA message (9). However, these studies examined t-PA secretion over periods of hours to days, not minutes.

Subsequently, a cell culture model of t-PA release was developed. In this model, it was found that t-PA is both constitutively secreted and can be released rapidly in a dose-dependent manner in response to agonists such as thrombin, isoproterenol, and prostacyclin. It was discovered that these agonists act in a dose-dependent manner, and preincubation of cells with sodium butyrate or retinoic acid amplifies the response to stimulation with agonists (10).

Little is known about the precise molecular mechanisms by which bradykinin induces t-PA release. It has been suggested that thrombin and prostacyclin induce t-PA release through independent G-protein–signaling pathways (7,11). Prostacyclin and isoproterenol couple Gs and likely signal through cAMP, whereas thrombin, which couples Go, likely signals through calcium-dependent pathways. The thrombin receptor couple G12, Gi, and Gq. The Gi, which inhibits adenyl cyclase, and Gq, which signals through Rho, seem unlikely candidates in facilitating t-PA release. Gq, which generates inositol triphosphate through phospholipase C-beta (PLC-beta), inducing intracellular calcium shifts, appears to be a likely downstream target. The G-protein subunits beta and gamma, which also activate PLC-beta, may also be responsible for these calcium effects (12). Furthermore, coupling of the thrombin receptor to Gi may explain the variable effects of pertussis toxin on t-PA release in the presence and absence of calcium (7).

**In vivo studies of t-PA release.** As alluded to earlier, t-PA release from endothelial cells is an area of much interest, as it is a distinct marker of endothelial function. Studies of in vivo t-PA release have revealed much about the mechanics of this phenomenon, as both endothelial-dependent agonists such as methacholine and endothelial-independent agonists such as isoproterenol induce rapid release of t-PA, independent of effects on vessel wall relaxation (13). In addition to these agonists, bradykinin was also found to induce t-PA release and increase flow in both the human forearm and coronary artery bed (14,15). Bradykinin-induced t-PA release is a bradykinin type-2 receptor (B2)–mediated phenomenon, as HOE-140, a specific B2 receptor antagonist, blocks bradykinin-mediated t-PA release. Because B2 stimulation results in prostacyclin and nitric oxide generation, it was hypothesized that inhibition of synthesis

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of these products with indomethacin, a nonselective cyclooxygenase inhibitor, and t-NAME, a nitric oxide synthase inhibitor, would prevent t-PA release from the vascular bed. However, neither indomethacin nor t-NAME, alone or in combination, inhibited bradykinin-induced t-PA release. This led to the hypothesis that it may be either an endothelial-derived hyperpolarization factor-mediated phenomenon or perhaps a calcium-mediated phenomenon, as the B2 receptor couples to the Gq/PLC-beta/calcium-signaling pathway (16).

Because bradykinin is rapidly degraded by angiotensin-converting enzyme (ACE), one would anticipate that ACE inhibition would augment bradykinin-induced t-PA. Treatment with quinapril enhanced bradykinin-induced t-PA release in the human forearm, whereas losartan, an angiotensin II type-1 receptor blocker, had no effect. Substance P-mediated t-PA release, however, was not augmented by treatment with quinapril or losartan. Labinjoh et al. (17) suspected that some of the beneficial clinical effects of ACE inhibitors may be the result of enhancement of bradykinin-mediated t-PA release.

In humans, t-PA release in response to bradykinin or Substance-P is impaired in smokers, both in the coronary vasculature and forearm (18,19). Methacholine-induced t-PA release is unchanged in borderline hypertensive subjects (20). Other conditions associated with impaired endothelial vasodilator function, such as hypercholesterolemia and diabetes, have not been specifically studied for their impact on arterial t-PA release in response to bradykinin or Substance P.

In this issue of the Journal, Witherow et al. (21) examined the effects of ACE inhibition on t-PA release in subjects with ischemic cardiomyopathy. The highlight of this study is the extremely robust response to bradykinin in the presence of ACE inhibition; this response was 4.5-fold greater than that seen in subjects without ischemic cardiomyopathy. The authors describe transient t-PA increases that approach those seen when t-PA is administered for brinolysis of patients with heart failure, much less is known about the clinical factors that impair endothelial t-PA release or therapies that restore this important function. Furthermore, it is possible that t-PA release may be a critical aspect of endothelial function in terms of preventing coronary thrombosis. Studies such as Witherow et al. (21) are likely to lead to a more comprehensive perspective of the complexities of endothelial function.

**REFERENCES**


