Plasminogen activator inhibitor-1 in cardiovascular cells: rapid induction after injecting mice with kainate or adrenergic agents

Bhuvarahamurthy Venugopal¹, Ronit Sharon², Rene Abramovitz, Ala Khasin³, Ruth Miskin*

Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel

Received 29 June 2000; accepted 27 October 2000

Abstract

Objectives: Plasminogen activator inhibitor-1 (PAI-1) is a major anti-fibrinolytic glycoprotein thought to promote vascular diseases. Recently we have shown that systemically injecting mice with kainate, an analog of the principal brain excitatory neurotransmitter glutamate, immediately induced PAI-1 mRNA in brain vascular cells which are not known to contain glutamate receptors. Here we further investigated whether: (a) kainate also increases PAI-1 gene expression in the cardiac vascular bed; (b) subunits of kainate/AMP A receptors could be expressed in cardiac and brain vascular cells; and (c) PAI-1 mRNA could be similarly induced by agonists of adrenergic receptors that are candidates to act downstream in kainate-activated pathways.

Methods: We analyzed cardiac and brain cryosections for ³⁵S-labeled specific riboprobes. PAI-1 activity was tested in cardiac homogenates using one-phase reverse zymography.

Results: Prominent PAI-1 mRNA hybridization signals were induced in the vascular cells of the heart, and unexpectedly, also in cardiocytes, within 1–2 h after injection of kainate (i.p., 11–25 mg/kg body weight); the signals persisted for at least 8 h and disappeared after 24 h. In addition, PAI-1 activity increased (~5 fold) 2–10 h after the treatment. In contrast, mRNAs encoding the kainate/AMP A receptor subunits could not be detected. The adrenergic agents adrenaline (3.5 mg/kg) and isoproterenol (200 mg/kg) exerted kainate-like effects in cardiovascular cells.

Conclusions: These results revealed, for the first time, that PAI-1 gene expression can be enhanced locally in the cardiovascular system by a fast-acting neurological mechanism triggered by glutamate receptors, whose pathway and relation to catecholamines, which exerted similar effects, have yet to be resolved. These findings raised the possibility that excessive glutamate, or stress-related catecholamines, may increase the risk of stroke and myocardial infarction.

Keywords: Adrenergic agonists; Gene expression; Neurotransmitters; Receptors; Thrombosis

1. Introduction

Plasminogen activator inhibitor-1 (PAI-1) is an inducible, secreted ~50-kDa glycoprotein of the serpin superfamily (reviewed in Refs. [1–3]). PAI-1 is considered the principal physiological inhibitor of tissue-type (tPA) and urokinase-type (uPA) plasminogen activators (PAs). These serine proteases specifically convert inactive plasminogen into active plasmin, the ultimate fibrinolytic enzyme. Plasmin is a non-specific trypsin-like protease that is also involved in tissue extracellular proteolysis (reviewed in Refs. [4–6]). In addition, components of the PA system (specifically PAI-1, uPA and its specific surface receptor uPAR) can modulate cell adhesion and migration in a proteolytic-independent manner [7]. In accordance with these multiple functions, the PA system has been implicated, apart from fibrinolysis, in various normal and pathological events such as inflammation, wound healing, angiogenesis [5,8], restenosis [9], cardiac rupture [10], tumor metastases [6,8], and brain plasticity [11] and toxicity [12].

*Corresponding author. Tel.: +972-8-934-3150; fax: +972-8-934-4118.
E-mail address: ruth.miskin@weizmann.ac.il (R. Miskin).

¹Present address: Department of Medicine, Renal Division, Harvard Institute of Medicine, 77 Louis Pasteur Ave, Boston, MA 02115, USA
²Present address: Center for Neurologic Diseases, Brigham and Women’s Hospital, 77 Louis Pasteur Ave, Boston, MA 02115, USA
³Present address: Internal Medicine Department 3, Kaplan Medical Center, Rehovot, Israel.

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PII: S0008-6363(00)00271-6

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Increased concentration of plasma PAI-1 has been associated with an increased risk for thrombotic diseases in humans, in particular myocardial infarction [3,13,14] and stroke [14,15]. Furthermore, the levels of PAI-1 antigen or transcripts in the vascular wall, which are low in healthy individuals, were elevated in endothelial and smooth muscle cells of atherosclerotic plaques [16–19], suggesting that PAI-1 may play a role in atherogenesis as well, perhaps by promoting matrix deposition and thrombotic complications associated with plaque rupture. Studies using rodent models, including transgenic and gene-deficient mice, have confirmed that PAI-1 can promote vascular thrombosis [20,21], but conversely, could not detect any PAI-1 gene influence on atherosclerotic plaques [22]. Yet, other studies have indicated that PAI-1 could inhibit neointimal thickening [9], and furthermore, it could protect against cardiac rupture, a uPA-dependent event that follows myocardial infarction [10].

PAI-1 gene expression could be modulated by a large group of hormones, growth factors, cytokines and inflammatory agents in diverse cell types in vitro [1]. Notably, bacterial endotoxins administered into mice elevated PAI-1 mRNA in the kidney and liver specifically in endothelial cells [23,24]. So far, only a few studies have reported on PAI-1 expression in cardiac cells. For example, transforming growth factor-β (TGF-β) activated the PAI-1 gene promoter in cultured chicken embryonic cardiac cells [25], and angiotatin II enhanced PAI-1 expression in fibroblasts derived from human hearts [26]. Still, very little is known about the physiological mechanisms controlling PAI-1 gene expression in cardiovascular cells in vivo. Specifically, it is yet unknown whether neurological or neuroendocrine mechanisms are involved in this control.

Previously we have studied the response of the PAI-1 gene in the brain to kainate [27], a potent excitatory and excitotoxic compound which is a glutamate analog. Glutamate is the most prevalent excitatory neurotransmitter in the central nervous system (CNS) [28,29], whose involvement in the autonomic nervous system (ANS) is poorly understood. In rodents, kainate causes extensive brain toxicity which involves activation of early and late genes (see Ref. [27] for a detailed list of references). These effects are thought to be mediated by kainate interaction with two subclasses of glutamate receptors, the kainate and AMPA subclases named after their preferred agonist. Within the complex family of glutamate receptors, the kainate/AMPA receptors comprise the non-NMDA ionotropic receptors that act as ligand-gated ion channels and are highly prevalent in the CNS (reviewed in Refs. [28–30]). We have previously found, through in situ mRNA hybridization experiments, that kainate systemically injected into mice, caused a rapid and robust induction of PAI-1 mRNA in brain nerve cells, and also, unexpectedly, in brain blood vessel cells [27] which are not known to contain glutamate receptors. These findings demonstrated, for the first time, that PAI-1 gene expression in vascular cells can be enhanced through a neurotransmitter. However, it was not clear whether PAI-1 induction in brain vascular cells occurred independently of neighbouring neuronal cells, or rather, was mediated through kainate-induced effectors released from these neuronal cells. Furthermore, because the vascular induction was detected after systemically injecting kainate, it was of interest to test whether it occurred exclusively in the brain or also encompassed the vascular bed of other organs.

Here we report that kainate intravenously injected into mice immediately induced PAI-1 mRNA in cardiac vascular cells and, unexpectedly, also in cardiocytes, and it also increased PAI-1 activity in cardiac homogenates. In addition, because adrenoceptors are candidates to act downstream in kainate-activated pathways, we also tested the effect on PAI-1 gene expression in the heart and brain of adrenoreceptor agonists (i.e. adrenaline, an α- and β-adrenoceptor agonist, and isoproterenol, a non-selective β-adrenoceptor agonist). Our results show that neurological and/or neuroendocrine mechanisms can enhance PAI-1 gene expression in the murine cardiovascular system in vivo.

2. Methods

2.1. Treatment of mice

FVB/N female mice (n=42), 3–4 months old, propagated and maintained as previously described [31], were injected i.p. with the following solutions: (i) PBS (250 μl, serving as control); (ii) kainate (a freshly prepared solution in PBS, at the indicated amount; Sigma); (iii) adrenaline (3.5 mg/kg body weight; Teva Pharmaceutical Industries, Israel); (iv) isoproterenol (200 mg/kg; Abbot, Chicago, USA). At the indicated times after treatment, mice were heavily anesthetized with ether. Their organs were immediately removed and frozen over dry ice and kept frozen at −70°C for up to 4 weeks. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH) publication no. 85-23, revised 1996.

2.2. In situ hybridization

Thin cryosections (12 μm) were prepared from hearts and brains and tested through in situ hybridization exactly as we have previously described [27,32], using 35S-labeled riboprobes at the antisense or sense orientations, specific for PAI-1 [27] or for the kainate receptor subunits GluR1, GluR3, and GluR5 (plasmids carrying GluR sequences were a kind gift from Professor V. Teichberg). Sections were maintained under photographic emulsion for 2–3 weeks, developed with Kodak D-19 developer and counterstained very lightly with hematoxylin (cardiac sections) or cresyl violet (brain sections).
2.3. One-phase reverse zymography

The mice were treated as indicated, lightly anesthetized with ether and then perfused. Their organs were excised and homogenized (10% w/v in PBS containing 0.5% Triton X-100). Homogenate samples were tested for PAI-1 activity using one-phase reverse zymography [33] as previously reported for the brain [27]. Briefly, samples were applied to mini SDS–PAGE gels containing purified human plasminogen and casein. After electrophoresis, the SDS was washed out with Triton X-100 and the gels were incubated for proteolysis with human uP A (0.5 IU/ml, American Diagnostica) for 4 h. Consequently, the uP A converted the plasminogen throughout the gels into plasmin, which in turn degraded the casein. Next, the gels were stained with Coomassie brilliant blue. On the lightly stained background, PAI-1 yielded dark bands resulting from inhibition of casein degradation. Parallel control gels were treated similarly, but they did not include casein; they served as a control to assure that the dark bands in the casein-containing gels did not result from just staining of sample proteins. Results were quantitated using the Bio-Rad imaging densitometer, model GS-690, via the Bio-Rad Multi-Analyst version 1.0.1.

3. Results

3.1. Kainate induced PAI-1 mRNA in vascular cells, and in cardiocytes

To test whether kainate could induce PAI-1 mRNA in cardiac vascular cells, we injected the drug i.p. into mice at several doses in the range of 8–25 mg/kg body weight. Cardiac cryosections were tested for PAI-1 mRNA through in situ hybridization using PAI-1-specific 35S-labeled riboprobes at the antisense and sense orientations. Hybridization signals were induced in vascular cells in the heart within 1–2 h after injecting kainate, and persisted for at least 6–8 h (Fig. 1B,C,G,H) as previously reported for vascular cells in the brain [27]. Signals returned to the basal level after 1 day. The signals were detected with the antisense but not with the sense riboprobe (Fig. 1D), indicating PAI-1 mRNA specificity. Induction was ob-

Fig. 1. PAI-1 mRNA hybridization signals in cardiac sections of kainate-treated mice. Cardiac sections were obtained from a control mouse (A) or from mice injected with kainate at 20 mg/kg and sacrificed after 2 h (B,D), or 12 mg/kg and sacrificed after 6 h (C–E). Sections were hybridized with PAI-1 35S-labeled riboprobes at the antisense (A–C, E) or sense (D) orientation. F–H are higher magnification photographs of selected fields in section B (F,G) or C (H). Photographs were taken in dark or bright field illumination at the following magnifications: A–D, 9×; E,G, 60×; F,H, 1000×. Arrows indicate blood vessels.
obtained at kainate concentrations of 11 mg/kg body weight or higher, with signals increasing in a dose-dependent manner. No PAI-1 mRNA induction was noted at non-convulsive concentrations of 10 mg/kg and lower. As in the brain, PAI-1 mRNA hybridization signals in cardiac vessels were associated with smooth muscle cells (Fig. 1H). Signals were also seen in endothelial cells as judged by morphological appearance.

Interestingly, PAI-1 mRNA was also detected in cardiocytes, including cells containing elongated nuclei typical of cardiomyocytes (Fig. 1B,C,E,F). In most cases, however, we could not conclusively distinguish between nuclei of cardiomyocytes and stromal cells. In cardiocytes, PAI-1 mRNA hybridization signals usually increased within 1–8 h after injecting kainate and were virtually undetectable after 24 h. Some change, however, was observed in the spatial distribution of the signals. Thus, 1–3 h after treatment, signals were associated with cells dispersed throughout the section, including the lower region of the cardiac muscle and atria (Fig. 1B). After 6 h, hybridization signals were more abundant in cells residing in the upper region of the ventricular myocardium and septum, whereas they were usually missing in the atria (Fig. 1C). Sometimes, the transition was sharp between areas densely packed with cells containing signals, and adjacent areas where such cells were rare (see Fig. 1E for an example). Interestingly, blood vessels containing strong hybridization signals were also localized in areas where the surrounding cardiocytes were devoid of signals, especially in the lower parts of the cardiac muscle (Fig. 1C). Notably, signals were also associated with cells in the endocardium.

3.2. Testing brain and cardiac sections for mRNAs encoding subunits of kainate receptors

PAI-1 mRNA induction in vascular and cardiac cells was unexpected, because these cells are not known to contain kainate/AMPA receptors. However, we decided to test this issue here, because it is not firmly established and our results raised a major question concerning the localization of the effective receptors. To date, several subunits of the kainate/AMPA receptors are known, designated GluR1–GluR7, K1 and K2, and assembled together in homomeric or heteromultimeric complexes [28–30]. Like all glutamate receptors, the kainate/AMPA receptors are abundant in the CNS, however they show different distribution patterns. These receptors could possibly account for the previously reported robust PAI-1 mRNA induction in brain nerve cells that propagated in a dynamic spatial pattern [27]. Here we have analyzed brain and cardiac sections of normal mice for glutamate receptor mRNAs through in situ hybridization, using 35S-labeled riboprobes specific for the GluR1, GluR3, and GluR5 subunits. For comparison, we also presented results obtained for PAI-1 mRNA in parallel sections from brains excised 1 h after kainate treatment. Clearly, strong PAI-1 mRNA signals were seen in blood vessels in these sections (Figs. 2A and 3Aa). In contrast, GluR1 mRNA or GluR3 mRNA hybridization signals were missing in brain vascular cells, while seen in neuronal cells (Figs. 2B and 3Bb), in agreement with previous results [34]. Additionally, no GluR1 or GluR3 mRNAs could be detected in the heart (Figs. 2C and 3Cc). Similarly, hybridization signals could not be detected in the heart and in brain blood vessels after hybridization with the GluR5 riboprobe, or with all three GluR riboprobes in spinal cord sections (results not shown). These results suggest that at least the three kainate receptor subunits tested here are not expressed in murine cardiovascular cells at appreciable levels.

3.3. Adrenaline and isoproterenol induced PAI-1 mRNA in brain and cardiac blood vessel cells and in cardiocytes

In principle, kainate could mediate PAI-1 mRNA induction in cardiovascular cells through glutamate receptors.
Homogenate samples were analyzed along with conditioned medium collected from the murine hepatic carcinoma Hepa 1c17, previously shown to secrete high PAI-1 activity after treatment with the phorbol ester PMA [33]. The Hepa PAI-1 inhibitory activity is seen in the gel as a dark band corresponding to an ~50-kDa protein (Fig. 5, lanes 1 and 10). Only marginal bands of PAI-1 activity could be detected in cardiac homogenates of control mice (lanes 2 and 3), while this band increased considerably 3 and 6 h after treatment with kainate (lanes 4 and 5), adrenaline (lanes 6 and 7) or isoproterenol (lanes 8 and 9). Densitometer scanning conducted for three mice for each drug indicated elevations of PAI-1 activity of 5.4±0.4, 4.0±1.4 and 4.7±1.6 (fold±S.E.) 6 h after treatment with kainate, adrenaline or isoproterenol, respectively.

4. Discussion

The findings that a glutamate analog and adrenergic agents can rapidly increase PAI-1 mRNA in cardiovascular cells have indicated, for the first time, that PAI-1 gene expression can be controlled in the cardiovascular system through neurological and/or neuroendocrine mechanisms. The rapid elevation of PAI-1 mRNA observed in the cardiovascular cells after kainate injection was unexpected, primarily because these cells are not known to contain brain 35 AMPA receptors. Thus, it appears that kainate could induce PAI-1 by acting upstream rather than directly at the vascular and cardiac target cells. Furthermore, the finding that PAI-1 mRNA was also induced in vascular cells in the heart strongly suggests that the induction in brain vascular cells occurred through a common mechanism rather than through effectors released from neighbouring brain neurons after kainate administration.

We do not yet know what is the anatomical localization of the glutamate receptors that communicate with cardiovascular target cells for PAI-1 induction, and through which pathway they transmit their effect. In principle, these glutamate receptors could reside in brain or spinal cord areas controlling or mediating ANS sympathetic and parasympathetic transmission or neuroendocrine pathways (i.e. the hypothalamic–pituitary–adrenal axis) [35–37]. Interestingly, very little is known about the role of glutamate in the ANS and neuroendocrine pathways. Recently it has been demonstrated that microinjection of glutamate, kainate or NMDA into specific spinal cord or hypothalamic sites elicited, within minutes, changes in arterial blood pressure, heart rate, and myocardial contractility [38–40], and also increased plasma catecholamines [39]. However, the influence on gene expression has not been tested in these cases. As far as we know, our results on the PAI-1 gene provide the first evidence that glutamate can also promote gene expression in ANS- innervated tissues, specifically the cardiovascular system.

The ANS and neuroendocrine pathways involve adren-
Fig. 4. PAI-1 mRNA hybridization signals in cardiac and brain sections of mice treated with adrenaline or isoproterenol. Cardiac sections (A–C,E,F) and brain sections (D,G,H) were derived from mice 2 h after treatment with adrenaline (A–D) or isoproterenol (E–G), or from a control mouse (H). Sections were hybridized with $^{35}$S-labeled PAI-1 antisense riboprobe. Photographs were taken at dark or bright field illumination at the following magnifications: A,E, 9×; B,D,F–H, 1000×; C, 400×.

Fig. 5. Effect of kainate, adrenaline, and isoproterenol on PAI-1 activity in the heart. Cardiac homogenates were tested for PAI-1 activity by one-phase reverse zymography. The following samples were applied to the gels: conditioned medium from PMA-treated Hepa 1c17 cells (lanes 1 and 10); molecular weight markers (BioLab, Prestained Protein Markers, Broad range #7701) (lane 11); cardiac homogenate samples (3 μg protein) from two control mice (lanes 2 and 3), or from mice treated with kainate (20 mg/kg) (lanes 4 and 5); adrenaline (lanes 6 and 7) or isoproterenol (lanes 8 and 9). Mice were sacrificed 3 or 6 h after the treatment, as indicated in the figure. Gel A contained plasminogen and casein, as substrates for uPA and plasmin, respectively. Gel B did not contain any substrate, serving as a control.
endothelial pools and decreased clearance in the liver, rather than to enhanced gene expression. Our results have shown, for the first time, that catecholamines, and thus the neuroendocrine mechanism, can affect the fibrinolytic capacity in cardiovascular tissues at the level of PAI-1 gene expression. Our results, however, are not sufficiently complete to indicate whether catecholamines are also involved in mediating the kainate effect on PAI-1 gene. To resolve the kainate-mediated pathway a comprehensive pharmacological study should be undertaken.

It is noteworthy that kainate, being a glutamate structural analog, can also inhibit glutamate transport through excitatory amino acid transporters (EAATs) [43]. Such inhibition may result in extracellular increase of glutamate, and, if occurring in cardiac neuronal elements in the heart, could in principle lead to PAI-1 induction in adjacent cells carrying any subclass of active glutamate receptors. At present, however, it is not known if such receptors are found in vascular smooth muscle cells and cardiomyocytes. Furthermore, we found that NMDA, an agonist of the NMDA receptor subclass, did not induce PAI-1 mRNA in cardiocytes (unpublished results), thus excluding this receptor subclass as a primary or secondary mediator of PAI-1 induction.

Interestingly, after kainate treatment we did not detect in vascular or cardiac cells an induction of the mRNA encoding plasminogen activator inhibitor-2 (PAI-2) (the second PA-specific inhibitor), uPA mRNA and activity, or tPA activity (unpublished results). These differences from PAI-1 indicate some specificity of the kainate/AMP A receptors regarding the capacity to induce in cardiovascular cells PAI-1, among the fibrinolytic components. Likewise, the adrenergic agents did not induce uPA mRNA, or uPA and activity, or tPA activity.

Our findings have raised the possibility that glutamate, through the kainate receptor, could act as a PAI-1 inducer in cardiovascular cells under normal conditions, and could lead to PAI-1 overproduction by excessive receptor triggering. Furthermore, catecholamines may exert similar effects. PAI-1 overproduced locally in the cardiac and brain vascular bed could increase the risk of myocardial infarction and stroke. In the latter context, it is of interest that elevated levels of extracellular glutamate are thought to mediate brain injury after stroke [29]. Therefore, the possibility is raised that this glutamate, if generated in central ANS sites, may be further deleterious by inducing PAI-1 in the brain vasculature, thereby interfering with the fibrinolytic clearance of the causal occlusion. Transplant arteriosclerosis could be another case reflecting the neurological control of the PAI-1 gene, or rather the loss of this control because of denervation. Thus, the exquisite intima thickening occurring in arteries of grafted organs [44] could perhaps result, in part, from the loss of PAI-1 induction through the ANS in the denervated graft. It is not clear, however, what could be the functional consequences of PAI-1 overproduction in the cardiac muscle. Here, PAI-1 could stabilize the extracellular environment, while excess PAI-1 could disturb the proteolytic balance in the extracellular milieu, thereby interfering with cell–matrix and cell–cell interactions. On the other hand, excessive PAI-1 could act more efficiently to prevent cardiac rupture after myocardial infarction, as has recently been learned through PAI-1 and uPA-deficient mice [10].

As previously mentioned, our study has provided the first indication that glutamate can rapidly elevate gene expression in cardiovascular cells. The PAI-1 gene is usually activated by biological effectors of pleiotropic influences, i.e. they lead simultaneously to the activation of several genes, rather than exclusively the PAI-1 gene. We therefore assume that the PAI-1 gene is only one representative of a group of genes that can be induced in vivo in cardiovascular cells through kainate/AMP A receptors.

Thus, our findings raise the possibility that cardiovascular homeostasis may be regulated, in part, through a fast, long-lasting neurological mechanism affecting gene expression. It would be interesting to search for such putative genes.

In summary, our findings revealed that glutamate, or adrenergic agents, can induce PAI-1 mRNA in cardiovascular cells and can elevate PAI-activity locally in the heart. These findings indicate that neurological or neuroendocrine mechanisms, or both, can control cardiovascular PAI-1 gene expression, and suggest that excessive glutamate or catecholamines may increase the risk of cardiovascular diseases, in particular myocardial infarction and stroke.

Acknowledgements

We thank Professor Vivian Teichberg for the gift of GluR plasmids. B.V. was an incumbent of the Feinberg Postdoctoral Fellowship, The Weizmann Institute of Science. This work was supported, in part, by the Leo and Julia Forchheimer Center for Molecular Genetics and the Y. Leon Benoziyo Center for Molecular Medicine at the Weizmann Institute of Science.

References

[2] van Meijer M, Pannekoek H. Structure of plasminogen activator fibrinolytic clearance of the causal occlusion. Transplant arteriosclerosis could be another case reflecting the neurological control of the PAI-1 gene, or rather the loss of this control because of denervation. Thus, the exquisite intima thickening occurring in arteries of grafted organs [44] could perhaps result, in part, from the loss of PAI-1 induction through the ANS in the denervated graft. It is not clear, however, what could be the functional consequences of PAI-1 overproduction in the cardiac muscle. Here, PAI-1 could stabilize the extracellular environment, while excess PAI-1 could disturb the proteolytic balance in the extracellular milieu, thereby interfering with cell–matrix and cell–cell interactions. On the other hand, excessive PAI-1 could act more efficiently to prevent cardiac rupture after myocardial infarction, as has recently been learned through PAI-1 and uPA-deficient mice [10].

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References

[6] Andreasen PA, Kjoller L, Christensen L, Duffy MJ. The urokinase