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Adenosine Potentiates Human Lung Mast Cell Tissue Plasminogen Activator Activity

Michal J. Sereda,* Peter Bradding,† and Catherine Vial*

We investigated whether adenosine, a potent contributor to the regulation of pulmonary function, can modulate human lung mast cell (HLMC) fibrinolytic activity. Tissue plasminogen activator (tPA) activity and tPA transcript expression levels from a human mast cell line (HMC-1) and HLMC were monitored following adenosine application. Adenosine potentiated mast cell tPA activity and tPA gene expression in a dose-dependent manner. Adenosine effects were abolished in the presence of adenosine deaminase. HMC-1 cells and HLMC predominantly expressed adenosine A2A and A2B receptor transcripts (A2B ≈ A2A > A3 >> A1). Pharmacological and signaling studies suggest that the A2A receptor is the major subtype accounting for adenosine-induced mast cell tPA activity. Finally, the supernatant from HMC-1 cells and HLMC treated with adenosine (for 24 h) significantly increased fibrin clot lysis, whereas ZM241385, an A2A receptor antagonist, abolished this effect. To our knowledge, this study provides the first data to demonstrate the potentiating effect of adenosine on mast cell tPA activity and fibrin clot lysis. The Journal of Immunology, 2011, 186: 1209–1217.

M ast cells are located in all tissues where they sense the local tissue environment, ready to respond to diverse tissue insults with an appropriate pattern of gene expression and mediator release required for the initiation of tissue inflammation and subsequent repair. However, when a tissue insult becomes chronic, ongoing mast cell activation may have deleterious consequences, resulting in tissue damage and remodeling (1, 2). With respect to the lung, mast cells contribute to host defense against bacterial infection (3) and play a major role in several diverse diseases, including asthma (2), pulmonary fibrosis (in which they interact intimately with fibroblasts) (4–6), and pulmonary vascular remodeling in response to pulmonary embolism and hypoxia (7, 8). Mast cells achieve these effects through the stimulus-specific release of autacoid mediators, proteases, and numerous chemokines and cytokines (9–14).

Tissue plasminogen activator (tPA) is one of the main components of the plasminogen/fibrinolytic system whose role involves fibrin homeostasis (i.e., blood clot resorption) and tissue remodeling through the activation of matrix metalloproteinases (15). The serine protease tPA converts proenzyme plasminogen into the active enzyme plasmin. In turn, plasmin degrades fibrin and can convert latent promatrix metalloproteinases into active matrix metalloproteinases known to degrade the extracellular matrix. Therefore, this pathway is implicated in the processes of tissue remodeling and angiogenesis, and tPA was shown to play a major role in the development of peritoneal and hepatic fibrosis (16, 17). These effects may be mediated, in part, by the ability of tPA to activate myofibroblasts and prevent myofibroblast apoptosis (18, 19). However, in some systems, tPA protects against fibrosis (20, 21), and so its biology with respect to fibrosis and remodeling is undoubtedly complex. The vascular endothelium is considered to be the major site of synthesis and release of tPA (22). However, perivascular cell types, such as mast cells, have also been exposed as an additional source of tPA. Endothelial tPA is both constitutively secreted and released rapidly in a dose-dependent manner following stimulation with thrombin, bradykinin, Substance P, isoproterenol, prostacyclin, and histamine (23, 24). Two studies also revealed that tPA transcription and secretion can be enhanced by protein kinase C and cAMP (25, 26). In contrast to endothelial cells, the factors regulating tPA release from mast cells remain undetermined. Mast cells constitutively release tPA in its free and enzymatically active form (11); however, with exposure to components of the complement system, such as C5a, mast cells can also produce plasminogen activator inhibitor (PAI)-1, which antagonizes tPA activity (27).

Adenosine is a ubiquitous purine nucleoside that accumulates during tissue inflammation (28) and in response to metabolic stress induced by hypoxia (29, 30). For example, pulmonary hypoxia triggers several adaptive mechanisms, including a rise in extracellular nucleotide release, enhanced ectonucleotidase activity, and attenuated adenosine uptake, consequently leading to a considerable accumulation of adenosine and increased adenosine receptor signaling (29). Adenosine concentrations are increased in the lungs of patients with asthma (31), and there is evidence of increased adenosine activity in chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis (28). Adenosine has complex activity on human lung mast cells, with both activation and inhibition of mediator release, depending on the receptors that are engaged. In view of this, we investigated the ability of adenosine to regulate tPA transcription, release, and activity in the human mast cell line HMC-1 and primary human lung mast cells.
To our knowledge, we show for the first time that adenosine is an activator of human mast cell tPA release and activity, suggesting that targeting the adenosine–mast cell–tPA axis may offer a novel approach to the treatment of a number of pulmonary diseases.

Materials and Methods

Reagents

Adenosine, 8-(4-chlorophenylthio)-2′-O-methyladenosine 3′, 5′-cyclic monophosphate monosodium hydrate (8CPTOMe), 8-bromoadenosine 3′:5′-cyclic monophosphate sodium (8Br-cAMP), H-89 dihydrochloride hydrate, and MRS1191 were purchased from Sigma-Aldrich (Gillingham, U.K.). CGS21680 hydrochloride, DPCPX, MRS1334, MRS1754, PSB603, and ZM241385 were from Tocris Biosciences (Bristol, U.K.). Adenosine deaminase (ADA), forskolin, and PKA Inhibitor 14-22 Amide (PKA inhibitor amide) were purchased from Calbiochem (Merck Chemicals, Nottingham, U.K.). Stem cell factor, IL-6, and IL-10 were from R&D Systems Europe (Abingdon, U.K.).

HMC-1 cell culture

The human mast cell line HMC-1 was a gift from Dr. J. Butterfield (Mayo Clinic, Rochester, MN) and was cultured as described previously (32).

HLMC isolation and culture

All patients gave written informed consent, and the study was approved by the Leicestershire Research Ethics Committee. HLMC were prepared and cultured as described previously (33). Final mast cell purity was >99%, and viability was >97%. Cells were used 1–8 wk after purification.

HMC-1 and HLMC stimulation

HMC-1 cells were centrifuged, resuspended in fresh media, and left to recover for 30 min at 37°C. Cells were then washed (twice for HMC-1 and once for HLMC) and resuspended in their corresponding media at 10⁶ cells/ml. For active tPA secretion measurements, cells were used at 5 × 10⁵ cells per assay. For measurement of tPA mRNA levels, 3 × 10⁶ and 1.5 × 10⁶ cells per assay were used for HMC-1 cells and HLMC, respectively. Cells were then incubated for 2 h (except where indicated) at 37°C in control media or following the addition of adenosine and/or other drugs. We chose 2 h as the stimulation period to assess changes between control and adenosine treatment because it was the minimal incubation interval giving robust, reproducible, and statistically different responses. After incubation, the cells were pelleted by centrifugation; the supernatant was used for active tPA-secretion measurements or clot-lysis assay, whereas the cell pellet was used for total RNA extraction. Many of the chemicals used in this study were dissolved in DMSO. DMSO (≤0.5%) had no effect on HMC-1 cell tPA activity or mRNA expression level (n = 3). Parallel controls were performed where appropriate. At a working concentration of 1 IU/ml ADA, ADA buffer contains 1.43 mM (NH₄)₂SO₄, which does not...
alter the pH of the stimulation media. In the experiments involving ADA, all conditions were performed in the presence of 1.43 mM (NH₄)₂SO₄ buffer.

**Active tPA-, total tPA-, and active PAI-1- secretion measurements**

Most cell tPA and PAI-1 activities, as well as total tPA, were measured using human tPA activity, PAI-1 activity, and tPA total Ag assays (Molecular Innovations, Novi, MI), according to the manufacturer’s recommendations. To circumvent variations inherent to each HMC-1 cell preparation/stimulation and each HLMC isolation (from different donors)/stimulation, changes in tPA activity and total tPA are expressed as the percentage of control response (unstimulated cells).

**Real-time PCR**

Total RNA from HMC-1 cells and HLMC were isolated with the RNeasy kit (Qiagen, Crawley, U.K.), treated with DNase I (Invitrogen), and subsequently reverse transcribed using iScript cDNA Synthesis Kit (Bio-Rad, Hemel Hempstead, U.K.), according to the manufacturer’s instructions, and Chromo4 DNA Engine thermocycler (MJ Research, Bio-Rad). Briefly, the reactions consisted of 40 cycles at 94°C for 45 s, 60°C for 30 s, and at 72°C for 1 min. The following primer sequences used for PCR amplification:

- **α-actin forward**: 5'-TCCATATGCGGCGACGAG-3'
- **α-actin reverse**: 5'-CTGTTCCTGCGCCAAGTTC-3'
- **PAI-1 forward**: 5'-CTGTTTGTCACGAAATCCC-3'
- **PAI-1 reverse**: 5'-TGCCACTCTGGTTCAC-3'
- **A2 receptor forward**: 5'-AAAGGGCCTTTATCCTTCC-3'
- **A2 receptor reverse**: 5'-GGTAATTTCACTTTCCAGCAG-3'
- **A2B receptor forward**: 5'-ATGGTGTCACGAATCC-3'
- **A2B receptor reverse**: 5'-GGGGACCACATTCTCAAAG-3'
- **tPA forward**: 5'-TCCTATGTGGGCGACGAG-3'
- **tPA reverse**: 5'-GATGTGTGCTAGTGCTTCGT-3'

All qPCR amplifications: 30 s at 94°C, 60°C for 30 s, and at 72°C for 1 min. The following primer sequences used for PCR amplifications:

- **Rad** forward: 5'-ACATCCATGCTCCATGGACC-3'
- **Rad reverse**: 5'-TCCTATGTGGGCGACGAG-3'

For each cycle, qPCR reactions were performed using MxPRO software (Stratagene, La Jolla, CA). Statistical analysis was performed using an unpaired Student’s t test and significance was accepted at p < 0.05.

**Results**

**Adenosine potentiates mast cell line HMC-1 tPA activity**

To test whether adenosine modulates tPA release from HMC-1 cells, tPA activity in stimulated HMC-1 cell media was measured after 2 h of incubation. Nontreated cell supernatant exhibited a tPA activity of 2.17 ± 0.12 IU/ml (n = 39), which increased significantly to 3.36 ± 0.16 IU/ml in the presence of 100 μM adenosine (n = 37; p < 0.0001). tPA activity in media alone was negligible (0.04 ± 0.02 IU/ml; n = 4). Adenosine-induced tPA activity in HMC-1 exhibited dose dependence in the range of 1–100 μM (Fig. 1A). ADA (1 U/ml), an enzyme that breaks down adenosine to inosine, significantly decreased basal tPA activity (73.3 ± 3.7% of control response; n = 9; p < 0.0001); it abolished adenosine-dependent tPA activity and reduced it below basal level (72.0 ± 3.5% of control response; n = 9; p < 0.0001) (Fig. 2A). Potentiation of tPA release by 100 μM adenosine was inhibited by 88% in the presence of adenosine receptor antagonist mixture (DPCPX [A1 receptor antagonist], ZM241385 [A2A], MR1754 [A3], and MRS1191 [A3], 1 μM each) (n = 18, p < 0.0001). This mixture also reduced the control response slightly, but significantly (6%; p = 0.0247). Interestingly, a similar pattern of dose-dependent potentiation by adenosine and inhibition by the adenosine receptor antagonist mixture was observed when measuring total tPA concentration (combination of free tPA [active form] and PAI-1-bound tPA [inactive form]) (Fig. 1B). Nontreated cell supernatant contained 3.01 ± 0.30 ng/ml of total tPA (n = 12). This value was significantly increased to 4.12 ± 0.27 ng/ml when cells were treated with 100 μM adenosine (n = 12). Total tPA in media

**FIGURE 2.** ADA abolishes adenosine-mediated potentiation of tPA activity and tPA transcript-expression level in HMC-1 cells. HMC-1 cells were stimulated with adenosine 100 μM in the presence or not of ADA (1 U/ml). ADA was added 10 min prior to adenosine. Conditioned media were assayed for tPA activity (A), whereas stimulated cells were harvested, and their tPA transcript-expression level was determined (B). **p < 0.01; ***p < 0.005, compared with control. *p < 0.05; ****p < 0.0005, compared with adenosine (100 μM).
alone was negligible at <0.01 ng/ml (n = 2). Basal HMC-1 PAI-1 activity (4.1 ± 0.2 U/ml; n = 3) was unaffected by treatment with 100 μM adenosine (4.3 ± 0.3 U/ml; n = 6; p = 0.6067).

Adenosine upregulates tPA transcript-expression level in HMC-1 mast cell line

Quantitative PCR was used to assess tPA transcript-expression level in HMC-1 (three to seven independent experiments). Adenosine upregulated tPA transcript expression in a dose-dependent manner by 2.1-fold (n = 4; p = 0.0349), 3.6-fold (n = 4; p = 0.0001), and 6.2-fold (n = 7; p = 0.0008) for 1, 10, and 100 μM, respectively (Fig. 1C). The adenosine receptor antagonist mixture (Fig. 1C) and ADA (Fig. 2B) abolished adenosine-evoked (100 μM) upregulation of tPA transcript level (n = 7; p = 0.0008 and n = 4; p = 0.0114, respectively). Interestingly, transcript levels for PAI-1 and PAI-2 remained unaffected by adenosine treatment (data not shown).

Adenosine upregulates A2A receptor subtype transcript-expression level in HMC-1 and HLMC

Based on their transcript-expression levels, the predominant adenosine receptor subtypes expressed on HMC-1 cells were A2A and...
A2B (n = 3). A3 and A1 receptor subtypes were less abundant (1.9 and 0.06% of A2A, respectively) (Fig. 3A, open bars). In HLMC, the adenosine receptor subtype distribution was slightly different. A2A and A3B receptor subtype transcripts remained predominantly expressed, and A1 receptor transcripts stayed poorly represented (1.1% of A2A). However, A3 receptor transcripts were more abundant (22.7% of A2A; three donors) (Fig. 3B, open bars). Interestingly, treatment with adenosine (100 μM) upregulated A2A receptor transcript expression by 5-fold (n = 3; p = 0.0043) and 2.2-fold (three donors, p = 0.0317) in HMC-1 cells and HLMC, respectively (Fig. 3, filled bars), but it had no effect on other adenosine receptor subtype transcript levels.

**Adenosine mediated-potentiation of mast cell tPA activity occurs mainly through the A2A receptor subtype**

**HMC-1 cells.** Selective adenosine receptor subtype antagonists (1 μM each) were added to HMC-1 cells 5 min before adenosine application (100 μM) (Fig. 4A). ZM241385, a selective antagonist for A2A receptors (7.8% inhibition; n = 15; p = 0.0078), and antagonist mixture (66.6%; n = 18; p = 0.0088) modestly diminished basal tPA activity (n = 36). ZM241385 dramatically reduced adenosine-induced mast cell tPA activity potentiation by 86.2% (n = 34 for adenosine alone, n = 21 for ZM241385 + adenosine; p < 0.0001), an inhibition similar to that obtained with the antagonist mixture (87.4%; n = 18; p < 0.0001). The A1 antagonist DPCPX and A2B antagonist MRS1754 reduced adenosine-evoked potentiation by 28.7% (n = 12; p = 0.0139) and 33.9% (n = 12; p = 0.0047), respectively. However, PSB603, another selective A2B antagonist, had no effect on adenosine-induced tPA activity potentiation, neither did the A3 receptor antagonists MRS1191 and MRS1334. We used the A2A receptor agonist CGS21680 to further confirm the major involvement of the A2A receptor subtype in adenosine-evoked tPA activity potentiation. CGS21680 evoked a dose-dependent increase in tPA activity in HMC-1 media, with significant differences observed with concentrations as low as 10 nM (lowest concentration tested) (Fig. 4C). Responses to 100 μM adenosine and 10 μM CGS21680 were not significantly different (n = 18). The A2A receptor antagonist ZM241385 and the antagonist mixture also abolished adenosine-induced tPA transcript upregulation (n = 3; p < 0.0001 for both). However, DPCPX, PSB603, MRS1754, MRS1191, and MRS1334 also markedly decreased tPA mRNA upregulation by 49.1 (p = 0.0038), 46.7 (p = 0.0002), 64.3 (p < 0.0001), 62.1 (p < 0.0001), and 16.7% (p = 0.0216), respectively (n = 3 for each) (Fig. 4B). In addition, only the higher concentrations of CGS21680 tested significantly upregulated tPA transcripts (by 4.5- and 10-fold for 1 μM CGS21680 [n = 9; p = 0.0052] and 10 μM CGS21680 [n = 6, p < 0.0001], respectively) (Fig. 4D).

**HLCM.** Adenosine increased primary HLCM basal tPA activity from 0.66 ± 0.23 IU/ml to 0.96 ± 0.28 IU/ml (five donors), but there were considerable variations among donors. In media alone, tPA activity was negligible (<0.001 IU/ml). However, similarly to HMC-1 cells, adenosine (100 μM) increased tPA release from HLCM by 53.0 ± 9.0% (p = 0.0007) (Fig. 5A). Potentiation by adenosine was inhibited by 57.5% (four donors, p = 0.0385) in the presence of 1 μM A2A receptor antagonist ZM241385, which, when used alone, did not affect the control response. Adenosine upregulated tPA transcript expression in HLCM by 4.4-fold (five donors, p = 0.0002), whereas ZM241385 totally abolished adenosine-evoked upregulation of tPA transcripts (five donors, p = 0.0005) (Fig. 5B). ZM241385, when used alone, did not affect tPA expression level.

Taken together, these results suggested that A2A is the major adenosine receptor subtype accounting for adenosine-induced human mast cell tPA activity, whereas more than one subtype seem to regulate tPA gene expression.

**Signaling pathway(s) involved in adenosine-mediated potentiation of mast cell tPA activity**

Because adenosine A2A receptors preferably couple to the Gs proteins (35), we used 8Br-cAMP, a metabolically stable analog of cAMP, and the adenylate cyclase activator forskolin to determine whether potentiation of tPA activity and mRNA upregulation by adenosine were mediated by cAMP. In HMC-1 cells, 8Br-cAMP (500 μM) increased tPA activity to 117.5 ± 2.9% (n = 9; p < 0.0001) of the control response, whereas forskolin (1 μM) increased it to 123.3 ± 4.7% (n = 9; p < 0.0001) (Fig. 6A). Interestingly, only high concentrations of forskolin (50 μM) significantly upregulated tPA transcripts (n = 3; p < 0.0001; Fig. 6B). 8Br-cAMP did not significantly increase tPA mRNA levels (n = 3). Increases in cAMP can lead to protein kinase A (PKA) and/or exchange protein directly activated by cAMP (Epac) activation. The PKA inhibitor H89 (10 μM added 40 min before adenosine) significantly reduced adenosine-induced tPA activity by 68.5% (n = 9; p < 0.0001), whereas the PKA inhibitor amide (25 μM; added 40 min before adenosine) reduced it by 34.8% (n = 9; p = 0.0320; Fig. 6C). H89 alone decreased basal tPA activity by 16.1% (n = 8; p < 0.0001). Both drugs considerably diminished upregulation of tPA transcripts induced by adenosine (inhibition of 63.3% for H89 [n = 4; p = 0.0028] and 41.8% for PKA inhibitor.

**FIGURE 5.** Adenosine potentiates HLCM tPA activity and transcript-expression level. HLCM were stimulated with adenosine 100 μM in the presence or not of adenosine receptor antagonist mixture (consisting of DPCPX, ZM241385, MRS1754 and MRS1191 [each at 1 μM]). A. Conditioned media were assayed for tPA activity. B. Stimulated cells were harvested for the measurement of tPA transcript-expression level. ***p < 0.005, compared with control. *p < 0.05; **p < 0.005, compared with adenosine (100 μM).
amide \([n = 4, p = 0.0293]\); Fig. 6D). The Epac activator 8CPTOMe (100 \(\mu\)M) increased tPA mRNA level by 3.7-fold \((n = 4; p < 0.0001)\), but it had no effect on basal tPA activity \((n = 12)\). These results suggested that A2A receptors control adenosine-induced tPA activity mainly through cAMP production and PKA activation. Comparatively, the signaling pathways controlling tPA up-regulation seem to be more complex, supporting the idea that multiple adenosine receptor subtypes are involved.

**Time course of adenosine-induced human mast cell fibrinolytic activity**

To assess the progress of adenosine-induced mast cell tPA activity over time, HMC-1 cells were stimulated with control buffer or 100 \(\mu\)M adenosine, and tPA activity was assessed over a 24-h period. Control and adenosine resulted in a continuous increase in tPA activity until 18 h, after which a plateau was reached. However, potentiation by adenosine was relatively steady \((39.7 \pm 5.3\% \text{ over control}; n = 3; p < 0.0001)\) across the different check points \((2, 4, 6, 12, 18, \text{ and } 24 \text{ h})\) (Fig. 7A). In addition, ZM241385 abolished adenosine-induced potentiation at 2 h \((n = 3; p = 0.0125)\) and reduced it by 63.6\% at 24 h \((n = 3; p = 0.0019)\). Adenosine also significantly upregulated tPA transcript level at each time point \((n = 3; p < 0.05 \text{ for all conditions}; \text{Fig. 7B})\). Interestingly, upregulation at 6 h was significantly lower than at 2 or 4 h \((n = 3; p = 0.0398)\), perhaps reflecting a change in HMC-1 cell cycle.

**Mast cell stimulation by adenosine potentiates clot lysis**

The physiological relevance of mast cell tPA secretion (fibrinolytic activity) was demonstrated using a clot-lysis assay. The fibrinolytic activity was assessed 10 h after the addition of HMC-1 cell supernatant on a fully formed tissue factor-induced fibrin clot. The supernatant from HMC-1 cells in culture for 18 and 24 h caused 15.7 \(\pm\) 1.9\% and 16.1 \(\pm\) 1.3\% of clot lysis compared with 9.9 \(\pm\) 1.2\% for media alone \((n = 3)\). The supernatant from cells treated with 100 \(\mu\)M adenosine for 18 and 24 h produced 23.6 \(\pm\) 2.5\% and 31.0 \(\pm\) 2.9\% of clot lysis \((n = 3)\), resulting in an increase of 50.4 and 92.6\% over their respective controls \((n = 3; p = 0.0386 \text{ and } p = 0.0004, \text{respectively}; \text{Fig. 7C})\). In addition, the A2A receptor antagonist ZM241385 abolished adenosine’s potentiating effect \((24 \text{ h stimulation})\) on clot lysis \((n = 3; p = 0.0002)\).

Because of the intrinsic lower level of tPA activity, HLMC fibrinolytic activity was assessed 24 h after the addition of the cell supernatant on a fully formed tissue factor-induced fibrin clot. The supernatant from HLMCs in culture for 24 h caused 18.0 \(\pm\) 2.5\% of clot lysis compared with 9.7 \(\pm\) 2.6\% for media alone \((n = 3)\). The supernatant from HLMC treated with 100 \(\mu\)M adenosine for 24 h
produced 28.3 ± 2.5% of clot lysis, revealing an increase of 57.2% over control (three donors; \( p = 0.0442 \); Fig. 7D). These results suggested that adenosine enhances human mast cell fibrinolytic activity.

**Discussion**

To our knowledge, this study provides the first data demonstrating the potentiating effect of adenosine on human mast cell tPA activity and fibrin clot lysis. Active and total tPA secretion, as well as tPA transcript level, were increased in a dose-dependent manner by adenosine in human mast cells. As described previously, mast cells exhibited a time-dependent accumulation of tPA activity for 12 h (11) but reached a maximum over longer incubation periods (≥18 h). Adenosine treatment displayed similar features, with a relatively steady potentiation over time, suggesting that adenosine-dependent mRNA upregulation leads to de novo synthesis and release of tPA rather than being limited to acute release from a preformed pool. Potentiation of secretion and tPA mRNA expression by adenosine were dramatically inhibited by ADA and the adenosine receptor antagonist mixture (DPCPX, ZM241385, MRS1754, and MRS1191), confirming the involvement of adenosine receptor(s). Interestingly, ADA, the adenosine receptor antagonist mixture, and the \( A_{2A} \) receptor antagonist ZM241385 used alone reduced basal mast cell tPA activity. This suggested that basal mast cell tPA activity might be induced, in part, by the endogenous release of adenosine and/or ATP/ADP (possibly metabolized to adenosine by endogenous ectonucleotidases).

Mast cells have long been known to express adenosine receptors. In our study, we found that HLMC and the HMC-1 cell line predominantly expressed the \( A_{2A}, A_{2B}, \) and \( A_3 \) receptor subtypes. \( A_1 \) receptor expression was negligible, in accordance with previous publications (36). Interestingly, adenosine treatment upregulated \( A_{2A} \) receptor transcripts but had no effect on the level of expression of other adenosine receptor subtypes. This is in accordance with our pharmacological studies in which \( A_{2A} \) seemed to be the major adenosine receptor subtype responsible for enhancing mast cell tPA activity. In contrast, multiple adenosine receptor subtypes seem to contribute to tPA transcript upregulation (35), and this possibility was supported by our signaling studies. \( A_{2A} \) receptors couple to the \( G_\text{s} \) protein, leading to the activation of adenylyl cyclase and, subsequently, to the production of cAMP. We showed that tPA transcript upregulation required higher intracellular cAMP concentrations than did tPA activity and that the Epac activator 8CPTOMe dramatically increased tPA mRNA ex-

**FIGURE 7.** Time course of adenosine-induced mast cell fibrinolytic activity. At each time point, conditioned media from HMC-1 cells were assayed for tPA activity (A) and used for fibrin clot-lysis assay (C). B, Stimulated HMC-1 cells were harvested for the measurement of tPA transcript-expression level. D, Conditioned media from HLMC (24 h) were used for fibrin clot-lysis assay. \( p < 0.05; *** p < 0.01; **** p < 0.005, \) compared with control. \( \# p < 0.05; \#\# p < 0.01; \#\#\# p < 0.005, \) compared with adenosine (100 \( \mu \)M).
pression level but had no effect on adenosine-dependent tPA activity, suggesting distinct signaling pathways. Nevertheless, PKA inhibitors, H89 and the PKA inhibitor amide, significantly reduced both cellular events, indicating that Epac and PKA are involved in adenosine-mediated tPA transcript upregulation, whereas increased tPA activity is only PKA dependent. Overall, our study suggests that A2A receptors control adenosine-induced tPA activity, whereas the adenosine receptor subtype(s) responsible for tPA mRNA upregulation remain to be elucidated.

Adenosine-induced mast cell tPA activity could result from an increase in the release of tPA in its free and enzymatically active form or from a diminution of its natural inhibitor PAI-1. In mast cells, it was shown that modulation of PAI-1 activity by components of the complement system, such as peptide C5a, PMA, or calcium ionophore, subsequently affected tPA activity (27, 37). However, we did not observe such regulation of PAI-1 (or PAI-2) following adenosine treatment, because PAI-1 activity and PAI-1 and PAI-2 transcript levels remained unaffected, indicating that adenosine increases the release of tPA in its free and active form.

These findings are supported by early studies suggesting that adenosine enhances mast cell mediator release (38). The ability of mast cells to dissolve a fibrin clot via the constitutive release of tPA was reported previously (11, 37). Using a similar approach, we showed that adenosine, primarily via A2A receptor activation, can enhance this process of fibrin clot dissolution, suggesting that mast cell tPA activity and, consequently, fibrinolytic activity can be modulated. In the acute setting, in which the tissue insult resolves rapidly, this activity may be seen as beneficial, restoring the normal status quo.

In the disease setting in which the tissue insult is chronic or repetitive, the role of mast cell production of tPA is unclear; conflicting evidence suggests that active tPA might be pro- or anti-fibrotic. In several systems, tPA contributes to tissue remodeling and fibrosis (16, 17, 39, 40). tPA activates myofibroblasts, prevents myofibroblast apoptosis, and activates matrix metalloproteinases (15, 18, 19, 41). The involvement of mast cells in pulmonary vascular remodeling following hypoxia was shown to involve matrix-degrading metalloproteinases in animal models (7, 8). Such hypoxic conditions cause a rapid accumulation of adenosine in the lungs and, consequently, increased adenosine receptor signaling (29). Similarly, increased adenosine and tPA activity are evident in chronic asthma, and enhanced adenosine activity is seen in idiopathic pulmonary fibrosis (28, 42–44). Mast cells are strategically placed in the vicinity of pulmonary vessels (45) and structural airway cells (46, 47), and they interact intimately with fibroblasts in fibrotic conditions (4–6). Therefore, chronic tPA release might be predicted to contribute to lung remodeling in chronic disease.

In contrast, in animal models, tPA activity was shown to protect against bleomycin-dependent pulmonary fibrosis, and aerosolized tPA reduces the fibrotic response. Studies involving tPA-deficient mice highlighted the protective role of tPA against pulmonary and skin fibrosis (20, 21), although it promoted kidney fibrosis (40). In addition, aerosolized tPA greatly improved the clearance of obstructed airways following acute lung injury (48) and airway hyperresponsiveness (49). Moreover, tPA displays anti-inflammatory properties. For example, the exogenous administration of tPA inhibits IL-1-induced lung leak (50) and reduces edema in rodent models, without affecting neutrophil infiltration (51, 52). It also prevents the production of reactive oxygen species by macrophages and neutrophils (53, 54), which seems to be independent from its proteolytic activity (55).

In the presence of hypoxia and/or tissue inflammation, the acute release of tPA by mast cells in response to adenosine accumulation may be beneficial and promote resolution. However, there are two conflicting views on whether tPA secretion is protective or deleterious with regard to chronic disease. In either case, targeting adenosine and its receptors in chronic disease has the potential to offer a novel therapeutic approach to the treatment of several diverse pulmonary diseases.

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Disclosures

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