Contribution of ecto-5′-nucleotidase to the inhibition of platelet aggregation by human endothelial cells

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We studied the role of adenosine (Ado), which is generated from adenine nucleotides via the activity of ecto-5′-nucleotidase (ecto-5′-NT), in the inhibition of platelet aggregation by endothelial cells (ECs). The enzymatic activity of nucleotidases on human umbilical vein endothelial cells (HUVECs) was examined with regard to (1) the inhibition of adenosine diphosphate (ADP)-induced platelet aggregation and (2) the liberation of inorganic phosphate from adenine nucleotides. Adenosine 5′-monophosphate (AMP) pre-incubated with HUVECs significantly inhibited ADP-induced platelet aggregation. This was completely blocked by the treatment of HUVECs with a specific inhibitor of ecto-5′-NT, 5′-[α,β-methylene] diphosphate (APCP), or by the addition of an A2a receptor antagonist. Neither nitric oxide nor prostacyclin was involved in this inhibitory activity, suggesting that Ado generated in the incubation medium by the activity of 5′-NT on HUVECs inhibited platelet aggregation. When ADP was incubated on HUVECs, it lost most of its agonistic activity for platelets. Pretreatment of HUVECs with APCP at a concentration that abolished ecto-5′-NT activity partially restored ADP-induced platelet aggregation. Ecto-5′-NT contributes to EC function by inhibiting platelet aggregation in cooperation with ATP diphosphohydrolase, which degrades ATP to AMP, thereby allowing Ado to be released from HUVECs.

Introduction

It has been well established that quiescent endothelial cells (ECs) exert anticoagulant effects both through their expression of thrombomodulin and heparan sulfate proteoglycans and by the release of tissue factor pathway inhibitor. ECs also inhibit platelet aggregation through the production of nitric oxide (NO) and prostacyclin (PGL) and through degradation of adenosine diphosphate (ADP) by adenosine triphosphate (ATP) diphosphohydrolase (ATPDase), which is expressed on the luminal surface of ECs. However, ATPDase can inhibit platelet aggregation and recruitment in the absence of NO and PGL. ADP released from activated platelets induces platelet recruitment followed by further platelet aggregation via binding to platelet P2Y1 and P2Y11 receptors. ATPDase (molecular mass, 70-100 kd) is a glycoprotein belonging to the E-type ATPase family. This enzyme hydrolyzes ATP and ADP to ADP and adenosine 5′-monophosphate (AMP), respectively. Enzyme activity is dependent on calcium (Ca2+) and magnesium (Mg2+), and is inhibited by chelating agents, azides, and ATP analogues, although it is not affected by inhibitors of P-, F- and V-type ATPases. Degradation of extracellular ADP by ATPDase has been recognized as important for the inhibition of platelet aggregation by ECs, because of the critical role ADP plays as an agonist for platelet aggregation.

Ecto-5′-nucleotidase (ecto-5′-NT) (CD73) also participates in adenine nucleotide metabolism on the surface of ECs. AMP, generated from ADP by the action of ATPDase, is subsequently hydrolyzed to adenosine (Ado) by ecto-5′-NT. Widespread distribution in bacteria, plant cells, and vertebrate tissues, 5′-NT is classified into 4 groups according to cellular location and biochemical properties: a membrane-anchored ecto-5′-NT, a soluble form derived from ecto-5′-NT, and 2 cytoplasmic forms. Ecto-5′-NT, anchored to the plasma membranes via glycosyl-phosphatidylinositol (GPI) moiety, is distributed in a variety of cells including hepatocytes, fibroblasts, endothelial cells, lymphocytes, and glial cells. Inhibition of 5′-NT controls intracellular and extracellular levels of AMP and Ado, thereby allowing Ado to be metabolized for the synthesis of adenine nucleotides in the purine salvage pathway. In addition to its enzymatic activity, ecto-5′-NT is involved in cell-cell and cell-matrix interactions and transmembrane signaling.

The disulfide-linked homodimer form of ecto-5′-NT is essential for its enzymatic activity. Although ecto-5′-NT hydrolyzes a variety of nucleoside 5′-monophosphates, it has greatest affinity for AMP, with Km values in the micromolar range. Enzymatic activity is not dependent on added divalent cations but is inhibited by metal ion chelating agents because of the presence of several potential zinc (Zn2+) binding sites that are important for its activity. Adenosine 5′-[α,β-methylene] diphosphate (APCP) is a potent inhibitor of ecto-5′-NT with Ki values in the nanomolar range. ATP and ADP also inhibit ecto-5′-NT with Ki values in the micromolar range.

Ado inhibits a variety of cellular functions including platelet aggregation, expression of tissue factor or adhesion molecules and cytokine release by activated ECs, and injury to ECs, and release of superoxide from neutrophils. However, Ado also enhances NO production by ECs. These effects are mediated through the binding of Ado to the A1, A2, and A3 receptors that are expressed on cells in a variety of tissues. The inhibition of platelet aggregation by Ado is thought to be mediated by the stimulation of adenylate cyclase through A3 receptors expressed on platelets. Although the inhibitory aggregation effect...
of Ado on platelet is evident in vitro,\textsuperscript{23} its effect in vivo has remained controversial because of its rapid transport into cells\textsuperscript{29,30} and its rapid degradation by Ado deaminase (ADA).\textsuperscript{31} However, studies showing that both platelet aggregation ex vivo and thrombosisis formation in vivo were inhibited by the administration of Ado analogues to humans and dogs\textsuperscript{32} and that an Ado receptor antagonist caused thrombosis in dogs\textsuperscript{33,34} suggest a critical involvement of Ado in the inhibition of platelet aggregation in vivo. In the present study, we examined ecto-5′-NT activity on the surface of human umbilical vein endothelial cells (HUVECs) and confirmed the cooperative function of ecto-5′-NT with ATPDase in the regulation of platelet aggregation.

Materials and Methods

Materials

We purchased the following materials: MCDB131 medium, gelatin, bovine serum albumin (BSA), aspirin, levamisole, and APCP (Sigma Chemical Co., St Louis, MO); AMP, Ado, malachite green, and N\textsuperscript{6}-nitro-L-arginine-methyl ester (L-NAME) (Wako Pure Chemical, Osaka, Japan); 5-(chlorostyryl) caffeine (CSC) and dipyrindamole (DIP) (Research Biochemicals International, Natick, MA); nitrate/nitrite colorimetric assay kit (Cayman Chemical, Ann Arbor, MI); fetal calf serum (FCS) (Nichirei, Tokyo, Japan); acidic fibroblast growth factor (aFGF) (Austral Biologicals, San Ramon, CA); heparin (Daichi Pure Chemicals, Tokyo, Japan); ADP ( Oriental Yeast, Tokyo, Japan); Urinary Prostacyclin Enzyme Immunoassay Kit (Assay Designs Inc., Ann Arbor, MI); and recombinant human tumor necrosis factor–α (TNF–α) (Peprotech EC, London, England).

Cell culture of HUVECs

HUVECs were prepared from umbilical cords according to the methods described previously.\textsuperscript{35} The cells were cultured in MCDB131 medium supplemented with 15% FCS, 10 ng/mL aFGF, and 5 μg/mL heparin on gelatin-coated flasks (Nalge Nunc International, Naperville, IL) at 37°C under 5% carbon dioxide (CO\textsubscript{2}). Second- to fourth-passage cells were used in the present study.

Inhibition of platelet aggregation by ADP preincubated on HUVECs

Nucleotidase activity. HUVECs were grown to confluence in a gelatin-coated 96-well plate. After removal of the culture medium, the HUVEC-containing wells or the cell-free (blank) wells were washed twice with the wash buffer (buffer B) containing 50 mmol/L Tris HCl (pH 7.4) with 0.25 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.125 mmol/L ethylene glycoltetraacetic acid (EGTA), 130 mmol/L sodium chloride (NaCl), and 5.5 mmol/L glucose. The wells were then washed with the incubation buffer (buffer C) containing 50 mmol/L Tris HCl (pH 7.4) with 130 mmol/L NaCl, 5 mmol/L MgCl\textsubscript{2}, and 5.5 mmol/L glucose. Buffer C, containing 1-100 μmol/L AMP, was then added to the wells and incubated at 37°C for 15 minutes. The amounts of Pi liberated into the supernatants were measured as described above. The inhibitory effect of the preincubated buffer on HUVECs or blank wells on ADP-induced platelet aggregation was also examined as described below. In some experiments, HUVECs were treated with one of the following inhibitors prior to incubation with AMP: 0.1-10 mmol/L L-NAME, an inhibitor of NO synthase; 0.01-1 mmol/L aspirin, an inhibitor of cyclooxygenase\textsuperscript{29,35}; 1-100 μmol/L DIP, an inhibitor of adenosine transporter\textsuperscript{36,37}; 0.1-10 mmol/L levamisole; or 0.01 μmol/L APCP.

Production of NO and PGE\textsubscript{2} by HUVECs

The concentration of NO in the incubation buffer was measured as total nitrate/nitrite concentration using a nitrate/nitrite colorimetric assay kit. In some experiments, HUVECs were pretreated with 0.01-10 mmol/L L-NAME. The production of PGE\textsubscript{2} during the incubation period was evaluated by measuring its stable breakdown products, 6-keto-prostaglandin F\textsubscript{1α}, and 2,3-dinor-6-keto-prostaglandin F\textsubscript{1α} in the incubation buffer using a urinary prostacyclin enzyme immunoassay kit. In some experiments, HUVECs were pretreated with 0.001-1 mmol/L aspirin.

Statistical analyses

The Student unpaired \textit{t} test was used for all statistical analyses.

Results

Inhibition of platelet aggregation by ADP preincubated on HUVECs

AMP incubated on the blank wells did not release Pi and induced aggregation of platelets (Figure 1). However, Pi was liberated from HUVECs in a dose-dependent manner relative to the concentration of AMP added (Figure 1B). Platelet aggregation induced by the AMP-containing buffer preincubated on HUVECs was inhibited compared with aggregation induced by the buffer preincubated on blank wells (Figure 1).

Inhibition of ADP-induced platelet aggregation by AMP preincubated on HUVECs

AMP was not hydrolyzed on blank wells (Figure 2B). Pi was liberated by the incubation of AMP on HUVECs in a dose-dependent manner relative to the concentration of AMP added.
Figure 2B). The AMP-containing buffer preincubated on HUVECs inhibited ADP-induced platelet aggregation compared with that preincubated on blank wells (Figure 2).

**Involvement of NO and PGI2 in the inhibition of ADP-induced platelet aggregation by AMP preincubated on HUVECs**

The 30 μmol/L AMP buffer preincubated on HUVECs did not contain detectable amounts of nitric oxide. Pretreatment with more than 10 μmol/L L-NAME, which completely inhibited NO production in the growth medium of HUVECs, did not affect the inhibition of ADP-induced platelet aggregation by the AMP buffer preincubated on HUVECs (Figure 3A). This buffer contained 0.76 ± 0.09 pg/10⁵ cells per hour (the mean ± SD) of PGI2, which is at the lowest limit of detection of the assay used. Pretreatment with more than 10 μmol/L aspirin completely blocked PGI2 production in the growth medium of HUVECs. Under these conditions, inhibition of ADP-induced platelet aggregation by AMP buffer preincubated on HUVECs was not affected (Figure 3B).

**Involvement of Ado receptor in the inhibition of ADP-induced platelet aggregation by AMP preincubated on HUVECs**

In the presence of CSC, inhibition of ADP-induced platelet aggregation by purified Ado was abrogated in a dose-dependent manner (data not shown). The inhibition of ADP-induced platelet aggregation by 30 μmol/L AMP buffer preincubated on HUVECs was blocked by more than 1 μmol/L CSC (Figure 3C).

**Involvement of ecto-5'-NT in the inhibition of ADP-induced platelet aggregation by AMP preincubated on HUVECs**

Levamisole (10 mmol/L) did not affect AMP degradation on HUVECs (data not shown). APCP inhibited AMPase activity on HUVECs in a dose-dependent manner, as measured by the mean ± SD: Pi release without APCP, 21.0 ± 1.9 μmol/L; Pi release with 1 μmol/L APCP, 11.6 ± 2.2 μmol/L; Pi release with 10 μmol/L APCP, 5.6 ± 1.3 μmol/L. Pi was not liberated with 100 μmol/L APCP (Figure 4B). Inhibition of platelet aggregation by the AMP buffer preincubated on HUVECs was also attenuated by APCP in a dose-dependent manner (data not shown) and was completely blocked by 100 μmol/L APCP (Figure 4A).

**Involvement of ecto-5'-NT in the inhibition of platelet aggregation by ADP preincubated on HUVECs**

Pretreatment of HUVECs with APCP partially inhibited Pi liberation from ADP preincubated on HUVECs (Figure 5B). Platelet aggregation induced by the ADP buffer preincubated on HUVECs was restored by the pretreatment of HUVECs with APCP (Figure 5A).
Discussion

The ability of HUVECs to inhibit platelet aggregation by ADP degradation was confirmed (Figure 1). The ADP- or AMP-treated buffers preincubated on HUVECs could contain not only adenine nucleotides but also NO and PGI2 produced in the incubation periods. Because both NO and PGI2 are major substances released from ECs that inhibit platelet aggregation, it was possible that NO and/or PGI2 were involved in the mechanisms that inhibited platelet aggregation under our experimental conditions. HUVECs released NO in the growth medium at 0.14 ± 0.02 (mean ± SD) nmol/10^5 cells per hour, at the lowest limit of detection of the assay. However, we removed the medium followed by 3 washes of the wells prior to incubation with ADP- or AMP-containing buffer for 15 minutes. These incubation buffers did not contain any detectable amounts of nitric oxide. Pretreatment of HUVECs with more than 10 μmol/L L-NAME, which was enough to block NO production in the growth medium, did not change the inhibitory effect on platelet aggregation of ADP buffer preincubated on HUVECs (data not shown). This suggested that NO produced in the buffer during the incubation phase did not affect platelet aggregation under our experimental conditions.

Basal levels of PGI2 in the growth medium of HUVECs were 1.34 ± 0.25 pg/10^5 cells per hour, and the incubation buffer contained 0.51 ± 0.07 pg/10^5 cells per hour, the limit of detection of the assay. Aspirin completely inhibited PGI2 production during the incubation of buffer at 0.01-1 mmol/L aspirin (data not shown). Because pretreatment of HUVECs with 1 mmol/L aspirin did not influence the inhibition of platelet aggregation by the ADP buffer preincubated on HUVECs (data not shown), it is likely that PGI2 in the incubation buffer did not affect the inhibitory effect on aggregation in our experiments.

On the surface of HUVECs, ATPDase, ecto-5'-NT, and ALP can hydrolyze adenine nucleotides. However, pretreatment of HUVECs with 0.1-10 mmol/L levamisole, a specific inhibitor of ALP, did not affect Pi liberation from ADP in our experiments. This may be due to our experimental condition being at a lower pH than the optimal pH for ALP. Therefore, the role of HUVECs in the inhibition of platelet aggregation shown in Figure 1 could be explained as ADP degradation by nucleotidases on HUVECs. Undoubtedly, hydrolysis of ADP by ATPDase

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Figure 3. Involvement of the NO, PGI2, and Ado receptor in the inhibition of ADP-induced platelet aggregation by AMP preincubated on HUVECs. HUVEC-containing wells and blank wells were treated with (A) 0.1-10 mmol/L L-NAME or (B) 0.01-1 mmol/L aspirin. After washing, the wells were incubated with 30 μmol/L AMP at 37°C for 15 minutes. The inhibitory activity of the AMP buffer preincubated on HUVECs against ADP-induced platelet aggregation was examined. ○ and ● denote the AMP buffer preincubated on HUVECs and blank wells, respectively. The concentrations of NO (panel A, ●) and PGI2 (panel B, ●) in the buffer incubated on HUVECs are also shown. The results are the mean ± SD of 3 separate experiments.

Figure 4. Involvement of ecto-5'-NT in the inhibition of ADP-induced platelet aggregation by AMP preincubated on HUVECs. HUVEC-containing wells and blank wells were pretreated with 100 μmol/L APCP, an inhibitor of ecto-5'-NT, then incubated with a phosphate-free buffer containing 1-100 μmol/L AMP at 37°C for 15 minutes. (A) The inhibitory activity of the AMP buffer preincubated on HUVECs for ADP-induced platelet aggregation (●) was completely blocked by the treatment of HUVECs with APCP (△) to the levels found for the blank wells (○). The results are the mean ± SD of 3 separate experiments. (B) Pi was liberated on HUVECs in a dose-dependent manner relative to the concentration of the AMP added (△). The treatment of the HUVECs with APCP completely inhibited Pi liberation from AMP (△), comparable to the results from the blank wells (○). The results are the mean ± SD of 3 separate experiments.
The ineffectiveness of L-NAME on the function of AMP buffer preincubated on HUVECs to inhibit ADP-induced aggregation (Figure 3A) indicated that NO was not a major contributor under our experimental conditions. This might be due to the conditions of HUVECs in the confluent layer because previous studies revealed that NO synthase expression and NO release declined in the confluent phase.\(^\text{52}\) As for PGI\(_2\), AMP buffer preincubated on HUVECs contained little PGI\(_2\), and pretreatment of HUVECs with aspirin did not affect the inhibitory effects of the incubation buffer (Figure 3B). This suggests that PGI\(_2\) produced during the incubation phase did not influence ADP-induced platelet aggregation in our experiments. The IC\(_{50}\) value of PGI\(_2\) for ADP-induced platelet aggregation is about 100-fold higher than the concentration of the preincubated buffer.\(^\text{43}\) The production of PGI\(_2\) by ECs may decrease in the subculture and confluent states in our experiments, as previously shown in several studies.\(^\text{44,45}\)

Because we hypothesized that the hydrolysis of AMP by ecto-5'-NT was the main mechanism by which AMP buffer preincubated on HUVECs inhibited ADP-induced platelet aggregation (Figure 2), we expected that the Ado receptor antagonists would block this effect. As expected, CSC, a selective A\(_{2a}\) receptor antagonist,\(^\text{40}\) abrogated this effect in a dose-dependent manner (Figure 3C). Therefore, it is likely that the inhibitory effect of AMP buffer preincubated on HUVECs is derived via an interaction of Ado with the A\(_{2a}\) receptor on platelets.

The remaining question was whether some portions of Ado produced on the luminal surface may be taken up by HUVECs or deaminated by ADA before exerting any biological effects. Pretreatment of HUVECs with 1-100 \(\mu\text{mol/L}\) DIP, an inhibitor of Ado transport,\(^\text{39}\) did not affect AMP degradation or the inhibitory effect of the buffer on platelet aggregation (data not shown). This indicated that transport of Ado across the membranes had a limited effect on the extracellular levels of Ado, at least in our experimental conditions. Taken together with the report by Aalto et al.,\(^\text{46}\) which showed that the effect of deamination of Ado by ADA on cultured HUVECs is limited, it is likely that in our experiments, the extracellular levels of Ado are regulated mainly by the generation of Ado from adenine nucleotides on HUVECs. Because treatment with levamisole, an inhibitor of ALP, did not interfere with the AMPase activity on HUVECs, we estimated that ecto-5'-NT was mainly involved in the hydrolysis of AMP. APCP, an AMP analogue modified on the phosphate chain by substituting a bridging oxygen with a methylene, is a specific inhibitor of 5'-NT.\(^\text{47}\) It was demonstrated that APCP is neither affected by the hydrolysis of ADP by ATPDase nor is it hydrolyzed by ATPDase.\(^\text{47}\) APCP displayed almost no affinity for the P\(_{2\alpha}\) receptor,\(^\text{48}\) which was also supported by our findings that ADP-induced platelet aggregation was not influenced by the addition of APCP (data not shown).

As shown in Figure 5, pretreatment of HUVECs with APCP resulted in a partial decrease in Pi liberation from HUVECs that were treated with ADP. In addition, platelet aggregation was partially restored at comparatively low concentrations of ADP. This might be explained by the fact that while a low concentration of ADP could be completely hydrolyzed by ATPDase on HUVECs, excessive ADP, which was undigested by ATPDase, inhibited the activity of ecto-5'-NT. Therefore, it seems reasonable to suppose that ecto-5'-NT is involved in the HUVEC function of inhibiting platelet aggregation in cooperation with ATPDase, especially at comparatively low concentrations of ADP. Inhibition of remnant ADP, which is not hydrolyzed by ATPDase, by Ado generated by the activity of ecto-5'-NT should lead to an increase in the threshold for platelet aggregation.

In conclusion, we identified a contribution of ecto-5'-NT to the inhibitory effect of HUVECs on platelet aggregation in cooperation with ATPDase at a comparatively low concentration of ADP.
Ecto-5'-NT seems to be effective for prevention of thrombosis formation by increasing the threshold for platelet aggregation, although its role in vivo remains to be clarified. Results from these studies verify the concept that platelets in motion and in close proximity to endothelial cells do not respond to standard platelet agonists. This is probably due to the combined action of ATPDase and ecto-5'-NT described in our studies.

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References

1. Esmon CT. The regulation of natural anticoagu-
2. Marcum JA, Rosenberg RD. Anticoagulant ac-
tive heparin-like molecules from vascular tissue.
3. Broge Jz Jr. The role of tissue factor pathway
inhibitor in a revised coagulation cascade. Semin
4. Ignarro LJ. Nitric oxide: a novel signal transduc-
tion mechanism for transcellular communication.
5. Moncada S. Biological importance of prostacy-
of platelet function by an aspirin-insensitive endo-
thelial cell ADPase: thromboregulation by endo-
cation and characterization of CD39/vascular ATP
diphosphohydrolase. J Biol Chem. 1996;271:
33116-33122.
al. The endothelial cell ecto-ADPase responsible
for inhibition of platelet function is CD39. J Clin
Jb, Kunapuli SP. Molecular basis for ADP-
induced platelet activation. Evidence for three
distinct ADP receptors on human platelets. J Biol
tor is necessary for adenosine 5'-diphosphate-
induced platelet aggregation. Blood. 1998;92:
152-159.
11. Plesner L. Ecto-ATPases: identities and func-
12. Komozynski M, Wojcik A, Apyrases (ATP
diphosphohydrolases, EC 3.6.1.5): function and
relationship to ATPases. Biochim Biophys Acta.
Inhibition of platelet function by recombinant
101:1851-1859.
14. Zimmermann H. 5'-nucleotidase: molecular struc-
15. Zimmermann H. Biochemistry, localization and
functional roles of ecto-nucleotidases in the ner-
Y. Primary structure of human placental 5'-nucle-
olase and identification of the glycolipid anchor
in the mature form. Eur J Biochem. 1990;191:
563-569.
ecto-5'-nucleotidase (CD73): cDNA cloning and tissue
18. Thompson LF. Ecto-5'-nucleotidase can provide
the total purine requirements of mitogen-stimu-
lated human T cells and rapidly dividing human B
lymphoblastoid cells. J Immunol. 1985;134:3794-
3797.
19. Stochaj U, Mannherz HG. Chicken gizzard 5'-
nucleotidase functions as a binding protein for the
involved in lymphocyte binding to the endothelium:
characterization of lymphocyte-vascular adhesion
182:1603-1608.
21. Knobel T, Strater N. X-ray structure of the Esche-
richia coli periplasmic 5'-nucleotidase containing a
dimetal catalytic site. Nat Struct Biol. 1999;6:
448-453.
22. Naito Y, Lowenstein JM. 5'-nucleotidase from rat
heart membranes: inhibition by adenine nucleo-
tides and related compounds. Biochem J. 1985;
231:665-641.
rivatives of adenosine-5'-N-ethyl-5'-ethylaminonate:
selective A2 adenosine receptor agonists with poten-
tial inhibitory activity on platelet aggregation.
24. Deeguchi H, Takeya H, Urano H, Gabazza EC,
Zhou H, Suzuki K. Adenosine regulates tissue
factor expression on endothelial cells. Thromb
Res. 1998;91:57-64.
25. Bouma MG, van den Wildenberg FJAM, Buurman
WA. Adenosine inhibits cytokine release and ex-
pression of adhesion molecules by activated hu-
man endothelial cells. Am J Physiol. 1996;270:
C529-522.
G, Hirschhorn RJ. Adenosine: an endogenous in-
hibitor of neutrophil-mediated injury to endothelial
27. Roberts PA, Newby AC, Hallett MB, Campbell AK.
Inhibition by adenosine of reactive oxygen me-
tabolite production by human polymorphonuclear
Adenosine enhances nitric oxide production by
vascular endothelial cells. Am J Physiol. 1995;
269:C519-523.
29. Pearson JD, Carleton JS, Hutchings A, Gordon
JL. Uptake and metabolism of adenosine by pig
aortic endothelial and smooth-muscle cells in cul-
30. Dieterle Y, Ody C, Ehrensberger A, Stalder H,
Juned AF. Metabolism and uptake of adenosine
triphosphate and adenosine by porcine aortic and
pulmonary endothelial cells and fibroblasts in cul-
31. Moser GH, Schroder J, Deussen A. Turnover of
adenosine in plasma of human and dog blood.
32. Bullough DA, Zhang C, Montag A, Mullane KM,
Young MA. Adenosine-mediated inhibition of
reactive oxygen metabolites for P2X-purinoceptors in rat
urethral bladder. Br J Pharmacol. 1999;142:1151-
1159.
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