How systemic inflammation modulates adenosine metabolism and adenosine receptor expression in humans in vivo

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Objective: Adenosine modulates inflammation and prevents associated organ injury by activation of its receptors. During sepsis, the extracellular adenosine concentration increases rapidly, but the underlying mechanism in humans is unknown. We aimed to determine the changes in adenosine metabolism and signaling both in vivo during experimental human endotoxemia and in vitro.

Design: We studied subjects participating in three different randomized double-blind placebo-controlled trials. In order to prevent confounding by the different pharmacological interventions in these trials, analyses were performed on data of placebo-treated subjects only.

Setting: Intensive care research unit at the Radboud University Nijmegen Medical Center.

Subjects: In total, we used material of 24 healthy male subjects.

Interventions: Subjects received 2 ng/kg Escherichia coli endotoxin (lipopolysaccharide) intravenously.

Measurements and Main Results: Following experimental endotoxemia, endogenous adenosine concentrations increased. Expression of 5'ectonucleotidase messenger RNA was upregulated (p = .01), whereas adenosine deaminase messenger RNA was downregulated (p = .02). Furthermore, both adenosine deaminase and adenosine kinase activity was significantly diminished (both p ≤ .0001). A<sub>1a</sub> and A<sub>2b</sub> receptor messenger RNA expression was elevated (p = .02 and p = .04, respectively), whereas messenger RNA expression of A<sub>1a</sub> and A<sub>2b</sub> receptors was reduced (both, p = .03). In vitro, lipopolysaccharide dose-dependently attenuated the activity of both adenosine deaminase and adenosine kinase (both p ≤ .0001).

Conclusions: Adenosine metabolism and signaling undergoing adaptive changes during human experimental endotoxemia promoting higher levels of adenosine thereby facilitating its inflammatory signaling. (Crit Care Med 2012; 40:2609–2616)

Key Words: adenosine; adenosine metabolism; inflammation; sepsis

The purine nucleoside adenosine is an important signaling molecule involved in tissue protection and repair in situations of impending tissue danger. After decades of pharmacological studies, it was the genetic in vivo approach that allowed to uncover the unique role of A<sub>1a</sub> adenosine receptors. The A<sub>1a</sub> adenosine receptor plays a critical and nonredundant role in the regulation of inflammation and subsequent tissue damage (1). During hypoxia, ischemia, and inflammation, the extracellular adenosine concentration rapidly increases, and subsequent stimulation of membrane-bound adenosine receptors induces various effects aimed to protect the affected tissue. This is accomplished by vasodilation, inhibition of thrombocyte aggregation, increasing intrinsic tolerance against ischemia and reperfusion, and modulation of the inflammatory response (2). The extracellular adenosine concentration is determined by the formation, transmembrane transport, and degradation of adenosine (3) (Fig. 1).

Under physiological conditions, adenosine is formed by dephosphorylation of adenosine monophosphate by ecto- and endo-5'-nucleotidase (5NT) and through hydrolysis of S-adenylhomocysteine. Adenosine degradation is confined to the intracellular compartment in which adenosine deaminase (ADA) and adenosine kinase (AK) are responsible for the degradation of adenosine. The equilibriative nucleoside transporter (ENT) controls the facilitated diffusion between extra- and intracellular adenosine depending on the concentration gradient (4).

It has been well-established in vitro that, during hypoxia, the rise in the extracellular adenosine concentration results from an activation of ecto-5NT, a reduced activity of AK and ADA, and a reduction in ENT capacity (5, 6). During systemic inflammation, the extracellular adenosine concentration increases rapidly as well (7–9), with concentrations increasing up to ten-fold in septic shock patients (7). How inflammation affects these enzyme activities in humans is unknown. Unraveling these mechanisms may be important because it allows for the development of pharmacological strategies to potentiate the increase in extracellular adenosine, which could limit tissue damage during inflammation.

The aim of the current study was to investigate the inflammation-induced changes in adenosine metabolism during systemic inflammation in humans in vivo and in isolated human lymphocytes and peripheral blood mononuclear cells (PBMCs) in vitro.
We hypothesized that, during inflammation, the adenosine metabolism is altered, resulting in an increased extracellular adenosine concentration. Furthermore, we hypothesized that the adenosine receptor expression changes during systemic inflammation, thereby potentiating the anti-inflammatory potential of adenosine. We studied the gene expression and protein activities of ecto-5’NT, ADA, AK, and the ENT, as well as gene expression of the adenosine A₁, A₂a, and A₂b receptors during systemic inflammation evoked by experimental human endotoxemia (administration of lipopolysaccharide [LPS] in healthy volunteers). Furthermore, in vitro studies were performed to confirm the adaptive changes in adenosine metabolism and rule out potential confounding by changes in circulating cell numbers and types during human endotoxemia.

**METHODS**

**Healthy Volunteers**

To investigate the effects of systemic inflammation on adenosine metabolism, blood samples were collected during three different human endotoxemia trials. These trials are registered at the Clinical Trial Register (NCT00513110, NCT00783068, and NCT01091571) (9, 10). In order to prevent confounding by the different pharmacological interventions in these trials, analyses were performed on data of placebo-treated subjects only. After approval by the local ethics committee of the Radboud University Nijmegen Medical Center, a total of 55 healthy volunteers signed written informed consent. All volunteers had a normal physical examination, electrocardiography, and routine laboratory values before the start of the experiment. Volunteers were asked not to take any prescription drugs and they refrained from caffeine intake for 48 hrs prior to the LPS administration. The subjects were admitted to our clinical research unit on the day of the experiment and were kept under close observation during 10 hrs. In total, 24 healthy subjects received placebo treatment.

**Experimental Protocol**

A cannula was inserted in a deep forearm vein for administration of 2.5% glucose/0.45% saline solution to ensure an optimal hydration status (11). Subjects received 1.5 L in 1 hr immediately before LPS infusion (prehydration), followed by 150 mL/hr until 6 hrs after LPS infusion, and 75 mL/hr until the end of the experiment. LPS was injected at t = 0 hrs, and blood was collected at various time points thereafter.

**Endotoxin**

U.S. reference *Escherichia coli* endotoxin (*Escherichia coli* 0:113, Clinical Center Reference Endotoxin, National Institute of Health, Bethesda, MD) was used. Ec-5 endotoxin, supplied as a lyophilized powder, was reconstituted in 5 mL 0.9% NaCl for injection and vortex-mixed for at least 10 mins after reconstitution. The endotoxin solution was administered as an intravenous bolus injection at a dose of 2 ng/kg of body weight.

**Cell Isolation**

Since neutrophils do not express 5NT (12), and previous animal studies demonstrated profound changes in adenosine metabolism in lymphocytes, we focused on changes in the lymphocyte population. PBMCs were collected at different time points (T = 0, 2, 4, 6, and 24 hrs) after LPS administration using Mononuclear Cell Preparation Tubes (BD Vacutainer CPT; Becton Dickinson and Company, Franklin Lakes, N.J). After centrifugation at 1800 × g at room temperature for 20 mins, mononuclear cells were harvested and washed twice with phosphate buffered saline.

For the separation of lymphocytes from the PBMC fraction, the magnetic-activated cell sorting separation technique was used (Miltenyi Biotec, Leiden, The Netherlands), according to manufacturer’s instructions. Lymphocyte purity was evaluated using flow cytometry (Beckman Coulter FC500, Woerden, The Netherlands).

**In Vitro Experiments**

PBMCs were collected from six healthy male volunteers who did not participate in the endotoxemia studies using Mononuclear Cell Preparation Tubes (BD Vacutainer CPT). PBMCs were washed three times in sterile PBS and resuspended in culture medium (RPMI 1640 Dutch modification, Flow Labs, Irvine, UK; supplemented with l-glutamine 2 mM, pyruvate 1 mM, gentamicin 50 mg/mL, and fetal calf serum 10%). Cells were seeded in 96-well culture plates at a density of 5 × 10⁴ cells/well in 200 μL and incubated with increasing concentrations of LPS (0, 1, 10, and 100 ng/mL, LPS Ultrapure E.coli, 0111:B4; Invivogen, Toulouse, France) for 24 hrs at 37°C, 95% O₂, and 5% CO₂. All experiments were performed in duplicate. After incubation, culture plates were centrifuged (1400 revolutions/min, 10 mins), and the supernatant was stored at −20°C until further analysis.

In addition, lithium–heparin anticoagulated blood (Vacutainer, BD Biosciences) blood was collected from three healthy male volunteers and diluted 1:5 in culture medium for experiments in whole blood. Diluted blood was incubated in 24-well culture plates (at a volume of 1 mL) with increasing concentrations of LPS (0, 1, 10, and 100 ng/mL) for 24 hrs at 37°C, 95% O₂, and 5% CO₂. All experiments were performed in duplicate. After incubation, culture plates were centrifuged (14000 revolutions/min, 5 mins), and supernatants were collected and stored at −80°C until further analysis. Erythrocytes were dissolved in 3-(N-morpholino)propanesulfonic acid buffer at a concentration of 20% and stored at 4°C until analysis of uridine transport. A small fraction of the samples was used to determine hematocrit levels.
Cytokine Measurements

Plasma concentrations of tumor necrosis factor (TNF-α) and interleukin-6 were determined using a simultaneous Luminex Assay (Bio-Plex cytokine assay, Bio-Rad, Hercules, CA) according to the manufacturer’s instructions. TNF-α and interleukin-6 concentrations in cell culture supernatants were determined by enzyme-linked immunosorbent assay (TNF-α: R&D duoset, R&D Systems, Minneapolis, MN; interleukin-6: Pelikine Compact, Sanguin, Amsterdam, The Netherlands) according to the manufacturer’s instructions.

Enzyme Activity Assays (ADA, AK, and 5NT)

Enzyme activity assays for ADA, AK, and ecto-5NT were performed as previously described (13, 14). In summary, PBMCs were washed three times in sterile PBS. Cell pellets were resuspended and lysed using mammalian protein extraction reagent in combination with the protease inhibitor HALT. For nucleotide activity 350 µL of incubation buffer (100 mM Tris/HCL pH 8, 20 mM β-glycerolphosphate, and 0.2% Triton X-100) was added to 150 µL suspension. For the measurement of ADA and kinase activity, the suspension was centrifuged, and 400 µL Tris-buffer was added to 150 µL supernatant. Samples were stored at −80 °C until analysis.

ENT Determination by Uridine Transport Measurements

We used uridine to reliably obtain the transport characteristics of the ENT because erythrocytes lack uridine kinases and phosphorylases. Contrary to uridine, adenosine is rapidly deaminated and rephosphorylated after uptake into the cell, a characteristic that will affect proper ENT measurement with the use of adenosine. For uridine transport measurements, a 50-µL uridine solution was added to 100 µL of 10% erythrocytes in 3-(N-morpholino)propanesulfonic acid buffer to obtain final concentrations of 1000 µmol/L. After 3 secs, uridine uptake was completely blocked by 100 µL of 25-µmol/L dipridamole, and erythrocytes were isolated by immediate centrifugation through a dibutylphthalate layer. After removal and washing of the upper layer, erythrocytes were lysed with Triton X-100 (Sigma-Aldrich BV, Zwijndrecht, The Netherlands), and proteins were precipitated using perchloric acid. After centrifugation, uridine concentrations in the supernatants were determined by high performance liquid chromatography.

RTQ-Polymerase Chain Reaction Procedures

All procedures were carried out according to the manufacturers’ instructions. Using Trizol reagent (Invitrogen, Breda, The Netherlands), total intact RNA was isolated from isolated lymphocytes stored in RNA later. RNA was reverse transcribed using pd(N)6 random hexamer primers and M-MLV reverse transcriptase (Invitrogen). RTQ-PCR was performed using the ABI PRISM 7900HT Gene Expression Micro Fluidic Card (Applied Biosystems, Bleuriswick, The Netherlands). cDNA amplification was performed in Taqman Universal PCR Master Mix, supplemented with 20x solution of the primer probe sets listed in Table 1 (all from Applied Biosystems). PCR reactions were analyzed using 700 System Sequence Detection Software (version 1.2.3, Applied Biosystems).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis, Blotting, and Antibodies

For Western blot, a fraction of the isolated lymphocytes was stored in RIPA-buuffer until analysis. Standard wet-blot procedures were used, and proteins were visualized as described previously (15). The primary antibodies mouse-anti-human CD73 (AbD Serotec, Dusseldorf, Germany) and mouse-anti-β-actin (Sigma-Aldrich), and secondary antibody donkey-anti-mouse alexa dye 680 (Invitrogen) were used.

Statistical Analysis

LPS-induced changes in time were analyzed using repeated measures analysis of variance, with post hoc tests for specific time points (Bonferroni). Nonparametric analyses were performed using the Friedman test followed by Dunn’s multiple comparison test. Since all gene expression levels of the enzymes, transporter, and adenosine receptor subtypes had a non-Gaussian distribution, data were log transformed before analyses with one-way analysis of variance, followed by Dunnett’s multiple comparisons posttest. For reasons of clarity, all data are presented as median (interquartile range) in the figures. Demographic characteristics are presented as mean ± sd. A p value < .05 was considered statistically significant.

RESULTS

The demographic characteristics of the healthy volunteers are presented in Table 2.

In Vivo Experiments

LPS-Induced Changes in Endogenous Adenosine Levels. During human endotoxemia, the endogenous plasma adenosine concentration increased from 9.5 [8.0–14.3] ng/mL at baseline to a peak level of 13.7 [10.9–17.7] ng/mL (an increase of 41±16%) 2 hrs after LPS administration (p = .043). In comparison, in sepsis patients who were admitted to our intensive care unit, we found a median (interquartile range) adenosine concentration of 29 [22–43] ng/mL (n = 31).

LPS-Induced Changes in Enzyme and Transporter Activity. Human experimental endotoxemia resulted in a profound increase in plasma concentrations of the proinflammatory cytokines, TNF-α and interleukin-6 (both p < .0001; Fig. 2). During the peak of the innate immune response, 5NT activity on peripheral blood mononuclear cells (PBMC), responsible for the formation of adenosine, did not increase, whereas it was significantly decreased 4 hrs after LPS administration (52% ± 7%, p < .0001; Fig. 3A). The degradation of adenosine was also attenuated: PBMC ADA activity, responsible for the deamination of adenosine, was reduced with a maximum of 59 ± 3% 4 hrs after LPS administration (p < .0001; Fig. 3B), and AK activity, responsible for adenosine phosphorylation, decreased with a maximum of 41 ± 4% (p < .0001; Fig. 3C) 4 hrs after LPS administration. ENT activity, measured in circulating erythrocytes, did not change after LPS administration (p = .27; Fig. 3D).

LPS-Induced Changes in Enzyme and Transporter Gene Expression on Purified Lymphocytes. The purity of the lymphocyte isolation averaged 88% ± 2% (n = 10). The expression of 5NT messenger RNA (mRNA) was significantly enhanced 2 hrs after LPS administration and returned to normal within 24 hrs (p = .010; Fig. 4A). The protein expression of 5NT, which was determined in isolated lymphocytes from five volunteers, showed a trend
towards an increase 2 hrs after LPS administration, and this increase tended to persist up to 24 hrs after LPS (p = .999). The gene expression of ADA decreased in the first hours after LPS administration, but returned to normal within 24 hrs (p = .024; Fig. 4B), whereas AK mRNA expression did not change significantly during endotoxemia (p = .52; Fig. 4C). Gene expression levels of ENT were enhanced 4 hrs after LPS administration (p = .034; Fig. 4D).

**LPS-Induced Changes in Gene Expression of Adenosine Receptor Subtypes.** In isolated lymphocytes, the A2b receptor mRNA expression was upregulated during experimental human endotoxemia (p = .022, Fig. 5C), whereas mRNA expression of the adenosine A1 and A2a receptors was attenuated (p = .029 and p = .026, respectively; Fig. 5, A and B). A2b receptor messenger RNA expression was significantly altered following endotoxemia (p = .043; Fig. 5D).

**In Vitro Experiments**

During experimental endotoxemia, the circulating cell population changed rapidly; therefore, we also studied changes in adenosine metabolism in isolated PBMCs and erythrocytes stimulated with LPS. In general, during human experimental endotoxemia, the total leukocyte count decreases in the first hour after LPS administration, followed by leukocytosis, with a peak leukocyte count 8 hrs after LPS administration. As depicted in Figure 6, incubation of both PBMC’s (Fig. 6A) and whole blood (Fig. 6B) (harvested from healthy volunteers not participating in the endotoxemia experiments) with increasing doses of LPS resulted in a significant and dose-dependent increase in TNF-α levels (p < .0001 and p = .017, respectively). In vitro stimulation of PBMC’s with LPS during 24 hrs did not change ecto-5NT activity (p = .95; Fig. 7A). ADA activity on the other hand was significantly decreased by LPS in a dose-dependent fashion, with a maximum reduction of 59 ± 7% after incubation with 100 ng/mL LPS (p < .0001; Fig. 7B). The rephosphorylation of adenosine by AK was also attenuated with increasing doses of LPS; AK activity decreased with a maximum of 67% ± 3% (p < .0001; Fig. 7C). Incubation of whole blood with increasing doses of LPS tended to increase ENT activity, with a maximum effect observed at 10 ng/mL LPS of 68% ± 29% (p = .075; Fig. 7D).

**DISCUSSION**

The main finding of our study is that during experimental human endotoxemia, the circulating endogenous adenosine concentration increases within 2 hrs after LPS administration, and that this rise appears to be caused by an inhibition of intracellular adenosine degradation rather than augmentation of adenosine formation. Concomitantly, the gene expression of the adenosine A2a receptor subtype, which upon activation has potent anti-inflammatory effects (16, 17), is increased, whereas the gene expression of the adenosine A1 and A2a receptor is decreased.
Knowledge of the effect of inflammation on adenosine metabolism is of great importance because endogenous adenosine can act as a potent negative feedback signaling molecule aimed to limit tissue damage in situations of hypoxia, ischemia, and inflammation. As such, (pharmacological) potentiation of this feedback mechanism, e.g., with ENT inhibitors, could be a promising strategy to limit tissue injury during inflammation (18, 19). For this approach, however, thorough knowledge of the mechanism mediating the increase in endogenous adenosine is essential. We showed that the rise in the extracellular adenosine concentration is initiated by a reduced intracellular degradation of adenosine. Consequently, the transmembrane adenosine concentration gradient will be reduced, and the uptake of extracellular adenosine via the ENT transporter is attenuated. Based on our findings, ENT inhibitors may increase the baseline adenosine concentration before initiation of inflammation, but will not potentiate the inflammation-induced increase in the extracellular adenosine concentration because the transmembrane adenosine concentration gradient is reduced. This fits into our observation that 7-day treatment with dipyridamole increases the baseline adenosine concentration, but does not potentiate the inflammation-induced rise in adenosine formation (20).

Our results suggest that the use of pharmacological activators of ecto-5NT may be more successful, as this will further increase the extracellular adenosine concentration, particularly since adenosine degradation is reduced. Only recently, Haskó and co-workers (21) demonstrated that ecto-5NT decreases mortality and organ injury during murine sepsis. Interestingly, recent studies in animals and in humans in vivo have shown that statins cause an activation of ecto-5NT activity (14, 22, 23). Furthermore, it was recently demonstrated that the use of statins during intensive care unit stay reduces hospital mortality (24), but whether an increase in extracellular adenosine accounts for the survival benefit needs to be further explored.

During human experimental endotoxemia, the enzymatic activities of ADA and AK were inhibited already 1.5 hrs after LPS administration. In addition, 4 hrs after LPS administration, ecto-5NT activity was modestly reduced. Interestingly, these changes in activity levels

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**Figure 4.** Enzyme/transporter messenger RNA (mRNA) expression levels in isolated lymphocytes. Following the administration of lipopolysaccharide (LPS), the mRNA expression of adenosine deaminase (ADA) (B) significantly decreased. Expression levels of both 5’-nucleotidase (5NT) (A) as well as equilibrative nucleoside transporter (ENT) (D) were significantly elevated following LPS administration. Adenosine kinase (AK) (C) expression did not change during endotoxemia. Data are presented as median [interquartile range]. Probability values refer to changes in mRNA expression, analyzed by repeated measures analysis of variance after log transformation of the data (n = 8). *p < .05.

**Figure 5.** Messenger RNA (mRNA) expression levels of three different adenosine receptor subtypes. Endotoxemia-induced changes in mRNA expression levels of the four different adenosine receptor subtypes in isolated lymphocytes, during endotoxemia. Data are presented as median [interquartile range]. Probability values refer to changes in mRNA expression, analyzed by repeated measures analysis of variance after log transformation of the data (n = 4–8). *p < .05. LPS, lipopolysaccharide.
were not caused by an alteration in gene transcription: ADA gene transcription was reduced, but only 6 hrs after LPS administration, transcription of AK was not affected, and transcription of ecto-5NT was even increased. Therefore, the changes in enzymatic activities of ADA, AK, and ecto-5NT are likely due to an effect on protein translation or a direct effect of inflammation on protein activity. The latter mechanism is most probable, given the findings that protein expression of ecto-5NT followed the change in its gene transcription (Fig. 4).

In parallel with the adaptive changes in adenosine metabolism, we demonstrated that the expression levels of the adenosine receptor subtypes also change during systemic inflammation. Expression of the predominantly anti-inflammatory adenosine $A_2a$ receptor (1, 25) increases in the first 2 hrs after LPS administration. Recent studies also demonstrate anti-inflammatory properties of the $A_2b$ receptor (26, 27). Herein we demonstrate significant changes in $A_2b$ receptor expression following endotoxemia. In contrast, both the adenosine $A_1$ and the $A_3$ receptor are downregulated during human endotoxemia. Whether these changes in expression levels are functional remains to be determined (28). Nonetheless, the changes in adenosine receptor gene transcription levels are in accordance with previous in vitro studies demonstrating that in LPS-stimulated macrophages, the gene transcription of the $A_2a$ and $A_3$ receptor is augmented, whereas $A_1$ and $A_3$ receptor expression is reduced. Furthermore, this increase in the number of $A_2a$ receptors correlated with an increase in the potency of a specific $A_2a$ receptor agonist to reduce TNF-$\alpha$ release (29). In accordance, in septic patients, the expression of the adenosine $A_2a$ receptor on circulating granulocytes is increased (30). However, receptor function appeared to be impaired because of reduced ligand-binding affinity, thereby diminishing the anti-inflammatory potential of adenosine (30).

Our results show that the effects of inflammation on adenosine metabolism differ to some extent from the reported effects of hypoxia/ischemia. Hypoxia results in a profound upregulation of ecto-5NT expression and function (5). Also, hypoxia reduces expression of human equilibrative nucleoside transporter 1. In contrast, during human experimental endotoxemia, the activities of these proteins do not explain the rise in extracellular adenosine. With regard to the degradation of adenosine, similarities between hypoxia and inflammation exist. In cultured pheochromocytoma cells, hypoxia decreased the gene expression of ADA and AK, but only after 24 hrs. In addition, hypoxia also induces an immediate inhibition of AK (31). In our study, we observed a reduction in ADA and AK activity already 1.5 hrs following LPS administration. As such, this reduced adenosine breakdown could well explain the rise in extracellular adenosine concentration observed 2 hrs after LPS administration.

Furthermore, our findings are in accordance with the recent finding that during murine peritonitis, the extracellular adenosine concentration increases rapidly due to changes in the adenosine metabolism. The expression levels of 5NT messenger RNA were enhanced 6 hrs after E. coli administration, followed by a peak 5NT protein concentration 24 hrs after induction of peritonitis. ADA and AK messenger RNA levels were reduced 12 hrs after induction of peritonitis, and the activity of ADA was significantly elevated within 2 hrs. Recent studies also demonstrate increased activity of AD and AK (32). Therefore, the results of hypoxia and inflammation are in accordance, and the role of adenosine in sepsis should be addressed in future studies.
decreased 12 hrs after induction of peri-
tonitis (32). Our findings are also supported
by the only previous human in vivo study
that demonstrated that in patients with
chronic pulmonary inflammation, ade-
osine metabolism changes rapidly (33),
resulting in an increase in sNT expres-
sion, reduction in ADA activity, and
increased A2 receptor expression levels
in pulmonary tissue. These data illustrate
that in humans both local (pulmonary) and
systemic (endotoxemia) inflamma-
tion results in similar changes in adenos-
ine metabolism.

During experimental human endotox-
emia, a shift in circulating cell popula-
tions occurs (34). It is characterized by
relative leucopenia in the first hour fol-
loowing LPS administration presumably
due to sequestration, followed by leucocy-
tosis in the hours thereafter. During leu-
cytosis, the cellular population almost
exclusively consists of neutrophils. At the
same time, the PBMC fraction is dimin-
ished and merely consists of lymphocytes
the first 6 hrs after induction of endotox-
emia. Shifts in lymphocyte populations,
both the total lymphocyte count as well as
possible functional changes, could have
influenced the in vivo measurements of
enzymes involved in adenosine formation
and metabolism. To further characterize
the intrinsic effects of inflammation on
adenosine metabolism, we therefore also
performed in vitro experiments in which
PBMCs were stimulated with LPS. Our in
vitro results demonstrate that 24-hr incuba-
tion with LPS causes a dose-dependent
inhibition of ADA and AK enzymatic activ-
ity, whereas ecto-sNT and ENT activities
were not significantly affected. Further-
more, these results demonstrate that the
effects observed in vivo are not attributed
to systemic changes in cell populations,
also for ADA, AK, and ENT, but are a
direct consequence of alterations at the
cellular level.

CONCLUSIONS

The elevation in plasma adenosine
during systemic inflammation evoked
by experimental human endotoxemia is
caused by reduced intracellular clear-
ance of adenosine, not by enhanced for-
mation. At the same time, the adenosine
A2 receptor mRNA expression is upregu-
lated which may further reinforce the
anti-inflammatory effects of adenosine.
Pharmacological modulation of these
alterations in adenosine metabolism
may potentiate the protective prop-
erties of adenosine during systemic inflamma-
tion.

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