Adenosine Inhibits Growth of Human Aortic Smooth Muscle Cells Via A_2B Receptors

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Abstract—Adenosine inhibits rat vascular smooth muscle cell (SMC) growth. However, the effects of adenosine on human vascular SMC proliferation and synthesis of extracellular matrix proteins, such as collagen, are unknown. The objective of this study was to characterize the effects of exogenous and endogenous (SMC-derived) adenosine on human aortic SMC proliferation and collagen synthesis. Growth-arrested SMCs were stimulated with 2.5% fetal calf serum (FCS) in the presence and absence of adenosine, 2-chloroadenosine (stable adenosine analogue), and with agents that increase endogenous adenosine levels, including erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), dipyridamole, and iodotubercidin. All of these agents inhibited in a concentration-dependent manner FCS-induced SMC proliferation as assessed by DNA synthesis (3H-thymidine incorporation) and cell counting, as well as collagen synthesis (3H-proline incorporation). EHNA, dipyridamole, and iodotubercidin increased extracellular levels of adenosine by 17-fold to 18-fold when added separately to SMCs, and EHNA + iodotubercidin + dipyridamole increased extracellular adenosine levels by more than 392-fold. Both KF17837 (selective A_2 antagonist) and DPSPX (A_1/A_2 antagonist), but not DPCPX (selective A_1 antagonist), blocked the antiproliferative effects of 2-chloroadenosine, EHNA, and dipyridamole on DNA and collagen synthesis, suggesting the involvement of A_2A and/or A_2B, but excluding the participation of A_1 receptors. The lack of effect of CGS21680 (selective A_2A agonist), excluded involvement of A_2A receptors and suggested a major role for A_2B receptors. A comparison of the inhibitory potencies of 2-chloroadenosine, N^6-cyclopentyladenosine (selective A_1 agonist), NECA (A_1/A_2 agonist), and MECA (A_1/A_2 agonist) were consistent with an A_2B receptor subtype mediating the inhibitory effects of adenosine on human aortic SMC proliferation. In conclusion, human aortic SMCs synthesize adenosine, and exogenous as well as endogenous (SMC-derived) adenosine inhibits SMC proliferation and collagen synthesis via activation of A_2B receptors (Hypertension. 1998;31[part 2]:516-521.)

Key words: adenosine, smooth muscle cells, hypertension, atherosclerosis, restenosis, proliferation, hyperplasia, extracellular matrix, collagen

Smooth muscle cells (SMCs) importantly contribute to the pathophysiology of vascular remodeling induced by hypertension, atherosclerosis and restenosis by proliferating, migrating into the intima, and depositing extracellular matrix (ECM) proteins, such as collagen. Endogenous factors that are generated in substantial amounts locally by the cells within the vessel wall, such as endothelial cells, SMCs, and fibroblasts, and that inhibit SMC proliferation, growth, and ECM production may play a major vasoprotective role in preventing vascular remodeling. In this regard, adenosine may be an important factor. Vascular SMCs, fibroblasts, cardiomyocytes, and endothelial cells, both vascular and cardiac, have several metabolic pathways for generating large amounts of adenosine. Moreover, we have recently shown that vascular SMCs can also synthesize adenosine from exogenous cAMP. Taken together, these findings suggest that substantial amounts of adenosine are synthesized locally within the vessel wall, thus assuring pharmacologically active levels of adenosine in the vasculature. Since both exogenous and SMC-derived adenosine inhibits fetal calf serum (FCS)-induced growth of rat aortic SMCs, we hypothesize that adenosine may similarly protect against vasoocclusive disorders in humans by regulating SMC growth and maintaining vascular homeostasis.

Accordingly, the aims of the present study were to determine (1) whether exogenous adenosine inhibits proliferation and collagen synthesis by human aortic SMCs (HASMCs), (2) whether specific adenosine receptor subtype(s) are involved in mediating any inhibitory effects of adenosine on proliferation and collagen synthesis by HASMCs, (3) whether HASMCs synthesize adenosine, and (4) whether HASMC-derived adenosine can effectively inhibit proliferation and collagen synthesis by HASMCs.

Materials

Dulbecco's Modified Eagle Medium (DMEM), DMEM/F12 medium, Hanks' Balanced Salt Solution (HBSS), penicillin, streptomycin, 0.25% trypsin-EDTA solution, and all tissue culture ware were purchased from GIBCO. FCS was obtained from HyClone Laboratories Inc. Adenosine, 2-chloroadenosine (Cl-Ad), erythro-9-(2-hydroxy-3-nonyl) adenine hydrochloride (EHNA), and dipyridamole (DIP), were purchased from Sigma Chemical Co.
N\(^{-}\)-cyclopentyladenosine (CPA), 2-p-(2-carboxyethyl) phenethylamino-5'-N-ethylcarboxamido adenosine-hydrochloride (CGS21680), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), idotubercidin (IDO), 1,3-dipropyl-8-p-sulfophenylxanthine (DPSPX), 5'-N-ethylcarboxamidoadenosine (NECA) and 5'-N-methylcarboxamidoadenosine (MECA) were purchased from Research Biochemicals International KF17837 was a generous gift from Dr. F. Suzuki, Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co Ltd., Suntou, Shizuoka, Japan H\(^{3}\)-thymidine and H\(^{3}\)-Proline was purchased from ICN Biomedicals. All other chemicals used were of best available grade.

Human Aortic Smooth Muscle Cell Culture

Arterial SMC cultured from adult thoracic aortas were obtained from male (n = 4) normal donor heart transplants. The cells were cultured by the explant method and cultured as described in detail previously. Briefly described, the medial layer of the aorta was removed surgically under the microscope, and minced sections of this layer were suspended in culture medium (DMEM/F12) supplemented with penicillin (100 U/mL), streptomycin (100\(\mu\)g/mL), NaHCO\(_3\) (13 mmol/L) and HEPES (25 mmol/L) containing 10% FCS, plated in tissue-culture flasks (75 cm\(^2\)) and incubated under standard tissue culture conditions. The SMC grew as explants from the medial tissue and were confluent in 12 to 14 days. Confluent monolayers of SMCs were diluted by treatment with 0.25% trypsin-EDTA solution (GIBCO) and passaged further. SMC purity was characterized by immunofluorescence staining of smooth muscle-specific anti-smooth muscle \(\alpha\)-actin monoclonal antibodies and by morphologic criteria specific for SMC as described in detail previously. SMCs in 3rd and 4th passage were used for all the studies.

DNA Synthesis Studies

H\(^{3}\)-thymidine incorporation studies were done to investigate the effects of agents on FCS-induced DNA synthesis. HASMCs were plated at a density of 2 \(\times\) 10\(^5\) cells/well in 24-well tissue culture dishes and allowed to grow for 48 hours in DMEM/F12 containing 10% FCS under standard tissue culture conditions. The HASMCs were then grown by feeding DMEM containing 0.4% bovine serum albumin (BSA, Sigma) for 48 hours. Growth was stimulated by treating growth arrested HASMCs for 20 hours with DMEM supplemented with 2.5% FCS and containing or lacking the following: (1) adenosine, (2) Cl-Ad (an analog of adenosine that is not metabolized and mediates its effects via both A\(_1\) and A\(_2\) receptors), (3) CPA (an adenosine agonist that mediates its effects selectively via A\(_1\) receptors), (4) CGS21680 (an adenosine agonist that mediates its effects selectively via A\(_1\) receptors), (5) NECA (an adenosine agonist that mediates its effects via both A\(_1\) and A\(_2\) receptors), (6) MECA (an adenosine agonist with affinity for both A\(_1\) and A\(_2\) receptors), (7) Cl-Ad plus DPCPX (a selective A\(_2\) receptor antagonist), (8) Cl-Ad plus KF17837 (a selective A\(_2\) receptor antagonist), (9) Cl-Ad plus DPSPX (a nonselective A\(_2\) adenosine receptor antagonist), (10) EHNA (an inhibitor of adenosine deaminase), (11) DIP (an inhibitor of adenosine transport), (12) IDO (an inhibitor of adenosine kinase), (13) EHNA plus DIP, (14) EHNA plus IDO, (15) DIP plus IDO, (16) DIP plus IDO plus EHNA, (17) KF17837, (18) EHNA plus KF17837, (19) DIP plus KF17837, (20) DPSPX, (21) EHNA plus DPSPX, (22) DIP plus DPSPX, (23) DPCPX, (24) EHNA plus DPCPX, or (25) DIP plus DPSPX. After 20 hours of incubation, the cells were pulsed with H\(^{3}\)-thymidine (1 \(\mu\)Ci/mL) for an additional 4 hours. The experiments were terminated by washing the cells twice with Dulbecco's phosphate buffered saline (PBS) and twice with ice-cold trichloroacetic acid (10%). The precipitate was solubilized in 500 \(\mu\)L of 0.3N NaOH and 0.1% SDS after incubation at 50°C for 2 hours. Aliquots from 4 wells for each treatment with 10mL scintillation fluid were counted in a liquid scintillation counter and each experiment was conducted using 4 separate cultures.

Cell Proliferation Studies

Trypsinized HASMCs in third passage were suspended in DMEM/F12 containing 10% FCS and plated in a 24-well culture dish at a density of 1 \(\times\) 10\(^5\) cells/well. After incubation for 18 hours, the cells were fed DMEM containing 0.25% FCS for 48 hours to growth arrest the cells. To study the effects of exogenous and endogenous adenosine on FCS-induced cytokinesis, we treated growth arrested HASMCs every 24 hours for 4 days with DMEM supplemented with 2.5% FCS and containing or lacking various treatments, as described above for DNA synthesis. The treatments were terminated on day 5 and cells were washed with trypsin-EDTA, diluted in Isoton-II and counted with a Coulter counter. Aliquots from three wells were counted for each group, and 4 separate cultures were used for each group.

Collagen Synthesis Studies

H\(^{3}\)-proline incorporation studies were done to investigate the effects of agents on FCS-induced collagen synthesis. HASMCs grown to confluence in 24-well culture dishes were made quiescent by feeding DMEM containing 0.4% bovine serum albumin for 48 hours. To study the effects of exogenous and HASMC-derived adenosine on collagen synthesis, we treated growth arrested HASMCs for 20 hours with DMEM supplemented with 2.5% FCS and 1 \(\mu\)g/mL proline. The supernatants were collected and collagen levels were estimated. To evaluate whether HASMCs catabolized adenosine, we assayed the levels of adenosine in the medium of confluent monolayers of HASMCs treated for 4 hours with exogenous adenosine in the presence and absence of EHNA, IDO, or EHNA plus IDO. Following the collection of supernatants, the monolayers of cells were inspected microscopically for intactness, the number of cells were counted, and the data were normalized to cell number.

Adenosine Synthesis Studies

Monolayers of HASMCs (3rd passage) grown to confluence in 12-well culture dishes were washed twice with PBS and then incubated with buffered (HEPES 25 mmol/L, NaHCO\(_3\), 13 mmol/L) PBS containing or lacking EHNA, DIP, IDO, EHNA plus DIP, EHNA plus IDO, DIP plus IDO, or DIP plus IDO plus EHNA. After 4 hours of incubation, the supernatants were collected and adenosine levels were estimated. To evaluate whether HASMCs catabolized adenosine, we assayed the levels of adenosine in the medium of confluent monolayers of HASMCs treated for 4 hours with exogenous adenosine in the presence and absence of EHNA, IDO, or EHNA plus IDO. Following the collection of supernatants, the monolayers of cells were inspected microscopically for intactness, the number of cells were counted, and the data were normalized to cell number.

Adenosine Analysis

Adenosine levels in the samples were analyzed by gradient elution using high pressure liquid chromatography (HPLC) as previously described. Adenosine levels were quantitated as the area under the chromatographic peak, and the absolute amount in each sample was calculated from a standard curve of adenosine.

Statistics

All growth and adenosine synthesis experiments were performed in triplicates or quadruplicates with 4 separate cultures, and all data are presented as mean±SEM. Statistical analysis was performed using ANOVA, paired Student's t-test, or Fisher's least significant difference test, as appropriate. A value of \(P\) < 0.05 was considered statistically significant.
Results

Treatment of growth-arrested HASMCs with 2.5% FCS stimulated thymidine incorporation by 7-fold to 10-fold (P<.001 versus 0.25% FCS; data not shown). Both adenosine and Cl-Ad inhibited FCS-induced thymidine incorporation in a concentration-dependent manner (Fig 1A; P<.05). Compared with adenosine, Cl-Ad was more potent in inhibiting FCS-induced thymidine incorporation. The lowest concentrations of adenosine and Cl-Ad that significantly inhibited FCS-induced thymidine incorporation were 10μmol/L and 0.1μmol/L, respectively. A 50% decrease in FCS-induced thymidine incorporation by adenosine and Cl-Ad was observed at 100μmol/L and 1μmol/L, respectively (Fig 1A).

FCS (2.5%) induced proliferation (cell number) of growth-arrested HASMCs by 9-fold to 11-fold (P<.05; data not shown). Adenosine and Cl-Ad inhibited FCS-induced increase in cell number in a concentration-dependent manner (Fig 1B; P<.05). Similar to the effects on DNA synthesis, Cl-Ad, the stable analog of adenosine, was more potent in inhibiting cell proliferation as compared to adenosine (P<.05). The lowest concentrations of adenosine and Cl-Ad which inhibited cell proliferation were 0.1μmol/L.

Treatment with 2.5% FCS stimulated proline incorporation by 6-fold to 8-fold (P<.001 versus 0.25% FCS; data not shown). Treatment of HASMCs with adenosine (10μmol/L) and Cl-Ad (10μmol/L) inhibited FCS-induced proline incorporation (Fig 1C; P<.05). Compared with adenosine, Cl-Ad was more potent in inhibiting FCS-induced proline incorporation (Fig 1C; P<.05). As shown in Fig 2A, the inhibitory effects of adenosine on DNA synthesis were significantly enhanced in presence of EHNA, and EHNA and IDO significantly increased the recovery of exogenous adenosine added to the medium (Fig 2B). Moreover, in HASMCs treated with EHNA plus IDO, almost all of the exogenously added adenosine was recovered in the medium (Fig 2B).

Treatment of quiescent HASMCs with EHNA, DIP, and IDO inhibited FCS-induced thymidine and cell number in a concentration-dependent manner (Fig 3; P<.001). The lowest concentrations of EHNA, DIP, and IDO that significantly inhibited FCS-induced thymidine incorporation were 10μmol/L, 100μmol/L, and 0.1μmol/L, respectively. A 50% decrease in FCS (2.5%) -induced thymidine incorporation by EHNA, DIP, and IDO was observed at 50μmol/L, 0.5 μmol/L, and 0.5 μmol/L, respectively. Similar to DNA synthesis and cell number, treatment of HASMCs with EHNA, DIP, and IDO significantly inhibited FCS-induced proline incorporation (Fig 4C). EHNA significantly enhanced the inhibitory effects of DIP and IDO on FCS-induced thymidine incorporation (Fig 4A), cell number (Fig 4B), and proline incorporation (Fig 4C). IDO significantly increased the inhibitory effects of DIP on thymidine incorporation, cell number, and proline incorporation. Moreover, in HASMCs treated with EHNA (10μmol/L) plus IDO (1μmol/L) plus DIP (0.1μmol/L), FCS-induced thymidine and proline incorporation and cell number were reduced to almost basal levels (Fig 4A, 4B and 4C).

In samples drawn at time zero, adenosine levels were nondetectable; however, the levels of adenosine were significantly increased in the medium of HASMCs collected after 4 hours of incubation and were 4.8±0.5nmol/L/10^6 cells (P<.001). Treatment of HASMCs with EHNA significantly increased the levels of adenosine in the medium to 89±8 nmol/L/10^6 cells (P<.001), an increase of ≥ 1800% (Fig 4D).

Similar to EHNA, treatment of HASMCs with DIP and IDO also increased the levels of adenosine in the medium (P<.05; Fig 4D). Moreover, in HASMCs treated with EHNA plus IDO, the levels of adenosine were increased from 88±8 nmol/L/10^6 cells and 8±0.1 nmol/L/10^6 cells in HASMCs
Concentration (pmol/L) Concentration (pmol/L)

Figure 3. Concentration-response relationships for the inhibition of 2.5% FCS-induced thymidine incorporation and cell number by EHNA, dipyridamole and iodotubericidin. Results are expressed as percentage of control, defined as thymidine incorporation or cell number in the presence of 2.5% FCS. Each data point represents mean ± SEM from 4 experiments, each conducted in quadruplicate. * P<.05 vs control.

treated with EHNA and IDO, respectively, to 1880±56 nmol/L/10^6 cells in HASMCs treated with EHNA+IDO, a 21-fold and 235-fold increase. In HASMCs treated with EHNA+IDO+DIP, the levels were also increased dramatically but were comparable to those observed in HASMCs treated with EHNA+IDO (Fig 4D).

High (10^-4 mol/L), but not low, concentrations of CPA inhibited FCS-induced thymidine incorporation (Fig 5) and cell proliferation (Fig 5). CGS21680 had little effect on FCS-induced thymidine incorporation and cell proliferation. NECA was more potent than CPA but less potent than Cl-Ad in inhibiting FCS-induced thymidine incorporation (Fig 5) and cell proliferation (Fig 5). MECA was more potent than NECA, CPA, and CGS21680 and as potent as Cl-Ad in inhibiting FCS-induced thymidine incorporation and cell proliferation. Cl-Ad inhibited thymidine incorporation and cell proliferation by 50% at a concentration of 5 and 1 µmol/L, respectively. At this concentration, CGS21680, CPA, NECA, and MECA inhibited DNA synthesis by approximately 6%, 21%, 28% and 50%, respectively.

With respect to the effects on collagen synthesis, we compared the effects of equimolar concentrations (10 µmol/L) of adenosine, Cl-Ad, CGS21680, CPA, NECA and MECA. Concentrations at which CPA and CGS21680 mediate receptor-specific pharmacological actions (1 µmol/L) did not inhibit FCS-induced collagen synthesis. However, 10 µmol/L of CPA and CGS21680 inhibited FCS-induced collagen synthesis (Fig 5). As compared to CGS21680 and CPS, NECA (10 µmol/L) was more potent in inhibiting collagen synthesis; however, it was significantly less potent than Cl-Ad and MECA, which inhibited collagen synthesis by 41% and 38%, respectively (Fig 5C).

KF17837 and DPSPX, but not DPCPX, significantly reversed the inhibitory effects of Cl-Ad on FCS-induced thymidine (Fig 6; P<.05) and proline (Fig 6; P<.05) incorporation. The inhibitory effects of EHNA, DIP, and IDO on FCS-induced thymidine and proline incorporation were significantly attenuated by KF17837 and DPSPX, but not by DPCPX (Fig 7).

To confirm that cell-death did not occur during various treatments and did not contribute to the observed inhibitory effects of the agents used in this study, trypan blue exclusion tests were carried out. At the concentrations used in this study, there was no loss in viability of cells treated with the various agents.

Discussion

The present study demonstrates that adenosine inhibits FCS-induced growth of HASMCs. Treatment of HASMCs with adenosine, with a stable adenosine analog (Cl-Ad), and with agents that elevate endogenous adenosine (EHNA, IDO, DIP) inhibit FCS-induced DNA synthesis, collagen synthesis, as well as cell proliferation. The inhibitory effects of adenosine are mimicked by MECA and partially by NECA, an adenosine agonists with affinity for both A1 and A2A receptors,3 but not by the adenosine agonists CPA and CGS21680, which are selective A1 and A2A receptor agonists, respectively. Thus, the inhibitory effects of adenosine are possibly mediated via A1 receptor and not via A2 or A2A receptors. Furthermore, the inhibitory effects of Cl-Ad, EHNA, DIP, and IDO were significantly reversed by KF17837, a selective A1 receptor antagonist, but not by DPCPX, a selective A1 receptor antagonist. Our findings provide the first evidence that exogenous as well as HASMC-derived adenosine inhibits HASMC growth and collagen synthesis via the A1 receptor.

Our observation that CPA (an adenosine analog which is highly selective for A1 receptors and mediates its effects at pharmacologically low doses [10^4 mol/L and lower]) was unable to inhibit FCS-induced growth and collagen synthesis by...
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Figure 5. Concentration-response relationships for the inhibitory effects of 5'-cyclopentyl-adenosine (CPA), CGS21680 (CGS), 5'-N-ethylcarboxamidoadenosine (NECA) and 5'-N-methylcarboxamidoadenosine (MECA) on 2.5% FCS-induced thymidine incorporation (top) and cell proliferation (cell number; middle); and the effects of NECA (10 μmol/L), MECA (10 μmol/L), CGS (0.01 and 10 μmol/L) and CPA (0.01 and 10 μmol/L) on 2.5% FCS-induced proline incorporation (bottom). Values for each point represent mean±SEM from 4 separate experiments conducted in triplicate or quadruplicate, using separate cultures. * Significant P<.01 vs control (25%FCS); $P<.05 vs CPA, CGS or NECA.

HASMC at low concentrations suggests that the inhibitory effects of adenosine are not mediated via A1 receptors. This conclusion is further supported by our observation that DPCPX, an adenosine receptor antagonist that is a 700-fold selective for A1 receptors, was unable to block the inhibitory effects of Cl-Ad on FCS-induced growth and collagen synthesis by HASMCs.

Figure 6. Inhibitory effects of 2-chloroadenosine (Cl-Ad; 10 μmol/L) on 2.5% FCS-induced thymidine (top) and proline (bottom) incorporation. Results (mean±SEM) from 4 separate experiments conducted in triplicates are expressed as percent of control, defined as thymidine or proline incorporation in HASMCs treated with 2.5% FCS; * P<.01 vs control (2.5%FCS); $P<.05 vs 2-chloroadenosine.

The observations that pretreatment of HASMCs with KF17837, a selective inhibitor of A2a adenosine receptors, and DPPSPX, an A1/A2 adenosine receptor antagonist, but not DPCPX, a selective A1 receptor antagonist, effectively reversed the inhibitory effects of Cl-Ad on DNA and collagen synthesis suggest that the inhibitory effects of adenosine are A1 receptor mediated. This conclusion is corroborated by the observations that NECA, an adenosine agonist with high affinity for A1 receptors, was as effective as Cl-Ad in inhibiting DNA as well as collagen synthesis by HASMC and NECA.

Our contention that the inhibitory effects of adenosine are mediated via A2a receptors is supported by the recently proposed and endorsed subclassification of A2 and A3 receptors. Gard et al. have recently demonstrated that the relative potencies of CGS21680 and NECA can be used as a reference to differentiate A2a from either A2b or A1 receptors. When the effects of CGS21680 are as potent as NECA, this implicates the A2a receptor subtype. In the present study, compared with CGS21680, NECA was more effective in mimicking the inhibitory effects of adenosine, which further substantiates our conclusion that the inhibitory effects of adenosine are mediated via A2a receptor.

Stimulation of HASMCs with adenosine has been shown to elevate cAMP levels, and cAMP in turn has antiproliferative effects on SMCs. Since A1 receptors are positively coupled with adenylyl cyclase and their activation results in a significant increase in cAMP levels, the inhibitory effects of adenosine on HASMC growth are most likely mediated largely via the second messenger cAMP. However, the participation of other mechanism(s) cannot be ruled out. Activation of A1 receptors by adenosine stimulates NO release from endothelial cells, and we have recently observed that adenosine via A1 receptors stimulates NO release from rat aortic SMCs. Since NO inhibits SMC
proliferation, this provides an additional pathway via which adenosine could inhibit HASMC growth. Elimination of adenosine from the interstitial space is mediated by facilitated transport of adenosine into cells and also by the metabolism of adenosine to inosine by adenosine deaminase, as well as by the metabolism of adenosine to adenosine monophosphate by adenosine kinase. Inhibition of the enzyme adenosine deaminase by EHNA and the enzyme adenosine kinase by IDO, as well as inhibition of adenosine transport and metabolism by DIP, has been shown to increase endogenous levels of adenosine. Hence, these three compounds were used in the present study to increase endogenous levels of adenosine so as to evaluate the effects of endogenously generated adenosine on FCS-induced growth.

Treatment of HASMCs with EHNA, IDO, and DIP elevated the levels of adenosine in the culture medium. Thus, the present study demonstrates that cultured HASMCs synthesize adenosine. In HASMCs treated with both EHNA plus IDO, the adenosine levels increased by more than 392-fold. This suggests that both adenosine deaminase and adenosine kinase regulate adenosine elimination in HASMCs. Direct evidence for this notion comes from our observation that, when HASMCs were treated with exogenous adenosine, only a fraction of adenosine, close to basal levels, was recovered in the medium. However, in the presence of EHNA plus IDO, but not in presence of EHNA or IDO alone, a dramatic increase in the recovery of adenosine was observed.

The inhibitory effects of EHNA, DIP, and IDO on DNA and collagen synthesis were significantly reversed by KF17837 and DPPX. In contrast, DPCPX did not reverse the inhibitory effects of EHNA, DIP, and IDO on HASMCs. These observations provide proof for our contention that the inhibitory effects of these modifiers of endogenous adenosine on HASMC-growth are mediated via generation of endogenous adenosine and via A1 receptors.

Can our in vitro findings be extrapolated to physiological situations in vivo and can adenosine prevent HASMC growth in vivo? Our finding that low concentrations of adenosine were able to inhibit HASMC growth in the presence, but not absence, of EHNA suggests that, although adenosine effectively inhibits HASMC growth, its effects are underestimated in the current state of experimentation, as they were conducted in the presence of FCS, which contains adenine deaminase. Under physiological conditions in vivo, most of the adenosine deaminase is localized within cells, and adenosine in the extracellular compartment will be available in active form to mediate the physiological inhibitory effects on HASMC growth. Additionally, since adenosine is synthesized by HASMCs, as well as endothelial cells, via multiple pathways, this would ensure pharmacologically active, steady-state levels of adenosine locally at the interface between endothelial and SMCs. In contrast, pathological conditions associated with decreased adenosine synthesis or increased adenosine deaminase leakage would reduce the pharmacologically active levels of adenosine, and this would result in decreased anti-growth effects of adenosine. Indeed, data from our laboratory suggests that adenosine deaminase may participate in at least two disease states associated with increased risk of cardiovascular disease, i.e., sickle cell anemia and angina/pneumocytic. However, future studies are needed to confirm or deny this role of adenosine deaminase.

In conclusion, we provide evidence that HASMCs synthesize adenosine and that both exogenous and HASMC-derived adenosine inhibit FCS-induced growth of and collagen synthesis by HASMCs. Our findings suggest, but do not prove, that adenosine produced by HASMCs may play a role as a local anti-growth agent and that decreased synthesis of adenosine by HASMCs or increased catabolism of adenosine by adenosine deaminase or adenosine kinase may contribute to the abnormal deposition of the extracellular matrix protein and growth of HASMCs in disease states, such as hypertension, atherosclerosis, and restenosis. Agents that elevate endogenous adenosine could be clinically important in preventing abnormal deposition of ECM proteins by, and proliferation of, HASMCs in coronary heart disease, thus exerting beneficial effects on coronary vascular structure.

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References

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