study, we analyzed effects of adenosine and analogues on the induction of gene expression in cultured human coronary artery smooth muscle cells (HCASMCs) as an early correlate for long-term effects of adenosine in vascular smooth muscle cells. Whole genome DNA array hybridization revealed that adenosine induced a set of early genes including the transcription factor NR4A1 (Nur77/TR3). The pattern of the effect of adenosine on gene expression resembles the change in expression induced by the direct activator of adenylate cyclase forskolin. Real-time reverse-transcriptase PCR confirmed that adenosine and its analogue *N*-ethyl-carboxamidoadenosine (NECA) elicited a strong induction of NR4A1. Moreover, NECA increased cellular cAMP levels and the cAMP response element (CRE)-dependent expression of luciferase in a reporter gene assay. The effects of adenosine and NECA on cAMP production, CRE-dependent luciferase expression, and the expression of NR4A1 were abolished or markedly blocked by the A_{2B} receptor-selective antagonist 8-[4-(4-benzylpiperazide-1-sulfonyl)phenyl]-1-propylxanthine (PSB-601), indicating that the effects were predominantly mediated by A_{2B} adenosine receptors. Platelet-derived growth factor (PDGF) is known to stimulate HCASMCs proliferation. Long-term studies (5 days) showed that 2-chloroadenosine counteracted the PDGF-induced proliferation of HCASMCs.

In summary, our results demonstrate strong effects of adenosine on gene induction in HCASMCs mediated by adenosine A_{2B} receptors which are possibly linked to anti-proliferative effects of adenosine on vascular smooth muscle cells.

Enhanced potency of nucleotide-dendrimer conjugates as agonists of the $P2Y_{14}$ receptor: multivalent effect in G-protein-coupled receptor recognition

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The $P2Y_{14}$ nucleotide receptor is a G-protein-coupled receptor activated by UPP-glucose to modulate immune function. Covalent conjugation of $P2Y_{14}$ nucleotide receptor agonists to polyamidoamine (PAMAM) dendrimers enhanced pharmacological activity. Uridine-5′-diphosphoglucuronic acid (UDPGA) and its ethylenediamine adduct were suitable functionalized congeners for coupling to several generations (G2.5–6) of dendrimers (both terminal carboxy and amino) to modulate potency of the intact conjugates. Prosthetic groups were attached for receptor detection and characterization, such as biotin for avidin complexation, chelating group for metal complexation (and eventual magnetic resonance imaging), and fluorescent moiety. The conjugates were assayed in stably transfected HEK293 cell expressing the human $P2Y_{14}$ receptor. A G3 PAMAM conjugate containing 20 bound nucleotide moieties (UDPGA) was 100-fold more potent (EC_{50} 2.4 nM) than the native agonist uridine-5′-diphosphoglucose. Larger dendrimer carriers and greater loading favored higher potency. A similar conjugate of G6 with 147 out of 256 amino groups substituted with UDPGA displayed an EC_{50} of 0.8 nM. Thus, biological activity was either retained or dramatically enhanced in the multivalent dendrimer conjugates in comparison with the monomeric $P2Y_{14}$ receptor agonists depending on size, degree of substitution, terminal functionality, and attached prosthetic groups.

Investigation of ATP-metabolizing enzymes and purinergic receptors in a new in vitro blood–brain barrier model system

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The proper functioning of the central nervous system would not be possible without the blood–brain barrier (BBB), consisting of the continuous layer of cerebral endothelial cells attached to each other by tight intercellular junctions and

completed by astrocytes and pericytes on the cerebral side. ATP can be released from endothelial cells during changes in flow (shear stress) or hypoxia, and it acts on P2Y receptors. The subsequent NO release results in relaxation. Moreover, nucleotides released from astrocytic end feet can influence brain capillary endothelial function through P2Y receptors on their basolateral membrane. Vascular endothelium also expresses very high level of NTPDase1, an ATP-hydrolyzing ectoenzyme, whereas the presence of NTPDase2 was demonstrated on astrocytes and pericytes. The use of a new in vitro model which contains all the three cell types forming the BBB [1] may help us understand the role of purinergic transmission in cell-to-cell communication and in BBB functioning in physiopathological conditions.

We have focused our study on the role and modulation of ectonucleotidase functions, on the possible role of the newly identified GPR17 receptor (which was suggested to be as a sensor of brain damage and a novel therapeutic target for stroke and brain repair) [2] and on $P2Y_{12}$ receptor subtype, a key player in platelet aggregation and an established target of antithrombotic drugs [3]. According to our preliminary results, both receptors are expressed at the mRNA level in all the three cell types. GPR17 and $P2Y_{12}$ were also demonstrated in endothelial cells at the protein level, while the sensitivity of NTPDases for oxygen–glucose deprivation showed differences among the three investigated cell types composing the BBB. Moreover, immunocytochemical analysis suggests an up-regulation of GPR17 receptor expression after oxygen–glucose deprivation in pericytes and endothelial cells, thus suggesting its possible role in the cell death processes following ischemic conditions in vitro.

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Sphingosine-1-phosphate receptors stimulate macrophage plasma membrane actin assembly via ADP release, ATP synthesis and $P2X_7$ receptor activation

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Eukaryotic plasma membranes assemble actin filaments within seconds following activation of many receptors, especially during chemotaxis. Here, serum or sphingosine-1-phosphate stimulation of J774 and RAW macrophages released ADP within seconds into the extracellular medium along with an adenylate kinase activity that converted ADP to ATP. ATP then activated the P2X7 receptor (P2X7R) that was necessary for a peak of plasma membrane actin assembly within 5–10 s in P2X7R expressing J774, RAW and primary macrophages. Neither actin assembly nor characteristic P2X7R channel activity was seen in response to ATP in P2X7R knockout macrophages, as detected by patch-clamp analysis. Since P2X7R has been shown previously to form a macromolecular complex with actin, we propose that it is involved in the membrane assembly of actin. Our data reveal a surprisingly rapid and complex relay of signalling and externalisation events that precede and control actin assembly induced by S1P. The overall model we present is strongly supported by the data presented in the accompanying papers [1, 2] that focused on latex bead phagosomes.

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