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PERSPECTIVE

A Tail of Two Signals: The C Terminus of the A_{2A}-Adenosine Receptor Recruits Alternative Signaling Pathways

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ABSTRACT

G protein-coupled receptors are endowed with carboxyl termini that vary greatly in length and sequence. In most instances, the distal portion of the C terminus is dispensable for G protein coupling. This is also true for the A_{2A}-adenosine receptor, where the last 100 amino acids are of very modest relevance to G_s coupling. The C terminus was originally viewed mainly as the docking site for regulatory proteins of the β-arrestin family. These β-arrestins bind to residues that have been phosphorylated by specialized kinases (G protein-coupled receptor kinases) and thereby initiate receptor desensitization and endocytosis. More recently, it has become clear that many additional "accessory" proteins bind to C termini of G protein-coupled receptors. The article by Sun et al. (p. 454) in the current issue of *Molecular Pharmacology* identifies translin-associated protein-X as yet another interaction partner of the A_{2A} receptor; translin-associated protein allows the A_{2A} receptor to impinge on the signaling mechanisms by which p53 regulates neuronal differentiation, but the underlying signaling pathways are uncharted territory. With a list of five known interaction partners, the C terminus of the A_{2A} receptor becomes a crowded place. Hence, there must be rules that regulate the interaction. This allows the C terminus to act as coincidence detector and as signal integrator. Despite our ignorance about the precise mechanisms, the article has exciting implications: the gene encoding for translin-associated protein-X maps to a locus implicated in some forms of schizophrenia; A_{2A} receptor agonists are candidate drugs for the treatment of schizophrenic symptoms. It is of obvious interest to explore a possible link.

Adenosine is a retaliatory metabolite. This catch phrase succinctly summarizes the concept that adenosine is a cellular signal of metabolic distress: hypoxia leads to a decline in cellular ATP levels and to the release of adenosine. On the extracellular side, adenosine affords tissue protection by eliciting both short-term effects (e.g., cellular hyperpolarization, inhibition of Ca^{2+} influx, vasodilation) and a delayed adaptive response (e.g., by triggering angiogenesis; see Linden, 2005). The widespread expression of adenosine receptors is also consistent with its role in mediating cellular protection: there are no tissues or organs that are not responsive to adenosine. The retaliatory action of adenosine results from the concerted stimulation of four adenosine receptors, termed A_1-, A_{2A}-, A_{2B}-, and A_3-adenosine receptors. These receptors differ in their affinity for adenosine, in the type of G proteins that they engage, and, hence, in the downstream signaling pathways that are activated in the receptive cells (Klinger et al., 2002a).

Adenosine and Neuroprotection

Adenosine, however, is not only released as a signal of cellular distress; it also participates in the purinergic synaptic signaling network. ATP is a constituent of neurotransmitter-containing vesicles and is thus subject to Ca^{2+}-dependent exocytosis. ATP can act, per se, on ionotropic and G protein-coupled receptors. In addition, neuronally released ATP is sequentially dephosphorylated by ectonucleotidases, apyrases, alkaline phosphatases, and 5′-nucleotidase to yield the two additional signaling molecules, ADP and adenosine, that act on a distinct set of G protein-coupled receptors (Zimmermann, 2006). Thus, ATP, ADP, and adenosine participate in neurotransmission; the activation status of neurons is believed to specify the relative contribution of individual receptors in this purinergic network (Moskvina et al., 2003).

Most of us are familiar with the immediate central nervous system depression that results from the accumulation of extra-

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ABBREVIATIONS: MAP, mitogen-activated protein; NGF, nerve growth factor; EGF, epidermal growth factor, TRAX, translin-associated protein-X.

cellular adenosine: we choose to antagonize it on a more or less regular basis by the intake of various caffeine-containing beverages. Everyday experience thus renders accessible the immediate effects that adenosine exerts on the brain via G protein-dependent signaling pathways. It is much less clear how adenosine receptors can also interfere with long-term decisions in the brain (i.e., proliferation, differentiation and outgrowth of neurite extensions that will give rise to axons and dendrites). Several observations have kindled interest in this area:

1. The A_{2B}-adenosine receptor was proposed to form a complex with DCC (deleted in colorectal cancer); axons require signaling of netrin-1 via DCC to cross the midline, and the A_{2B} receptor is the coreceptor that supports binding of netrin-1 (Corset et al., 2000). It should be noted that this model has been questioned (Stein et al., 2001).
2. In kainate-induced neurodegeneration, adenosine exerts neuroprotective effects in the hippocampus via A_{2A} receptors rather than via A_1 receptors (Jones et al., 1998). This observation is surprising, because the A_1 receptor mediates depression of neuronal activity via G_i/G_o-induced K^+-channel activation and inhibition of neuronal Ca^{2+} channels, and this is likely to alleviate excitotoxicity (kainate acts via ionotropic glutamate receptor). In contrast, it is intuitively less evident how the G_s-coupled A_{2A} receptor allows neurons to survive
3. Adenosine transactivates the neurotrophin receptors TrkA and TrkB (Lee and Chao, 2001). Transactivation refers to the fact that tyrosine kinase receptors can be recovered in active (i.e., phosphorylated) form from cellular lysates, although the cells have not been stimulated by their cognate ligand(s) but rather by a G protein-coupled receptor. The A_{2A} receptor triggers transactivation of TrkA and TrkB in PC-12 cells and hippocampal neurons, respectively (Lee and Chao, 2001). The precise mechanism is not clear, but transactivation requires the nonreceptor tyrosine kinase src, which also plays a prominent role in A_{2A} receptor mediated activation of mitogen-activated protein (MAP) kinase in several cell types, including PC-12 cells (Klinger et al., 2002b).

PC-12 Cell Differentiation As a Model System

The rat pheochromocytoma cell line PC-12 is a popular model to investigate the actions of the A_{2A} receptor in neuronal cells for two reasons: first, the A_{2A} receptor is endogenously expressed to high levels; second, upon serum withdrawal, nerve growth factor (NGF) initiates a differentiation program in PC-12 cells. Growth arrest is followed by the formation of growth cones and abundant sprouting of neurite extensions. At first, differentiation of PC-12 cells was proposed to be fully accounted for by the ability of NGF to induce a sustained stimulation of MAP kinase (Cowley et al., 1994). In this model, epidermal growth factor (EGF) failed to induce differentiation, because it only caused a transient increase in MAP kinase activity (Traverse et al., 1992). The deficiency of EGF is remedied if the receptor is overexpressed (Traverse et al., 1994). It has long been known that A_{2A} receptor stimulation may cause growth arrest in PC-12 cells (Huffaker et al., 1984) and synergize with NGF in the induction of differentiation markers (Guroff et al., 1981). It is noteworthy that these earlier observations indicated that stimulation of cAMP was not required, because the adenosine-induced growth arrest was not abolished by 3′,5′-dideoxyadenosine, a direct ("P-site") inhibitor of adenylyl cyclase (Huffaker et al., 1984). However, it is still possible to reconcile these findings with the above model, in which sustained activation of MAP kinase is the crucial signal for differentiation: the A_{2A} receptor can stimulate MAP kinase in both a G_s-dependent and -independent manner (Sexl et al., 1997; Seidel et al., 1999).

An Alternative Signaling Pathway and Its Implications

In the current issue of *Molecular Pharmacology*, Sun et al. (2006) propose an alternative pathway: the A_{2A} receptor generates two signals, one that relies on cAMP and protein kinase A-dependent phosphorylation of cAMP response element-binding protein (Cheng et al., 2002) and a second one that relies on a hitherto-unappreciated interaction partner, translin-associated protein-X (TRAX). In the absence of functional p53, differentiation of PC-12 cells is abrogated and activation of p53 is downstream of NGF-induced activation of $p21^{ras}$; p53-dependent induction of the cyclin-dependent kinase inhibitor $p21^{cip1}$ is likely to account for the NGF-induced cell cycle arrest (Hughes et al., 2000). Sun et al. (2006) show that stimulation of the A_{2A} receptor can bypass the requirement of p53 provided that TRAX is present.

It is not clear how TRAX works. In fact, its biochemical activities are poorly understood; TRAX can form complexes with translin (the mouse ortholog of which is referred to as TB-RBP, testis-brain RNA-binding protein). This may interfere with the ability of translin to bind (dendritic) mRNA (Chennathukuzhi et al., 2001). In view of our ignorance, why is the interaction between translin-associated protein-X and the A_{2A} receptor exciting news? The gene encoding translin-associated protein-X maps to a region on chromosome 1 (1q42) that is disrupted in some instances of schizophrenia (Millar et al., 2000); some haplotypes of the TRAX gene are in fact associated with the disease (Cannon et al., 2005). On the other hand, in rodents, A_{2A} receptor agonists elicit effects that are predictive of an antipsychotic action (i.e., a therapeutic efficacy in schizo-

TABLE 1
Accessory proteins that interact with the C-terminus of the A_{2A}-receptor
Accessory proteins are proteins other than G proteins, regulatory kinases, and β-arrestins that support signaling by the receptor and receptor desensitization.

Interacting Protein	Reported Effect on A_{2A} Receptor	Reference
D_2-Dopamine receptor	Cross-talk—mutual antagonism	Fuxe et al. (2005)
α-Actinin	Tethering to the actin cytoskeleton—receptor recycling	Burgueno et al. (2003)
ARNO	GEF for ARF6—required for sustained MAP kinase stimulation	Gsandtner et al. (2005)
USP4	Deubiquitinating enzyme—accelerates ER-export of the receptor	Milojevic et al. (2006)
Translin-associated protein-X	Binding partner of translin—rescues p53-deficiency in PC-12 cell differentiation	Sun et al. (2006)

GEF, guanine nucleotide exchange factor; ARNO, ADP-ribosylation factor nucleotide site opener; ER, endoplasmic reticulum.

phrenia) (Kafka and Corbett, 1996; Rimondini et al., 1997). Thus, it is tantalizing to suspect that this may be more than a fortuitous coincidence.

The A_{2A} Receptor C Terminus—A Crowded Place

The C terminus of the A_{2A} receptor is >120 amino acids long. The juxtamembrane segment immediately adjacent to the seventh transmembrane helix is required for proper folding of the receptor. The rest of the C terminus (100 amino acids) is dispensable for ligand binding (Piersen et al., 1994) and for G protein coupling (Klinger et al., 2002c). G protein-coupled receptors bind proteins other than G proteins, G protein-coupled receptor kinases, and β-arrestins (which support the eponymous signaling processes and initiate receptor desensitization). The list of these accessory proteins is rapidly growing (Bockaert et al., 2004). Table 1 gives an overview over accessory proteins that have been found to bind to the A_{2A} receptor. Although the length of the C terminus (~120 amino acids) may provide a lot of binding sites, the size of the individual binding partners [e.g., ADP-ribosylation factor nucleotide site opener (ARNO), ~47 kDa; TRAX, ~33 kDa] makes it unlikely that there is enough space for the simultaneous binding of all interaction partners. It is substantially more probable that any given interactor binds transiently and in a regulated manner. This may allow the C terminus to serve as a coincidence detector (the binding of agonist and a second signal must occur simultaneously for interactor recruitment/release) or as a signal integrator (several inputs must accumulate sequentially before interactor recruitment/release). Finally, the presence of absence of these accessory proteins may explain conflicting results. As mentioned above, A_{2A} agonists afford neuroprotection; this effect has been exploited for devising therapeutic strategies in spinal cord injury (Okonkwo et al., 2006). However, there is also evidence that A_{2A} antagonists—rather than agonists—are neuroprotective. In humans, consumption of caffeine protects against the development of Parkinson's disease; this effect has been confirmed in large prospective cohort studies and has been linked to blockage of A_{2A} receptors in the striatum (Ross et al., 2000; Ascherio et al., 2001). Neuroprotection by A_{2A}-antagonists can also be recapitulated in experimental models of toxin-induced degeneration of dopaminergic nigrostriatal projections (Pierri et al., 2005). It is therefore conceivable that the outcome of adenosine receptor stimulation (neuroprotection versus neurotoxicity) is contingent on the interaction partners of the receptors.

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