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A tail of two signals:

the C-terminus of the A_{2A}-adenosine receptor recruits alternative signaling pathways

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Running Title: The C-tail of the A_{2A}-receptor

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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. Originally the C-terminus was viewed mainly as the docking site for regulatory proteins of the β-arrestin family. These β-arrestins bind to residues which 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. 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. In spite of our ignorance about the precise mechanisms, the paper 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.

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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 adapative 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 isn't any tissue or organ which is 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- receptor. These receptors differ in their affinity for adenosine, in the type of G proteins, which they engage, and hence in the downstream signaling pathways, which 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 per se act 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, which 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 thought to specify the relative contribution of individual receptors in this purinergic network (Moskvina et al., 2003).

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Most of us are familiar with the immediate CNS-depression that results from the accumulation of extracellular 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 the interest in this area: (i) 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 co-receptor that supports binding of netrin-1 (Corset et al., 2000). It should be noted that this model has been questioned (Stein et al., 2001). (ii) 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. (iii) 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 PC12 cells and hippocampal neurons, respectively (Lee and Chao, 2001). The precise mechanism is not clear but transactivation requires the non-receptor tyrosine kinase src, which also plays a prominent role in A_{2A}-receptor mediated activation of mitogen-activated protein kinase (MAP kinase) in several cell types including PC12 cells (Klinger et al., 2002b).

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PC12 cell differentiation as a model system

The rat pheochromocytoma cell line PC12 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. In addition, upon serum withdrawal, NGF (nerve growth factor) initiates a differentiation program in PC12 cells: growth arrest is followed by the formation of growth cones and abundant sprouting of neurite extensions. Originally, differentiation of PC12 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, EGF (epidermal growth factor) fails to induce differentiation, because it only causes 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 PC12 cells (Huffaker et al., 1984) and synergize with NGF in the induction of differentiation markers (Guroff et al, 1981). Interestingly, 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 a G_s-independent way (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 (PKA)-dependent phosphorylation of CREB (Cheng et al., 2002) and a second one that relies on a hitherto unappreciated interaction partner, namely translin-associated protein-X

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(TRAX). In the absence of functional p53, differentiation of PC12 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. 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 schizophrenia (Kafka et al., 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 7^{th} transmembrane helix (TM7) 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). It has been appreciated that G protein-coupled receptors bind proteins other than G proteins, GRKs 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

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gives an overview over accessory proteins that have been found to bind to the A_{2A}-receptor. While 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. 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 of an) 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 people, consumption of caffeine protects against the development of Parkinson's disease; this effect has been confirmed in large prospective cohort studies and 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 *vs* neurotoxicity) is contingent on the interaction partners of the receptors.

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Table 1.
Accessory proteins* that interact with the C-terminus of the A_{2A}-receptor

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	guanine nucleotide exchange factor for ARF6 - required for sustained MAP kinase stimulation	Gsandtner et al. (2005)
USP4	deubiquinating enzyme - acceler-ates ER-export of the receptor	Milojevic et al. (2006)
Translin-associated protein-X	binding partner of translin - rescues p53-deficiency in PC12 cell differentiation	Sun et al. (2006)

**Accessory proteins are proteins other than G proteins, regulatory kinases and β-arrestins which support signaling by the receptor and receptor desensitization.*