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Adenosine receptors

Knockouts anxious for new therapy

Solomon H. Snyder

Over the past two decades, putative neurotransmitters have proliferated, from biogenic amines to amino acids, neuropeptides and, most recently, gases such as nitric oxide and carbon monoxide. Amidst the ferment over recently discovered messengers, scant attention has been paid to an atypical neurotransmitter whose candidacy dates back almost 30 years — adenosine. But the field may now be rejuvenated, thanks to a report by Ledent *et al.*¹ (page 674 of this issue) showing that there are behavioural and physiological alterations in mice lacking one adenosine-receptor subtype, A_{2a} .

Neurotransmission by adenosine and ATP — designated 'purinergic' by Burnstock² — is difficult to study because both molecules are involved in many pathways of general cellular metabolism. Nonetheless, the evidence for adenosine as a neurotransmitter is as persuasive as for any other substance: immunohistochemical studies have shown that adenosine is found in discrete neuronal populations in which those cells that use it as a neurotransmitter have much higher localized densities³; an efficient, energy-requiring uptake system exists which could account for synaptic inactivation of adenosine; and adenosine has prominent functions outside the brain. Studies by Berne⁴ have shown that oxygen starvation leads to a massive accumulation of adenosine, which causes vasodilation and increased blood flow.

The most compelling evidence for the specific actions of adenosine has come from the characterization of its receptors. The influences of adenosine on levels of cyclic AMP in brain slices provided the first molecular evidence for the function of receptors

and differentiation of subtypes. At nanomolar concentrations, adenosine lowers the activity of adenylyl cyclase (which converts ATP to cAMP) through adenosine A_1 receptors, whereas micromolar levels augment adenylyl-cyclase activity at A_2 receptors⁵.

Xanthines and caffeine both block adenosine receptors⁶, and this ability could account for their effects as behavioural stimulants. Xanthine derivatives can block adenosine receptors in direct proportion to their potencies as behavioural stimulants, and at concentrations similar to blood levels of caffeine after a few cups of coffee^{7,8}. The bronchodilating, antiasthmatic effects of theophylline and other xanthines may involve adenosine-receptor blockade, although the evidence is controversial.

Molecular-biological techniques have further discriminated receptor subtypes, specifically A_1 , A_{2a} , A_{2b} and A_3 (ref. 9). The A_{2a} receptor (which stimulates adenylyl cyclase) is particularly abundant in the basal ganglia, blood vessels and platelets, so it was selected by Ledent *et al.*¹ for targeted deletion.

The A_{2a} -receptor knockout mice seem to be normal on a gross level, and they breed successfully. But whereas the A_2 -agonist drug CGS-21680 reduces locomotor activity in wild-type mice, it has no behavioural effect in the knockouts. Strikingly, caffeine, which normally stimulates locomotor activity, substantially depresses activity in the knockout mice. These findings cement the conclusion that the stimulant effects of caffeine are derived from adenosine-receptor blockade. But why should caffeine depress behaviour in the knockouts? Adenosine analogues and caffeine can either stimulate or depress locomotor activity

depending on the dose⁷. So, in the knockouts, a stimulatory action of adenosine — perhaps through A_1 receptors — may be unmasked.

Ledent *et al.* also found that the knockout mice seem to be more anxious and aggressive than wild-type animals. This fits with abundant clinical evidence that caffeine increases anxiety, and that stimulation of the adenosine receptor can relieve anxiety. Adenosine modulates pain pathways in complex ways. There is evidence that stimulation of A_{2a} receptors on sensory pain fibres increases pain perception. The knockout mice show reduced pain responses, suggesting that — at least at A_2 receptors — adenosine normally augments pain perception. This also fits with observations that caffeine can be analgesic.

Adenosine has many effects in the cardiovascular system; it inhibits platelet aggregation and dilates several vascular beds. The knockout mice show more efficient platelet aggregation than wild-type animals, and they are resistant to inhibition of platelet aggregation by adenosine analogues. Ledent *et al.* also found that the knockout mice have increased blood pressure, supporting a tonic vasodilatory role for adenosine.

Pinning down the diverse biological functions of A_{2a} receptors should provide a route for the pharmaceutical industry to develop related therapeutic agents (Table 1). One possibility might be new cognitive stimulants. Although the relative merits of caffeine's stimulant effects are a source for debate, its cognition-enhancing actions are well documented. Agents that elicit these beneficial influences, without side-effects such as tachycardia, increased urination and agitation, may treat cognitive impairment of the elderly. At present, despite the wealth of available analgesics, none is entirely satisfactory. Moreover, the complex effects of adenosine on pain perception have thwarted efforts to develop adenosine-based analgesics¹⁰. The findings by Ledent *et al.*¹ from the knockout mice favour selective A_{2a} antagonists as non-addictive analgesics. Finally, antagonists to the A_{2a} receptor might improve defects in blood clotting and vascular shock, whereas A_2 agonists should decrease clotting and guard against low blood pressure. □

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Table 1 Potential adenosine-receptor drug development based on the A_{2a} knockout phenotype

Phenotype	Drug
Altered locomotor response to caffeine	A_2 antagonists as cognition enhancers
Apparent anxiety	A_2 agonists as anxiolytics
Lesser pain responses	A_2 antagonists as analgesics
Increased platelet aggregation	A_2 agonists as anticoagulants; A_2 antagonists for clotting defects
Hypertension	A_2 agonists as antihypertensives; A_2 antagonists for shock

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