The following is the abstract of the article discussed in the subsequent letter:

Kilpatrick EL, P Narayan, RM Mentzer, and RD Lasley. Adenosine A3 agonist cardioprotection in isolate rat and rabbit hearts is blocked by the A1 antagonist DPCPX. Am J Physiol Heart Circ Physiol 281: H847–H853, 2001.—Adenosine A3 agonists have been shown to protect ischemic rat and rabbit myocardium. However, these agonists have been reported to exert A1 independent effects, and no cardiac A3 receptor has yet been identified. We thus tested whether A3 agonist protection is due to A1 receptor activation. Isolated rat and rabbit hearts were subjected to 25 and 45 min of global ischemia, respectively. Rat hearts pretreated with adenosine (100 μM), the A3 agonist 2-chloro-N6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide (Cl-IB-MECA, 50 nM), and vehicle recovered 73 ± 2%, 75 ± 4%, and 46 ± 4%, respectively, of preischemic left ventricular developed pressure (LVDP) after 30 min of reperfusion. The A1 antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 100 nM) blocked the beneficial effects of Cl-IB-MECA (51 ± 5%) and adenosine (47 ± 6%). In rabbit hearts, the beneficial effects of the A3 agonist N6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide (50 nM) and the A1 agonist 2-chloro-N6-cyclopentyladenosine (100 nM) on postischemic LVDP (75 ± 4 and 74 ± 5%, respectively) were blocked by DPCPX (34 ± 4 and 36 ± 3%, respectively). The reduction in infarct size with both agonists was also completely blocked by DPCPX. These results suggest that these A3 agonists protect ischemic myocardium via A1 receptor activation.

ischemia; reperfusion; cardiac receptor

Apparent Activation of Cardiovascular A1 Adenosine Receptors by A3 Agonists

To the Editor: In the August 2001 issue of the American Journal of Physiology-Heart Circulatory Physiology, Kilpatrick and colleagues (9) examined the ability of selective and nonselective adenosine receptor antagonists to abrogate cardioprotection with A3-selective agonists in the rat and rabbit. Cardioprotective functions of adenosine receptor subtypes remain a source of much debate, and various groups continue to probe the roles of A1, A2A, A2B, and A3 adenosine receptors in ischemic and reperfused myocardium. In terms of the cardiac effects of A3 receptors, the weight of evidence from the literature supports A3-mediated cardioprotection via pathways distinct from those for A1 receptors in multiple species (3, 8, 10, 17). Even discounting studies employing A3 agonists, expression of A3 receptors themselves confers tolerance to injury in cardiac myocytes (6). Nonetheless, the interesting study by Kilpatrick et al. does address an important issue: which receptor subtypes are activated by so-called “A3-selective” agonists employed in these varied studies?

Through comparing responses to A3 agonists [N6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide (IB-MECA) and 2-chloro-N6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide (Cl-IB-MECA)] in the absence or presence of A1-selective antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) or nonselective antagonist 8-sulfoisophenyltheophylline (SPT), the authors conclude that the supposedly A3-selective agonists in fact protect via A1 receptors. However, the absence of appropriate control experiments renders this conclusion highly questionable. Specifically, effects of the antagonists themselves were not determined in ischemic-reperfused hearts.

To identify effects of exogenous receptor agonism in a tissue in which background endogenous responses exist, the appropriate “control” experiment is treatment of ischemic hearts with receptor antagonist alone. This is particularly important when endogenous agonist levels (and therefore responses) are enhanced, as during ischemia. If addition of agonist under these conditions no longer elicits a response, it can be concluded that the agonist acts via the targeted receptor. Importantly, a considerable literature reveals that A1- or nonselective adenosine receptor antagonism impairs ischemic or postischemic function in different species and models (7, 12, 14–16, 20). This demonstrates that endogenous adenosine serves a protective function in ischemic-reperfused myocardium. The recoveries observed by Kilpatrick et al. in hearts cotreated with agonist (adenosine, CCPA, Cl-IB-MECA, or IB-MECA) plus antagonist (DPCPX or SPT), which in all instances equal recoveries for untreated hearts, are likely to exceed the recoveries for ischemic-reperfused hearts treated with the antagonists alone (the appropriate control group). This would lead to the equivocal conclusion that: 1) effects of agonists are not dependent on the targeted receptor, and/or 2) competitive antagonism fails to effectively counter responses to applied agonist. Stated another way, the apparent lack of protection during cotreatment with A3 agonist and A1 antagonist may reflect a balance between beneficial effects of A3 agonism and injurious effects of antagonism of an endogenous A1 response.

With respect to the selectivity of the antagonist employed, DPCPX is selective for A1 receptors but also inhibits A2B and A2A receptors with an inhibitory constant (Ki) from 50 to 150 nM (11). It can therefore be argued that effects of DPCPX are complicated by A2B antagonism. However, this is not a likely explanation for the observations of Kilpatrick et al., because there is little evidence of A2-mediated protection in isolated asanguinous hearts, and A2-mediated protection from ischemia in vivo is resistant to DPCPX (18).
A final point relates to their experiments employing 100 μM exogenous adenosine. In these studies it is predicted that protection should occur via receptor-mediated (A1, potentially A2 and A3) and nonreceptor-mediated mechanisms. We and others (4, 13) have demonstrated a metabolic component to adenosine-mediated protection involving purine salvage. Nonetheless, selective A1 blockade apparently abolished protection in their study, implicating a single protective mechanism for adenosine (A1 activation). This unexpected observation might again reflect combined effects of inhibition of A1-mediated protection and protection via A1-independent pathways.

These uncertainties cannot be resolved in the absence of data on effects of receptor antagonism in ischemic-reperfused hearts. Studies examining adenosine antagonism reveal protection by endogenous adenosine in human (14), canine (19), rabbit (16, 20), rat (7, 15), and mouse myocardium (12). Interestingly, failure to identify effects of adenosine antagonism alone is not uncommon. For example, investigators often apply antagonists to test adenosine role in modulation of functional A1 activation. This unexpected observation might again reflect combined effects of inhibition of A1-mediated protection and protection via A1-independent pathways.


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currently available A3 antagonists and the lack of information on the effects of these agents in the heart. Despite these limitations, it is well known that rodent adenosine A3 receptors are relatively insensitive to methylxanthine-based antagonists. It has been reported that cloned rat and rabbit A3 receptors exhibit $K_i$ values for DPCPX $> 5 \mu$M and 1 $\mu$M, respectively (6, 7). In addition, although high doses (100 $\mu$M) of 8-SPT have been used to implicate A3 cardioprotection in the rabbit myocardium [$K_i$ for cloned rabbit A3 receptors $\geq 38 \mu$M (6)], we were not able to document any published reports of the use of low doses of this methylxanthine in the presence of an A3 agonist. These deficiencies in the literature provided the basis for our study. The methylxanthine DPCPX, at doses (100 nM) similar to what we used in our study, has been used extensively in A1 receptor radioligand binding studies and to study the adenosine A1 anti-adrenergic effect. Although this dose of DPCPX may have exerted some effects on A2a, we have previously reported that preischemic treatment with an A2 agonist in this same model is not cardioprotective. At the present time there is no evidence supporting a role for A2b receptors in adenosine cardioprotection. Thus it is likely the effects associated with low-dose DPCPX and 8-SPT in our study were due to A3 receptor antagonism.

Although we recognize the concerns of Dr. Headrick regarding the potential effects of DPCPX alone, we disagree with his conclusion that our omission of such a group “renders [our] conclusion highly questionable.” As Dr. Headrick pointed out, there are reports on the modulation of ischemia-reperfusion injury by various adenosine antagonists; however, we did not include this group based on several reports (in multiple species) documenting the lack of effect of DPCPX alone on ischemia-reperfusion injury when administered only before ischemia (3, 4, 12, 13, 18, 20). Hearts in our study were exposed to DPCPX (100 nM) only for 10 min immediately before ischemia. Two reports (14, 16) indicate that low doses of DPCPX (100–200 nM), even when administered only before ischemia, did exacerbate early recovery of ventricular function in isolated perfused mouse hearts. However, these hearts were only reperfused for 30 min, and in the latter study (16), during the final 10 min of the 30-min reperfusion period, both DPCPX groups exhibited a more rapid rate of recovery of preischemic function than control hearts. Whether this effect would have persisted for the duration of reperfusion remains unknown. In addition to potential differences between murine myocardium and that of other species, these conflicting reports indicate that the effects of adenosine receptor antagonists in ischemic-reperfused myocardium may be dependent on the time of administration, as has been widely documented for adenosine agonists.

Dr. Headrick’s final point related to the “unexpected observation” that the beneficial effects of 100 $\mu$M adenosine in our study were essentially completely blocked by DPCPX. He apparently interpreted this to indicate that we were excluding the metabolic effects of adenosine, such as purine salvage. In fact our initial studies (2, 22) on adenosine cardioprotection were based on the hypothesis that adenosine would be protective via this mechanism. However, purine salvage is an oxygen-dependent, reperfusion-related process of ATP resynthesis, and we observed beneficial metabolic effects of exogenous adenosine during ischemia. We subsequently reported that the ability of adenosine to retard the rate of ATP depletion during ischemia could be mimicked by an A1 agonist (9, 11), and there have been numerous reports of metabolic effects of adenosine agonists during ischemia. Given that we infused adenosine for only 5 min before ischemia, it is unlikely that purine salvage would play a role in this protection. Although we cannot discount potential metabolic effects of DPCPX during ischemia, there is substantial evidence that preischemic treatment with DPCPX does not exacerbate ischemia-reperfusion injury (3, 4, 12, 13, 18, 20) in several species. As we discussed in the paper, this does not appear to be the case with another antagonist BWA1433 (15), which has been used to implicate A3 receptors in adenosine cardioprotection.

As we concluded in our final statement in the paper “until the A3 receptor is identified in cardiac myocytes and more selective A3 agonists and antagonists become available, the exact role, if any, that A3 receptors play in adenosine cardioprotection remains to be determined.” This statement is further reinforced by the results of two studies indicating that knockout of the A3 receptor in the mouse confers resistance to myocardial ischemia-reperfusion (1, 5).

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