LETTERS TO THE EDITOR

Cardioprotection by adenosine: Is it a question of effective dose, timing, or the target compartment?

To the Editor:

We read with great interest the excellent study by Randhawa, Lasley, and Mentzer,¹ which tested the cardioprotective effect of preischemic (pretreatment) intracoronary adenosine versus postischemic (i.e., onset of reperfusion) intracoronary adenosine administration in a porcine model of contractile "stunning." This study was based on the authors' hypotheses that (1) the cardioprotective effects of adenosine were mediated principally via the A₁ receptor subtype located on the myocyte and (2) adenosine-induced cardioprotection required an accumulation of the purine in the interstitial fluid compartment. Adenosine was administered by intracoronary infusion either before coronary occlusion (either 5 or 50 $\mu\text{g/kg}$ per minute for 10 minutes) or immediately on the onset of reperfusion (50 $\mu\text{g/kg}$ per minute for 30 minutes). The investigators found that pretreatment adenosine at both low and high doses significantly attenuated postischemic contractile stunning in the ischemic-reperfused area, although accumulation of adenosine in the interstitial fluid compartment (microdialysis) was significant only in the group receiving 50 $\mu\text{g/kg}$ per minute. In contrast, adenosine administered at reperfusion had no salutary effect and failed to increase interstitial fluid adenosine. The authors concluded that (1) intracoronary adenosine is cardioprotective optimally when given before ischemia, (2) adenosine levels in the interstitial compartment must be augmented to exert this protection, and (3) the mechanism underlying this cardioprotection is likely related to an interaction of adenosine with its A₁ receptor subtype on the myocyte. A mechanism related to A₂ receptor activation was thought not to be involved.

The requirement for adenosine accumulation in the interstitial compartment has recently come under vigorous investigation. Van Wylen² has shown that the critical concentration of intravascular adenosine needed to overcome the endothelial "sink" or barrier and augment interstitial adenosine concentrations is approximately 5 to 10 $\mu\text{g/kg}$ per minute, which is close to the lowest dose used in the study under discussion. The failure of the 5 $\mu\text{g/kg}$ per minute dose to augment interstitial fluid adenosine above preinfusion levels may be related to the inability of adenosine to traverse this barrier. However, the enhanced recovery of contractile function in this low-dose group in the absence of augmented interstitial levels is inconsistent with a requirement for interstitial accumulation. As recently demonstrated by Lasley and colleagues,³ cardioprotection cannot be explained by simple augmentation of interstitial adenosine concentrations.

In addition to the interstitial (myocyte) compartment, a major and alternative target for adenosine is the vascular compartment. Adenosine, via activation of A₂ receptors, has potent inhibitory effects on neutrophils, resulting in attenuated production of superoxide anions, inhibition of

adherence to the vascular endothelium (purportedly a major initiating step in the inflammatory cascade involved in reperfusion injury), and a reduction in neutrophil-mediated endothelial damage. The importance of the intravascular space as a site for adenosine-mediated cardioprotection, independent of interstitial actions, has recently been shown by Todd and associates.⁴ They showed that macromolecular adenosine, confined to the intravascular compartment, reduced infarct size in a rabbit model of myocardial reperfusion injury. Therefore the intravascular compartment may be a significant site of action of adenosine, in addition to the myocyte interstitial compartment.

When does adenosine exert cardioprotection in the two compartments mentioned? Randhawa, Lasley, and Mentzer¹ found that a beneficial effect of adenosine was exerted in their nonlethal model only when adenosine was administered as a pretreatment, and not when infused during reperfusion alone. However, the literature is rich with studies in which adenosine (both exogenous and endogenous) exerts cardioprotection during the reperfusion phase. In addition, activation of the A₂ receptor specifically, rather than the A₁ receptor, was found in a number of studies to be primarily involved in protecting the heart from ischemic-reperfusion injury. Zhao and associates⁵ found that *endogenous* adenosine released during ischemia reduced infarct size predominantly by an A₂-mediated effect, that is, activation of adenosine A₂ receptors. This finding has been extended to exogenous adenosine administered only during reperfusion. The cardioprotection by A₂ receptor activation is likely due to inhibition of neutrophil activities involved in the pathogenesis of reperfusion injury, as discussed earlier. Therefore A₂-mediated effects exerted during reperfusion, in addition to A₁-mediated mechanisms active during reperfusion, are key factors in the overall cardioprotection exhibited by adenosine.

The apparent questions raised by the study of Randhawa, Lasley, and Mentzer¹ regarding the optimal timing of adenosine administration and the receptor mechanisms involved may be related to the intended target of therapy and the compartment in which that target resides. A pretreatment administration of adenosine, as used by Randhawa, Lasley, and Mentzer,¹ both reduces infarct size and enhances functional recovery by predominantly A₁-mediated mechanisms, possibly by activation of adenosine triphosphate-sensitive potassium channels. On the other hand, adenosine or analogs administered exclusively at the time of reperfusion significantly reduce infarct size (lethal injury), probably by inhibiting neutrophil by A₂ receptor-mediated effects through activation of A₂ receptors. However, neutrophils have not been clearly implicated in the pathogenesis of postischemic contractile stunning (nonlethal injury) because stunning occurs in neutrophil-free models. Therefore targeting neutrophil activation during the reperfusion

phase will likely not alter postischemic stunning, although other mechanisms of injury would be engaged. This explanation is consistent with the data presented in the study by Randhawa, Lasley, and Mentzer¹ and is consistent with other studies related to the cardioprotective mechanisms of adenosine.

The optimal timing of adenosine administration may span all three windows of protection: before treatment, during the ischemic event, and during reperfusion. In cardiac surgery, these three windows of protection are open. First, adenosine can be given as a pretreatment before the operation. Second, adenosine may be included as an adjunct to either crystalloid or blood cardioplegia,⁶ in which case it is administered during surgical "protected" ischemia. Third, adenosine may be administered after aortic unclamping and reperfusion. Which "window" of administration is *most* optimal, what dose is optimal, which receptors and effector systems are targets of adenosine, and in which compartment(s) protective effects are exerted in the surgical setting are questions that will keep young basic scientists and physician-investigators at the bench for some time.

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Reply to the Editor:

We thank Drs. Vinten-Johansen and Hammon for their interest in our work entitled "Salutary Effects of Exoge-

nous Adenosine Administration on In Vivo Myocardial Stunning."¹ We reported that adenosine's cardioprotective effects were related to the preischemic elevation of adenosine in interstitial fluid and subsequent activation of adenosine A₁ receptors located on the cardiac myocytes. We also concluded that adenosine must be administered before ischemia to attenuate stunning, because postischemic infusion of adenosine only transiently improved systolic wall thickening.

As pointed out by Vinten-Johansen and Hammon, in one protocol in this study we observed attenuation of stunning with an adenosine pretreatment dose (5 µg/kg per minute) that did not elevate preischemic dialysate adenosine concentrations. Although we acknowledged that the cardioprotective effect of this dose could have occurred independent of increased interstitial fluid adenosine, we concluded that the lack of increase in dialysate adenosine may have been due to the limitations of the dialysis technique. This technique provides only an estimate of interstitial fluid metabolites and is dependent on blood flow. In subsequent studies in the pig (unpublished data) we observed that the same intracoronary adenosine infusion (5 µg/kg per minute) increased coronary venous adenosine fivefold (from 0.31 ± 0.09 µmol/L to 1.51 ± 0.32 µmol/L). We detected a much smaller increase in dialysate adenosine—from 0.55 ± 0.05 µmol/L to 0.71 ± 0.11 µmol/L. We thus agree with Van Wylen² that the threshold intracoronary adenosine dose for overcoming the coronary endothelial barrier is approximately 5 µg/kg per minute.

Because both doses of adenosine undoubtedly increased plasma adenosine levels, adenosine A₂ receptors were presumably activated. However, it is unlikely that the cardioprotective effects of adenosine pretreatment are related to preischemic activation of adenosine A₂ receptors. This hypothesis is based on the results of numerous studies in which adenosine A₁ and A₂ receptor agonists and adenosine receptor antagonists were used. The preischemic activation of adenosine A₁ receptors appears to be critical for the cardioprotective effects of adenosine against both reversible and irreversible injury. In our recent study in a model of irreversible injury, we³ observed that a transient intravenous adenosine infusion (i.e., adenosine preconditioning) briefly increased preischemic levels of myocardial dialysate adenosine and reduced infarct size. The adenosine preconditioning did not, however, alter dialysate concentrations during the prolonged occlusion. These results are consistent with our working hypothesis that the cardioprotective effects of adenosine pretreatment are mediated by the preischemic activation of adenosine A₁ receptors, irrespective of ischemic interstitial fluid adenosine levels.

Although the cardioprotective effects of adenosine pretreatment in models of stunning and infarction are thought to be mediated by adenosine A₁ receptor activation, we agree with Drs. Vinten-Johansen and Hammon that adenosine exerts beneficial effects during reperfusion of the irreversibly injured heart. As suggested by several studies from Dr. Vinten-Johansen's laboratory, these effects appear to be mediated by activation of adenosine A₂ receptors located on endothelial cells and neutrophils. In fact, there is evidence that, in contrast to the beneficial