produced in the peripheral circulation. We agree with the speculation that some or most of the plasma cGMP is derived from the endothelium, which is in direct contact with the bloodstream. Although ANP receptors coupled to guanylate cyclase also exist in the vascular endothelium, the role of the increase of endothelial cGMP by ANP has not been clarified. Recently, Kohn et al. have indicated that the increase of cGMP by ANP inhibits secretion of endothelin-I, a potent endothelium-derived vasoconstrictor peptide, suggesting that endogenous ANP regulates the vascular tone due to the regulation of endothelin-I production of endothelium by the increase of cGMP. Actually, the plasma endothelin-I level was increased in patients with heart failure and may act as a vasoconstricor. In this context, we believe that cGMP production, even that mainly derived from the endothelium, may contribute to vasodilation by the regulation of endothelin-I production in patients with mild heart failure. We emphasize not the origin of plasma cGMP but the uncoupling of ANP extraction and cGMP production in patients with severe heart failure.

Hirooka et al. have shown that the vasodilative action of exogenous ANP is attenuated in patients with heart failure. However, they should not simply suggest a connection between the response of forearm vascular flow and the change of plasma cGMP levels in the antecubital vein, which is derived from various organs including the lungs, because the possibility of the difference of response in the tissue or organs to increase the plasma cGMP level by ANP cannot be excluded. The impaired production of cGMP in our patients with severe heart failure may represent an endothelial dysfunction, as shown by Jansen et al. in the elderly compared with the young. They have shown that the forearm vasodilative response to exogenous ANP is attenuated in the elderly despite the same cGMP production as in the young and concluded that changes in postreceptor processes are involved in the age-related reduction in vascular sensitivity for ANP. In our study, we also have observed the response of the plasma cGMP level in patients with New York Heart Association (NYHA) class I or II and in patients with NYHA class III before and after the administration of ANP, and the slope of the linear regression line between the plasma cGMP and ANP levels in NYHA class III patients was about one half of the slope of that in NYHA class I or II; suggesting a difference of the cGMP response in relation to the severity of heart failure. Therefore, we hypothesized the possibility of downregulation of ANP receptor coupled to guanylate cyclase. Moreover, even if the impaired cGMP production is due to the dysfunction of endothelium in patients with chronic severe heart failure, we believe that at least part of the endothelial damage is functional because we have observed that the plasma cGMP level is higher in patients with acute severe heart failure than in patients with chronic severe heart failure despite the same ANP levels and that the impaired cGMP production is reversible after the treatment in our preliminary data. Therefore, we suggest the possibility of downregulation of ANP receptors in the present study.

Although further studies are required to test our hypothesis on patients with heart failure, the report suggests that a high plasma ANP level is a strong prognostic predictor may support the inability of endogenous ANP to play a compensatory role in the pathogenesis of heart failure.

Regarding the contribution of plasma cGMP activated by soluble guanylate cyclase, we have reported previously that plasma cGMP production by nitroglycerin was very small compared with cGMP production by ANP but was detected in patients with heart failure. Therefore, we speculate that some part of the plasma cGMP level is derived from intracellular cGMP activated by EDRF or nitrates. However, further studies are also required to verify this hypothesis.

References


Ischemic Preconditioning and Adenosine Release

In a recent study, Kitakaze et al. reported that in the dog heart, ischemic preconditioning increased 5’-nucleotidase activity and adenosine release. This interesting study provides new valuable information on the effects of ischemia-reperfusion on purine catabolism. However, I have some difficulties in interpreting the time course of adenosine release during the preconditioning procedure.

Adenosine release increased immediately after each coronary occlusion, as expected, and the authors seem to imply that it returned to the baseline after 5 minutes of reperfusion because venous adenosine concentrations were not significantly different from control. However, the rate of adenosine release should be calculated as (venous adenosine concentration minus arterial adenosine concentration) times coronary flow. The average values of this variable can be estimated on the basis of the data reported in Tables 1 and 2, although the calculation is not strictly correct. In particular, 1) the mean of a product must be obtained from the raw data and is not necessarily equal to the product of the means, but the latter should be a close estimate of the former; 2) whole blood concentrations should be determined, but the use of plasma concentrations should not bias the comparison of different measurements because it seems reasonable to assume that differences in hematocrit were minimal, and in addition, the turnover of plasma adenosine is rather slow (a few minutes) in dog blood so that adenosine degradation or uptake by erythrocytes during the transit time in the coronary circulation should be negligible.

If we accept these limitations, it can be estimated that in the control group, baseline adenosine release averaged 209 pmol·min⁻¹·100 g⁻¹ and increased to 635 pmol·min⁻¹·100 g⁻¹ after 40
minutes of "stabilization." In the preconditioned group, adenosine release was much higher before the preconditioning procedure (at "0 minutes") adenosine release averaged 1592 pmol ⋅ min⁻¹ ⋅ 100 g⁻¹ and after each cycle of 5 minutes of coronary occlusion and 5 minutes of reperfusion (range for the four cycles, 1387 to 2354 pmol ⋅ min⁻¹ ⋅ 100 g⁻¹).

It is not easy to find a satisfactory interpretation for these results. Had baseline adenosine release been similar in the two groups, it could be speculated that 5 minutes of reperfusion did not allow complete washout of the catecholamines accumulated in the tissue during ischemia, as suggested also by the results that we obtained in a different experimental model. However, if I understand correctly that "0-minute" values in Table 1 correspond to "baseline" values in Table 2, it should be concluded that baseline adenosine release was not comparable between the two groups and that the difference persisted through the experimental protocol. Since the values of the hemodynamic variables and oxygen consumption were superimposable in the two groups, this is hard to believe.

I think that if the authors would clarify these points, the implications of their article could be better understood.

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References

Reply
We are grateful to Dr. Zucchi for his interest in our recent work entitled "Ischemic preconditioning increases adenosine release and 5'-nucleotidase activity during myocardial ischemia and reperfusion in dogs: implication for myocardial salvage." This study revealed that ischemic preconditioning increases adenosine release during reperfusion and 5'-nucleotidase activity and forwarded the hypothesis that increases in adenosine release contribute to the infarct size-limiting effects of ischemic preconditioning. Furthermore, recent reports from Dr. Downey's laboratory (Liu et al. and Thornton et al.) show that exposure to adenosine during ischemic preconditioning also makes myocardium resistant to ischemic injury. The issues that Dr. Zucchi has raised are very important and fundamental to understand the basic mechanisms of ischemic preconditioning. His questions are 1) whether or not the basal adenosine release at the beginning of the experiments in the control and ischemic preconditioning groups are identical, and 2) if adenosine release at 5 minutes of reperfusion during ischemic preconditioning procedures gradually increases.

We calculated adenosine release from the raw data of adenosine concentration of coronary arterial and venous blood according to the equation to calculate the amount of adenosine release. Although the mean value of adenosine release (1.5±0.79 nmol ⋅ 100 g⁻¹ ⋅ min⁻¹) in the basal condition in the ischemic preconditioning group was larger than the mean value of the control group (0.37±0.43 nmol ⋅ 100 g⁻¹ ⋅ min⁻¹), there was no significant difference between these two values, and it was concluded that there were no significant differences in the myocardial metabolic condition as well as coronary hemodynamic condition between the two groups. Furthermore, we could not conclude that adenosine was significantly released in the baseline normoxic condition in both groups from our measurement of plasma adenosine concentration and calculation of adenosine release. This very small amount of adenosine release in the baseline condition may account for the unimportant role of adenosine in the regulation of coronary blood flow in the unstressed myocardium. On the other hand, during reperfusion after sustained ischemia, release of adenosine in the control and ischemic preconditioning groups was 40.4±9.2 and 110.7±19.6 nmol ⋅ 100 g⁻¹ ⋅ min⁻¹, respectively, which revealed that adenosine was significantly and markedly released and that release of adenosine in the ischemic preconditioning group was significantly (P<.01) larger than that in the control group. This observation also strengthens our hypothesis that ischemic preconditioning increases adenosine release in the reperfused myocardium.

Second, as Dr. Zucchi pointed out, release of adenosine appears to increase at baseline conditions after 5 minutes of myocardial ischemia. In the control group, adenosine release at 0 and 40 minutes of the steady state was 0.37±0.43 and 0.71±0.38 nmol ⋅ 100 g⁻¹ ⋅ min⁻¹, respectively, and adenosine release at 0 and 40 minutes of ischemic preconditioning procedure was 1.51±0.79 and 1.32±0.79 nmol ⋅ 100 g⁻¹ ⋅ min⁻¹, respectively. However, there were no significant differences between the changes in adenosine release for 40 minutes in both groups tested by two-way repeated measures ANOVA. As Dr. Zucchi suggested, there still remains the possibility that interstitial adenosine concentration was higher at the baseline condition in the ischemic preconditioning group because of the incomplete washout of adenosine, and this delayed washout of adenosine may continuously stimulate adenosine receptors of myocardium and may make myocardium more resistant to sustained myocardial ischemia. This hypothesis from Dr. Zucchi's letter is worth testing and may constitute an important target for the future research of cardioprotection in the ischemic and reperfused heart. I believe that we need to combine our results and wisdom to solve the mystery of ischemic preconditioning and to obtain the strategy to limit infarct size.

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