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LETTER TO THE EDITOR

Ischaemic Preconditioning and Postconditioning do not Affect Adenosine A_1 and A_{2A} Receptor Sensitivity

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To the Editor:

Endogenous adenosine is an important mediator of ischaemic preconditioning and postconditioning. The protective effect of ischaemic preconditioning is dependent on activation of adenosine A_1 receptors in the first few minutes of reperfusion [1]. In contrast, the infarct size-limiting effect of ischaemic postconditioning is mediated by the activation of adenosine A_{2A} and A_{2B} receptors at the time of reperfusion [2, 3]. Paradoxically, although the cardioprotective effect of preconditioning and postconditioning are critically dependent on adenosine receptor stimulation immediately after reperfusion, several research groups could not demonstrate an infarct size-limiting effect of administration of exogenous adenosine at the moment of reperfusion [4–6]. A possible explanation is that the pre- and postconditioning stimuli increase the sensitivity of a specific adenosine receptor sub-type. Indeed, this mechanism was recently proposed for the adenosine A_{2B} receptor [7]. The A_{2B} receptor has a K_m value for adenosine that is much higher than the endogenous adenosine concentration reached during ischaemia. Kuno *et al* have recently shown in the rabbit heart that brief preconditioning ischaemia markedly lowered the threshold for an adenosine A_{2B} agonist to increase the phosphorylation of the kinases, *Akt* and *Erk1/2* [7].

In this study, we test the hypothesis that ischaemic preconditioning also increases the sensitivity of the adenosine A_1 receptor at the time of myocardial reperfusion, and that ischaemic postconditioning increases the sensitivity of the adenosine A_{2A} receptor, using an isolated perfused rat heart model. As adenosine A_1 receptor stimulation has a negative inotropic effect and adenosine A_{2A} receptor stimulation induces coronary vasodilation, we used heart rate and coronary flow as read-out parameters for adenosine A_1 and A_{2A} receptor sensitivity, respectively. Male Sprague-Dawley rats were obtained from the Biological Services Unit of University College London (United Kingdom). All experiments were conducted in accordance with UK Home Office Guide on the Operation of Animal (Scientific Procedures) Act of 1986. After anaesthesia with pentobarbital, hearts were excised and mounted on a constant pressure (80 mmHg) Langendorff-apparatus and perfused with modified Krebs–Henseleit bicarbonate buffer (gassed with 95% O_2 / 5% CO_2, pH7.35–7.5). Heart rate and coronary flow were monitored at regular intervals. To study the sensitivity of the adenosine A_1 receptor, hearts were perfused with incremental concentrations of the selective A_1 receptor agonist 2-chloro-N6-cyclopentyl-adenosine (CCPA) (10^{-10} to 10^{-6} mmol/l) for 5 min per concentration. Heart rate was measured in the last minute of perfusion of each concentration and compared to the baseline heart rate. To study the sensitivity of the A_{2A} receptor, the hearts were perfused with

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the selective A_{2A} receptor agonist CGS21680 (10^{-10} to 10^{-6} mmol/l) and coronary flow rate was measured in the last minute of each concentration. These measurements were performed at baseline (after 30 min of equilibration), at 20 min reperfusion after 35 min of regional ischaemia in control hearts and hearts treated with a standard ischaemic preconditioning protocol (two cycles of 5 min of global ischaemia and reperfusion) or a standard ischaemic postconditioning protocol (6 cycles of 10 s of global ischaemia and reperfusion). The measurements were performed 20 min after reperfusion to allow the heart rate and coronary flow to return to baseline values after the ischaemic insult.

Perfusion with CCPA dose-dependently lowered heart rate (Fig. 1). This reduction in heart rate was similar at baseline and at 20 min myocardial reperfusion (data not shown), and was not affected by preconditioning (P=0.9 and P=0.4 for comparison of EC_{50} and V_{max} values, respectively, univariate analysis of variance). Perfusion with CGS21680 dose-dependently increased coronary flow at baseline (Fig. 2). However, after ischaemia, this effect was blunted, and did not differ between the postconditioning and control groups (P=0.8, ANOVA for repeated measures on absolute flow values).

In conclusion, the present study demonstrates that ischaemic preconditioning and postconditioning do not increase the sensitivity of the adenosine A_1 and A_{2A} receptor, respectively. Therefore, this mechanism does not explain the apparent discrepancy that the cardioprotective effect of ischaemic pre-, and postconditioning is critically dependent on adenosine receptor sub-type stimulation during reperfusion, whereas administration of exogenous adenosine during reperfusion does not reduce infarct size. An alternative explanation could relate to the role of the coronary endothelium in the metabolism of adenosine. As the endothelium acts as a strong metabolic barrier for circulating adenosine by its rapid uptake and intracellular degradation, intravascular administration of adenosine does not affect the myocardial interstitial adenosine concentration, whereas ischaemic pre- and postconditioning do also increase the interstitial concentration [8]. Additional studies are needed to test this hypothesis.

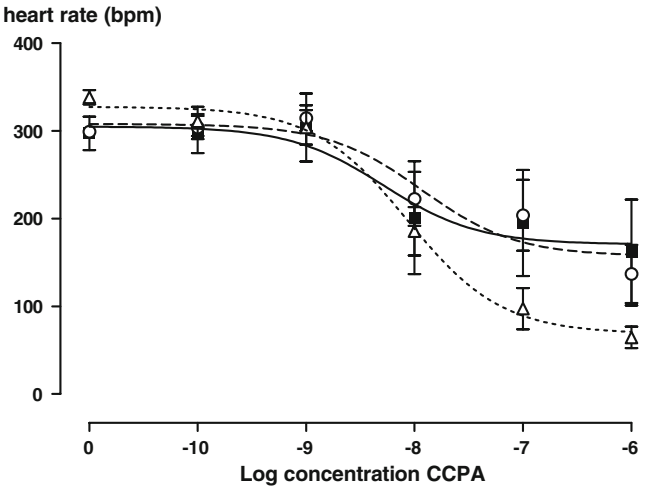

Fig. 1 The heart rate response to perfusion with incremental concentrations of CCPA at baseline (before any intervention, Δ, n=5), after 35 min of regional ischaemia and 20 min of myocardial reperfusion (■, n=5), after ischaemic preconditioning, 35 min of regional ischaemia and 20 min of myocardial reperfusion, ○, n=5)

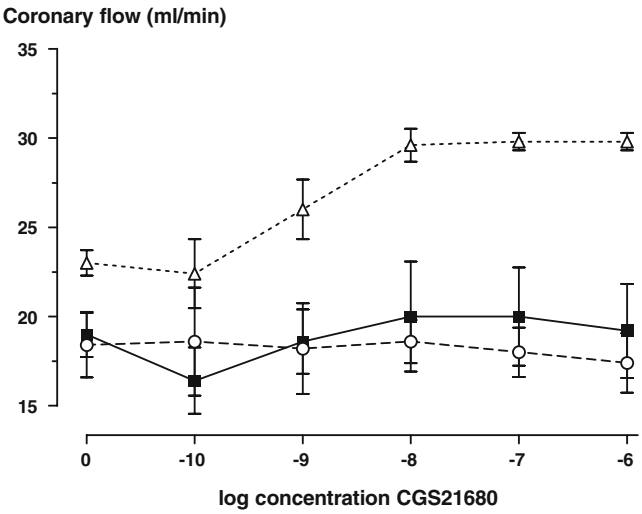

Fig. 2 The coronary flow rate response to perfusion with incremental concentration of CGS21680 at baseline (before any intervention, Δ, n=5), after 35 min of regional ischaemia and 20 min reperfusion (■, n=5), and after 35 min of ischaemia and 20 min of reperfusion incorporating the ischaemic postconditioning protocol, ○, n=5)

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