Historical note

Improved functional recovery by ischaemic preconditioning is not mediated by adenosine in the globally ischaemic isolated rat heart

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Keywords: Endothelial factors; Endothelial function; Nitric oxide; Shock; Veins

The experiments described in this paper were designed and performed between 1991 and 1992 when I was in the final year of my Ph.D studentship. I was introduced to preconditioning at my first American Heart Association meeting in 1989 and was fascinated that evolution had devised an endogenous protective mechanism for the heart, which was far more effective than any intervention developed by man. Not surprising really, considering that evolution has been working on stress adaptation for a couple of a million years! Since the aim of my PhD project was to extend the window of preservation for donor hearts prior to transplantation, after the AHA meeting, I began experiments to explore whether ischaemic preconditioning could provide protection over and above that provided by cardioplegia.

For the transplanted heart, we felt the most important parameter for assessment was contractile function. For a heart to be weaned off cardiopulmonary bypass, contractile function must recover sufficiently to support the body. It would be somewhat irrelevant for the transplanted heart if preconditioning reduced cell necrosis but left the heart so stunned that it could not maintain a sufficient cardiac output. However, in 1989, there were no published studies specifically investigating preconditioning-induced protection against contractile dysfunction. Therefore, my initial work characterised the phenomenon of preconditioning using the isolated ejecting rat heart and contractile function as the end-point of assessment [1] before proceeding to investigate whether preconditioning could improve on the protection provided by cardioplegia [2]. Since subjecting a donor heart to additional ischaemia, however short, is clearly not ideal, an obvious extension to these studies was to examine the possibility that the protection provided by ischaemic preconditioning could be mimicked by pharmacological means.

The first studies investigating the mechanism of preconditioning-induced protection were reported by Downey and colleagues in 1990 [3,4]. Liu et al. [5] reported that a 5-min intracoronary infusion of adenosine was as effective as 5 min of ischaemia in protecting parabiotically perfused isolated hearts against infarction from a 45-min ischaemic insult. Similarly an infusion of the A₁-selective agonist N⁶-1-(phenyl-2R-isopropyl) adenosine limited infarction at a dose which had no vasodilatory activity. Furthermore, blockade of A₁-receptors with 8-SPT abolished protection. Similar results had also been reported in abstract form in the pig [6] and dog [7] again using cell necrosis as an end-point of protection. None of these studies had investigated the impact of pharmacological preconditioning against contractile dysfunction nor had they demonstrated the involvement of adenosine in the mechanism of preconditioning in the rat.

Therefore our studies were designed to investigate whether a 5-min infusion of adenosine, at three different doses (10, 50 and 100 mmol/l), could mimic the protection provided by 5 min of ischaemia in the isolated working rat heart. All three doses resulted in a profound chronotropic effect and therefore hearts were paced throughout these protocols. The results clearly demonstrated that when compared to a control infusion of saline, none of the doses of adenosine provided any additional protection in terms of protection against contractile dysfunction. However, there was some evidence of protection against cell necrosis as creatine kinase release was significantly reduced at the highest dose of adenosine (100 μmol/l). Since it was possible that exogenously supplied adenosine had different effects to endogenously produced adenosine, we extended the study to determine whether 8-SPT could block ischaemic preconditioning in this model. A dose of 8-SPT was chosen (10 μmol/l) which blocked the negative chronotropic effect of exogenously supplied adenosine without effect on pre-ischaemic contractile function. The results demonstrated that the pres-
ence of 8-SPT depressed functional recovery in both the preconditioned and control group but these differences did not reach a level of significance (Fig. 1). We therefore concluded that while there was evidence that adenosine could be protective in the rat [8], adenosine release did not appear to be primarily involved in the mechanism of preconditioning against contractile function.

The results with adenosine were initially presented in abstract form at the 1991 International Society for Heart Research in Cincinnati, Ohio, USA. Presentation of these results met with considerable opposition and I clearly remember having to defend the results and conclusions to a packed and skeptical auditorium. The main criticism was that I had failed to show in my model that adenosine itself was protective when given as a pre-treatment without washout. I argued then, and still do, that such results would be irrelevant as an intervening washout period was essential in order to fully mimic preconditioning. More support for our hypothesis came the following year when Liu and Downey [9] demonstrated that the adenosine antagonist PD115,199 failed to block preconditioning-induced protection against cell necrosis in the rat. Although this group could produce protection against infarct size with an intracoronary infusion of an $A_1$-agonist (2-chloro-$N^6$-cyclopentyladenosine), they concluded that adenosine was not an initiator of preconditioning in the rat. Banerjee et al. [10] subsequently demonstrated that stimulation of $\alpha_1$-adrenergic receptors was primarily involved in the initiation of preconditioning in the rat.

Several years on, the preconditioning picture with regard to the initiation of preconditioning is clearer. We now know that several agonists are able to initiate protection and the relative contributions of each of the agonists will vary among species and models. The rat as a species is not dependent upon adenosine as an initiator but despite this it is clear that the phenomenon is effective in this species. The controversy still continues as to which of the three end-points of assessment are most relevant and whether the same mechanism of action exists for each of the different end-points. It is probably fair to say that infarct size as an end-point of assessment is the ‘gold standard’ and that the assessment of preconditioning-induced protection against contractile dysfunction is likely to represent a combination of protection against cell death and stunning. However, having said this, assessment of infarct size can be complicated by the choice of staining [11] and the degree of collateral flow.

In conclusion, 13 years after the first paper on preconditioning was published [12] and despite intense research activity, the end-effector of preconditioning-induced protection still eludes us. We can take small comfort from the fact that we have worked out how preconditioning is initiated and that, pharmacologically, we can mimic it, but it is clear that, as we enter the new Millennium, evolution still holds a few tricks up her sleeve!

**Fig. 1.** Effect of 8(p-sulphophenyl) theophylline (8-SPT) on preconditioning-induced protection of function. Post-ischaemic recovery of aortic flow, expressed as a percentage of the individual preischaemic values, after 20 min of ischaemia in the absence ($C_1$ and $P_1$) and presence ($C_2$ and $P_2$) of 8-SPT (10 $\mu$mol/l). Preconditioned hearts ($P_1$ and $P_2$) are represented by the dark-hatched columns and control hearts ($C_1$ and $C_2$) by the light hatched columns. Preconditioned hearts were subjected to 5 min if ischaemia and 5 min of reperfusion before the main period of ischaemia. Columns are means, bars=SEM, $n=6$/group.

**References**