Mast cell degranulation does not contribute to ischemic preconditioning in isolated rabbit hearts

Abstract Preconditioning the heart with a short period of ischemia makes it resistant to infarction from a subsequent ischemic insult. We have proposed that preconditioning is triggered by the release of endogenous substances including adenosine which activate protein kinase C through receptor-mediated cell signaling pathways. However, it has also been proposed that the initial brief ischemia may result in mast cell degranulation without significant myocardial damage, making it less likely that the toxic granule contents could be released to irreversibly damage vulnerable myocardial cells during the subsequent prolonged ischemia. To study the role of mast cells in ischemic preconditioning (PC) isolated rabbit hearts were subjected to 30 min of regional ischemia followed by 120 min of reperfusion. Infarct size was measured with triphenyltetrazolium chloride. In control hearts infarction was 31.9 ± 2.6 % of the risk zone. Preconditioning with 5 min of global ischemia and 10 min of reperfusion reduced infarct size to 5.6 ± 6.1 % (p < 0.01). When disodium cromoglycate (DSCG) (10 μM), a mast cell stabilizer, was infused shortly before the long ischemia it did protect the heart (12.8 ± 2.9 % infarction, p < 0.01 vs control) which supports the mast cell theory. However, a mast cell degranulating agent, compound 48/80 (24 mg/L), added to the perfusate prior to the 30 min ischemic period could not mimic PC (39.7 ± 5.6 % infarction). Mast cell granules are rich in histamine, and the latter was assayed in myocardium by immunoassay as a marker of intact granules. In homogenized left ventricle from normal rabbit hearts and those following a standard PC protocol of 5-min global ischemia/10-min reperfusion, histamine contents were 9.3 ± 1.4 and 8.9 ± 1.4 ng/g wet tissue, respectively. Compound 48/80 reduced histamine levels to 2.9 ± 0.6 ng/g (p < 0.05 vs control). Although baseline histamine contents were 10-fold higher in rats, PC also had no effect, but compound 48/80 reduced content by 91 %. Therefore, histamine tissue content and presumably mast cell granules were unaffected by a PC protocol which successfully protected ischemic myocardium, while pharmacological myocardial histamine depletion was not associated with protection. Hence, mast cells do not appear to be important in ischemic preconditioning. Although a mast cell stabilizer such as DSCG can protect ischemic myocardium, it may do so by one of its other properties, e.g., membrane stabilization.

Key words Compound 48/80 – disodium cromoglycate – histamine – mast cell degranulation
**Introduction**

Since the original description of ischemic preconditioning in dogs by Murry et al. (32), many investigators have confirmed that brief periods of ischemia before more prolonged ischemia can salvage jeopardized myocardium with resulting improvement of left ventricular function during the post-ischemic reperfusion phase (3, 33), smaller infarcts (24, 50), and reduction of arrhythmias (43). This salutary effect of ischemic preconditioning has been observed in hearts of many experimental animal species including rat (20, 26), rabbit (24, 50), pig (41), and dog (19, 32) as well as in human ventricular myocytes (15), ventricular muscle (59), and intact hearts (5, 6, 11, 17, 51). The role of brief ischemia in preconditioning is mimicked by 5-min infusions of ligands of protein kinase C (PKC)-coupled receptors including A1- (24, 50) and A2- (23) adenosine, α1-adrenergic (48, 52), B2-bradykinin (8, 54), M2-muscarinic (12, 36, 49, 58), AT1-angiotensin II (27), and ET1-endothelin (55) agonists. These data have suggested that PKC activation is critical to preconditioning. Indeed, studies with PKC activators and antagonists in rat (21, 31, 44) and rabbit (25, 28, 60) have clearly demonstrated PKC’s role in this phenomenon. However, data in larger animals [pigs (53) and dogs (37)] have not generally confirmed the importance of PKC in preconditioning. Reasons for this discrepancy are not yet clear, but inability to deliver adequate doses of PKC antagonists to these large animals without causing hypotension or true species differences may be involved.

PKC represents only part of the signal transduction pathway to the true effector of protection in preconditioning. Therefore, there has been much speculation as to what that effector may be. For example, some investigators have postulated that opening of K_ATP channels may be involved (9, 10), while others have suggested that stabilization of cytoskeletal proteins may minimize irreversible cell damage (2). Linden has proposed PKC-mediated mast cell degranulation as the key part of the preconditioning process (22). At least two studies in which a mast cell stabilizer was infused shortly before prolonged cardiac ischemia (35) or hypoxia (16) have demonstrated significantly less post-ischemic myocardial dysfunction. Additionally, mast cells are known to have many surface adenosine A3 receptors (22), stimulation of which activates PKC and leads to degranulation (39). Recently, A3 adenosine agonists have been documented to trigger cardioprotection (23). According to Linden's proposal adenosine released from the myocardial cell during brief preconditioning ischemia causes mast cells to degranulate, thus liberating several potentially toxic substances, including biogenic amines, prostaglandins, proteases, proteoglycans, leukotrienes, platelet-activating factor, cytokines (1, 42, 45, 56). These substances would be in contact with myocardial cells for only a few minutes before they are washed out because of the short duration of ischemia in the preconditioning phase. During the subsequent prolonged ischemia further release would not be possible since the mast cells had already been degranulated, thus sparing the tissue. In contrast, in the absence of preconditioning ischemia mast cells would be intact and the onset of prolonged ischemia would be associated with a release of destructive enzymes and toxins which would be in contact with the ischemic tissue for the duration of the ischemic period, thus contributing to the necrosis.

To critically evaluate the involvement of mast cells in preconditioning, the effect of both mast cell stabilizers and degranulating agents on infarct size in rabbits was examined and tissue from non-preconditioned and preconditioned hearts was assayed for a mast cell marker. The results reveal that mast cell stabilizers may indeed have some protective effect, but preconditioning’s protection does not appear to correlate with the integrity of mast cells.

**Methods**

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No 85-23, revised 1985).

**Surgical preparation of animals**

New Zealand White rabbits of either sex weighing from 1.2 to 2.4 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg). The trachea was intubated through a cervical incision. Mechanical ventilation was achieved with a positive-pressure respirator (MD Industries, Mobile, AL) using 100 % O2. A left thoracotomy was performed in the fourth intercostal space and the pericardium was opened to expose the heart. A 2-0 silk suture on a curved taper needle was passed through the myocardium and around a branch of the left coronary artery, and the ends were passed through a small vinyl tube to form a snare. The heart was rapidly excised and mounted on a Langendorff apparatus by the aortic root. The heart was perfused with Krebs-Henseleit bicarbonate buffer containing 118.5 mM NaCl, 1.2 mM MgSO4, 2.5 mM CaCl2, 24.8 mM NaHCO3, 1.3 mM KH2PO4, and 10 mM glucose. The perfusate was bubbled with a 95 % O2 – 5 % CO2 gas mixture and perfusate temperature was...