Pergamon

Biochemical Pharmacology, Vol. 49, No. 8, pp. 1023–1034, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(94)00467-6

COMMENTARY

ACUTE PHARMACOLOGIC PRECONDITIONING AS A NEW CONCEPT AND ALTERNATIVE APPROACH FOR PREVENTION OF SKELETAL MUSCLE ISCHEMIC NECROSIS

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Key words: skeletal muscle; ischemic injury; adenosine treatment

There are many clinical situations in which skeletal muscles are subjected to warm global ischemia. Some of the common examples are autogenous muscle transplantation for wound coverage or restoration of function, replantation of amputated limbs, post-traumatic compartment syndrome, application of vascular clamps or tourniquets in vascular and musculoskeletal reconstructive surgery, and embolism and thrombosis of major blood vessels. Human skeletal muscles are known to tolerate warm global ischemia for up to 2.5 hr with minimal risk of irreversible ischemic injury [1–6]. However, prolonged and/or repeated ischemic insults to skeletal muscles sometimes occur in these clinical situations as a result of unexpected operative or post-operative complications or delay in surgery. In some instances, although revascularization is established, various degrees of irreversible muscle injury (infarction) may still occur [2, 7–10]. Muscle infarction may require additional surgery or cause morbidity. Hyperkalemia, acidosis, myoglobinuria and renal failure can occur if muscle necrosis is extensive [2, 8, 11–13]. In recent years, intensive effort has been directed to studying the pathophysiology of ischemic injury in skeletal muscles with the goal of identifying effective pharmacologic agents for prevention or treatment of ischemic and or reperfusion injury. Furthermore, with the development of immunopharmacology, it is likely that donor tissue/organ rejection can be controlled with minimal side-effects in the future; thus, heterogenous muscle or limb transplantation for restoration of form and function may be a reality. An effective pharmacologic agent for augmentation of ischemic tolerance will permit procurement of donor muscles or limbs for transfer from great distances and performance of more complicated reconstructive operations requiring a longer period of warm global ischemia.

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† Abbreviations: I/R, ischemia/reperfusion; O_2^-, superoxide anion radical; OH·, hydroxyl radical; XD, xanthine dehydrogenase; XO, xanthine oxidase; cDNA, complementary deoxyribonucleic acid; mRNA, messenger RNA; SOD, superoxide dismutase, CAT, catalase; 8-SPT, 8-*p*-sulphophenyl theophylline, R-PIA, N^6-1-(phenyl-2*R*-isopropyl)-adenosine; DPCPX, 8-cyclopentyl-1,3-dipropyl xanthine; K_{ATP} channels, ATP-sensitive potassium channels; A_1 receptor, adenosine$_1$ receptor; PKC, protein kinase C; PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5-biphosphate; IP_3, inositol 1,4,5-triphosphate; and DAG, diacylglycerol.

PATHOGENESIS OF SKELETAL MUSCLE INFARCTION

The pathogenesis of skeletal muscle infarction in musculoskeletal and vascular reconstructive surgery is unclear. However, there is experimental evidence to indicate that when skeletal muscles are subjected to prolonged warm ischemia, injury occurs during sustained ischemia as well as reperfusion, a phenomenon known as I/R† injury [14]. The pathophysiology of ischemic injury and pathophysiology of reperfusion injury in skeletal muscles are different although they may be interrelated.

Ischemic injury

Little is known about the pathophysiology of ischemic injury in skeletal muscles. However, it has been observed in dog myocardium that long, sustained ischemia causes irreversible ATP depletion and excessive metabolite accumulation [15, 16]. These metabolites act as an osmotic load on the cell, causing cell swelling and damage of the sarcolemma and cytoskeletal membrane. Recent experimental evidence indicates that excessive high-energy phosphate depletion and osmotic load are also associated with ischemic injury in skeletal muscles in dogs [17].

Reperfusion injury

Reperfusion injury of the microvasculature and myocytes in ischemic skeletal muscles has been observed in rats [18–23], rabbits [24], dogs [25–28], and pigs [29–31]. It has also been observed that the extent of reperfusion injury is related to the duration of ischemia time [28, 30, 32] and oxygen content of the blood during reperfusion [33–35]. However, the pathogenic mechanism of reperfusion injury in skeletal muscle is unclear. The general consensus is that reperfusion injury in skeletal muscles is mediated by oxy-radicals (e.g. O_2^-, OH·) and hydrogen peroxides, involving phospholipid peroxidation as

indicated by the formation of hydroxy-conjugated dienes [27, 36].

STRATEGY IN PHARMACOLOGIC INTERVENTION OF I/R INJURY IN SKELETAL MUSCLES

Ideally, pharmacologic treatment for skeletal muscle I/R injury should aim at prevention/mitigation of ischemic as well as reperfusion injury. These areas of research are discussed briefly below.

Prevention of reperfusion injury

In the past decade, considerable attention has been focused on the prevention/attenuation of injury caused by reperfusion of ischemic skeletal muscles with the following approaches: (a) use of XO inhibitors or ATP-$MgCl_2$ for inhibition of O_2^- generation [21, 23, 26, 37]; (b) use of inhibitors, antagonists or adhesion molecule antibodies to prevent sequestration and adhesion of neutrophils on the endothelium of the microvasculature because neutrophils are known to occlude microvessels and produce oxy-radicals to cause reperfusion injury [38, 39]; (c) use of iron chelators to prevent $OH^\cdot$ formation [20, 40]; and (d) use of scavengers or anti-oxidants to prevent oxy-radical-mediated injury [23, 33].

There are potential problems or difficulties in these approaches for protection or attenuation of reperfusion injury. Using a radioenzymatic technique, we have demonstrated that XD and XO activities in human and pig skeletal muscles were minute (<0.05 mU/g wet weight), and allopurinol or oxypurinol were not effective in the mitigation of I/R injury (muscle infarction) in pig skeletal muscles [31]. More recently, a cDNA encoding human XD was cloned. Using this probe, little XD mRNA was detected in the human skeletal muscle [41]. All these observations are taken to indicate that the XD/XO enzymatic system is unlikely to be a major source of oxy-radicals in human skeletal muscles; therefore, XO inhibitors are unlikely to be effective drugs for the prevention/mitigation of skeletal muscle I/R injury. Furthermore, oxy-radical scavengers such as SOD and CAT have a short biological half-life, but reperfusion injury in skeletal muscles is known to occur up to 48 hr from the start of reperfusion. Therefore, continuous or repeated intravenous administration of drug is required. Conjugated SOD and CAT have long biological half-lives, but they are not cell permeable [42]. Deferoxamine, an iron chelator, has the potential to prevent $OH^\cdot$ formation and skeletal muscle ischemic injury, but it also has a short biological half-life and is cell impermeable when conjugated to polymers [43]. The efficacy of anti-oxidants (e.g. vitamin E) for the prevention of reperfusion injury in skeletal muscles has not been established. Last but not least, there is convincing evidence implicating neutrophils as a major source of oxy-radicals in skeletal muscle reperfusion injury in dogs [44] and pigs [31, 45]. If neutrophils are also a major source of oxy-radicals in human skeletal muscles, use of scavengers or anti-oxidants *per se* may not provide optimal protection against reperfusion injury. Specifically, neutrophils are known to adhere on the surface of endothelial cells and, because of cell-to-cell contact, these neutrophils release cytotoxic oxy-radicals and proteases directly onto the surface of the endothelium, causing cell injury. In addition, accumulated neutrophils may occlude microvessels, causing regional no-reflow.

In recent years, it has become increasingly evident that neutrophil recruitment and adherence on endothelial cells are dependent on the expression of multiple adhesion molecules on the cell surface of activated neutrophils (e.g. CD11b/CD18, L-selection) [46, 47]. Furthermore, there is experimental evidence to indicate that the complement system may play an important role in activation or up-regulation of neutrophil and endothelium adhesion molecules during reperfusion [47, 48]. It is too early to predict if selective inhibitors of the complement system or specific antibodies to adhesion molecules are feasible clinical treatment modalities for skeletal muscle reperfusion injury.

Prevention of ischemic injury

Our idea of augmentation of skeletal muscle ischemic tolerance as an alternative approach for the prevention of ischemic injury is derived from the phenomenon of ischemic preconditioning of myocardium for ischemic tolerance described by Murry *et al.* in 1986 [49]. Specifically, preconditioning of dog myocardium with four cycles of 5-min ischemia and 5-min reperfusion reduced the infarct size when the myocardium was subsequently subjected to 40 min of sustained warm ischemia. Since then, various laboratories have reported that only one cycle of ischemia and reperfusion is required for preconditioning of myocardium in dogs [50], rats [51], rabbits [52, 53], and pigs [54]. There is also clinical evidence to indicate that myocardial preconditioning can be induced in humans [55, 56]. More important, the protective effect of ischemic preconditioning has been demonstrated in human cardiomyocytes [57]. Recently, we obtained results demonstrating for the first time that the protective effect of ischemic preconditioning can also be induced in pig skeletal muscles, but the threshold for ischemic tolerance is much higher in the skeletal than in the cardiac muscle in pigs.* Specifically, at least three cycles of 10-min ischemia and 10-min reperfusion, a total of 60 min, were required to precondition pig latissimus dorsi muscles for augmentation of ischemic tolerance when latissimus dorsi muscles were subsequently subjected to 4 hr of warm global ischemia and 48 hr of reperfusion (Fig. 1). This protective effect of preconditioning against muscle infarction was confirmed in gracilis muscles in pigs (Fig. 2). However, this ischemic preconditioning was not effective when the muscle ischemia time was extended beyond 5 hr. At the present time, we seek to identify the mediator of ischemic preconditioning of skeletal muscles and plan to use the mediator to induce ischemic tolerance in skeletal muscles. This treatment modality will reduce the time required for preconditioning of skeletal muscles and may also be used for

* Pang CY, Zhong, A, Yang RZ, Xu N, Boyd B and Forrest CR, Manuscript submitted for publication.

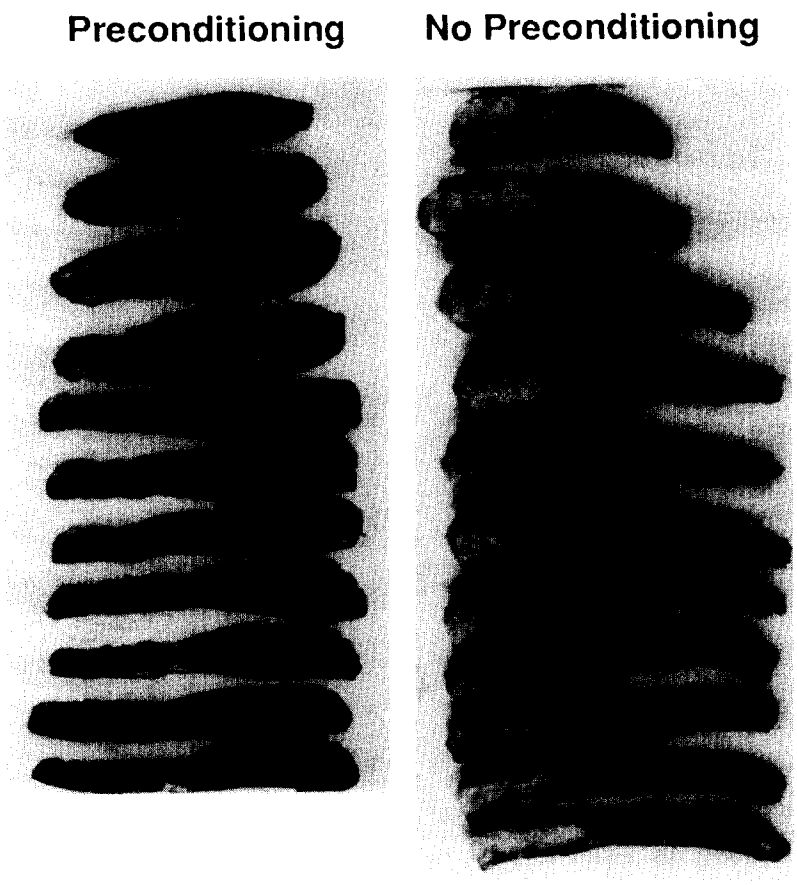

Fig. 1. Pattern of muscle infarction in the pig latissimus dorsi muscle subjected to 4 hr of warm global ischemia and 48 hr of reperfusion. Viable muscle was stained dark blue by nitroblue tetrazolium dye. Non-viable muscle (infarction) was stained red and is indicated by asterisks. Preconditioning reduced muscle infarction.

procurement of skeletal muscles for heterogenous muscle transplantation in the future.

MECHANISM OF ISCHEMIC PRECONDITIONING

Thus far, there is no publication on the mechanism of ischemic preconditioning in skeletal muscles. The proposed mechanisms of myocardial ischemic preconditioning summarized in Table 1 may provide insights into the mechanism of preconditioning in skeletal muscles. The validity of each of these proposed mechanisms has been discussed in detail by various investigators elsewhere [58–60]. Briefly, experimental evidence available thus far indicates that the protective effect of ischemic preconditioning is unlikely the result of increasing collateral blood flow [49, 50, 52], mitochondrial ATPase inhibitor protein activation [61–63], glycolytic flux [64], stunning [65, 66], decrease in oxy-radical generation or anti-oxidant defenses [67, 68], neutrophil-related mechanism [64], synthesis of stress protein [69], or increase in prostacyclin or nitric oxide synthesis [51, 70, 71]. On the other hand, consistent and convincing experimental evidence is available to indicate that adenosine is most likely the candidate of an endogenous mediator of myocardial ischemic preconditioning in several species of laboratory animals, and the adenosine action is mediated by A_1 receptors. The mediator and effector mechanisms of myocardial ischemic preconditioning are summarized below.

Mediator mechanism

The first indication of adenosine as a mediator of ischemic preconditioning came when Liu *et al.* [72] observed in rabbits that a non-selective adenosine receptor antagonist, 8-SPT or PD 115, 199, blocked the cardioprotective effect of preconditioning, and intracoronary infusion of adenosine or an A_1 receptor agonist, R-PIA, mimicked the cardioprotective effect of preconditioning. Since 8-SPT is cell impermeable, the adenosine receptors involved in mediating ischemic preconditioning must be cell-surface membrane receptors. An ensuing study from the same laboratory further demonstrated that an intravenous A_1 receptor agonist, R-PIA, or 2-chloro-N^6-cyclopentyladenosine (CCPA) also mimicked the cardioprotective effect of preconditioning, but that CGS 26180, an A_2 receptor agonist, failed to provide any cardioprotective effect in rabbit myocardium

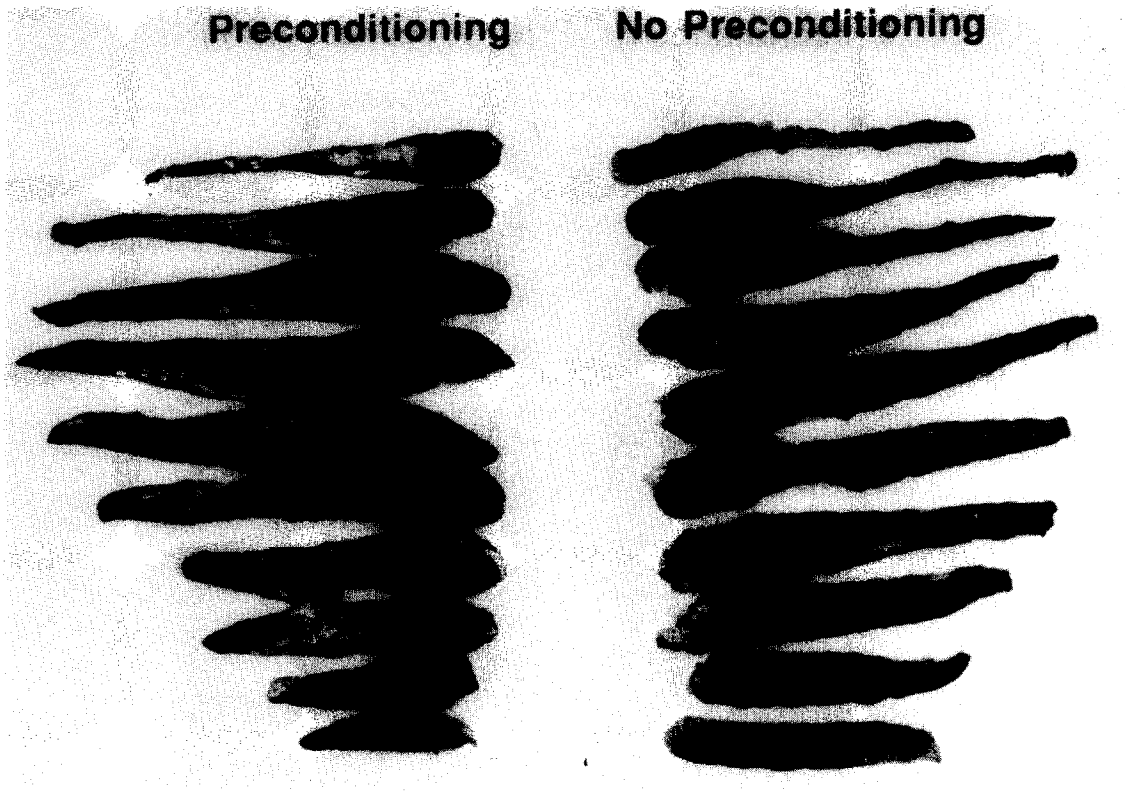

Fig. 2. Pattern of muscle infarction in the pig gracilis muscle subjected to 4 hr of warm global ischemia and 48 hr of reperfusion. Viable muscle was stained dark blue by nitroblue tetrazolium dye. Non-viable muscle (infarction) was stained red and is indicated by asterisks. Preconditioning reduced muscle infarction.

Table 1. Proposed mechanisms for ischemic preconditioning (IPC) in cardiac muscle

1.	Increase in collateral blood flow IPC induces an increase in collateral blood flow, thus maintaining an adequate blood supply to the ischemic zone.
2.	Mitochondrial ATPase IPC inhibits mitochondrial ATPase, thus reducing ATP hydrolysis and preserving intracellular pH.
3.	Glycolytic flux IPC induces an increase in glycolytic flux to provide ATP during ischemia, thus overcoming mitochondrial dysfunctioning.
4.	Stunning IPC induces reversible depression of contractile function, thus reducing energy demand during ischemia.
5.	Free radicals IPC produces free radicals, which induce stunning.
6.	Neutrophils IPC reduces neutrophil-related contractile dysfunction or infarction.
7.	Stress proteins IPC stimulates synthesis and release of stress proteins, which increase myocardial resistance to infarction.
8.	Prostanoids and nitric oxide IPC induces endothelial release of prostacyclin and/or nitric oxide, which provides cardioprotective effect against ischemia.
9.	Adenosine IPC causes accumulation of adenosine, which acts as an endogenous mediator of preconditioning through activation of adenosine$_1$ receptors, which, in turn, trigger intracellular biochemical changes.
10.	ATP-sensitive potassium (K_{ATP}) channels IPC causes a persistent or more rapidly recurring opening of K_{ATP} channels resulting in the shortening of cardiac action potential and the reduction of Ca^{2+} influx, thereby limiting contractile activity and ATP depletion.

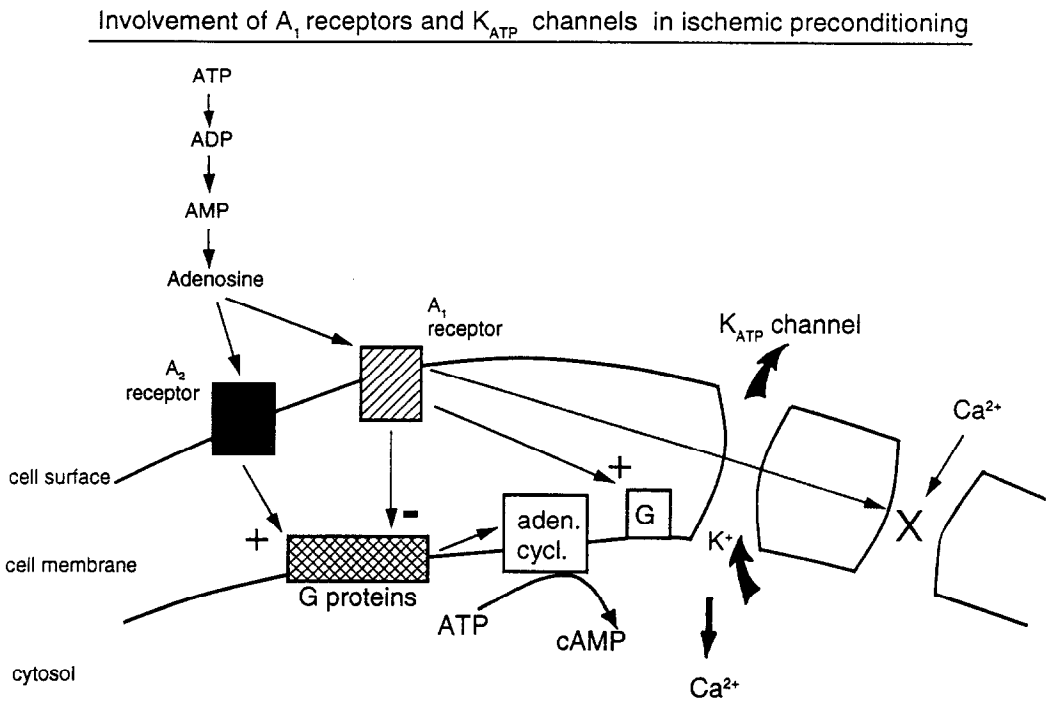

Fig. 3. Proposed ATP-sensitive potassium (K_{ATP}) channel effector mechanism for ischemic preconditioning. Brief cycles of ischemia and reperfusion result in stepwise catabolism of ATP to adenosine, which couples to A_1 receptors. Activated A_1 receptors couple to adenylate cyclase through an inhibitory G-protein, resulting in inhibition of conversion of ATP to cyclic AMP (cAMP). Activated A_1 receptors also couple to K_{ATP} channels through yet another G-protein, resulting in opening of K_{ATP} channels and increasing K^+ efflux and membrane hyperpolarization. Membrane hyperpolarization results in shortening the duration of action potential and opening time for L-type Ca^{2+} channels, thus reducing contractility, energy metabolism and accumulation of toxic metabolites. The end result is an increase in ischemic tolerance.

[73, 74]. In addition, this and other laboratories observed in rabbits that adenosine exerted its cardioprotective effect mainly during ischemia; thus, adenosine predominantly protects against ischemic injury rather than reperfusion injury [75–77]. It has also been confirmed in dogs [78–80] and pigs [81] that adenosine or an A_1 receptor agonist could mimic the cardioprotective effect of ischemic preconditioning.

Effector mechanism

A_1 receptors are known to couple to a variety of effector systems including adenyl cyclase [82], K_{ATP} channels [83], voltage-dependent Ca^{2+} channels [84], Na^+-Ca^{2+} exchange system [85], acetylcholine-sensitive potassium channels [86], and phospholipase A_2 and C systems [87]. At the present time, there are two major schools of thought regarding the effector mechanism for ischemic preconditioning.

A_1 receptor-K_{ATP} channel-linked effector mechanism. Murry *et al.* [16] demonstrated that ischemic preconditioning causes preservation of high-energy phosphates in dog myocardium. Subsequently, Kida *et al.* [88] observed that ischemic preconditioning conserves not only high-energy phosphates but also intracellular pH. To date, experimental evidence is accumulating to indicate that the protective effect of myocardial ischemic preconditioning involves an A_1 receptor-K_{ATP} channel-linked mechanism, which induces preservation of ATP and intracellular pH. For example, adenosine or CCPA (an A_1 receptor agonist) activates K_{ATP} channels via a G-protein in rat and guinea pig ventricular myocytes [89]. K_{ATP} channel antagonists block the cardioprotective effect of ischemic preconditioning in dogs [78, 90, 91], pigs [81], and rabbits [92]. K_{ATP} channel antagonists also block the cardioprotective effect of adenosine and A_1 receptor agonists in dogs [78–80], pigs [81], and rabbits [93]. Conversely, K_{ATP} channel activators mimic the cardioprotective effect of ischemic preconditioning in dogs [94, 95], and rabbits [96]. The proposed mechanism by which K_{ATP} channel activation causes preservation of high-energy phosphates is illustrated in Fig. 3. Specifically, K_{ATP} channel activation has been shown to shorten the duration of the action potential and antagonize membrane depolarization [97]. These effects would reduce the opening time of voltage-regulated (L-type) Ca^{2+} channels, thus reducing Ca^{2+} influx, muscle contractility and ATP catabolism. Indeed, it has been observed that activation of K_{ATP} channels caused shortening of the action potential and slowing

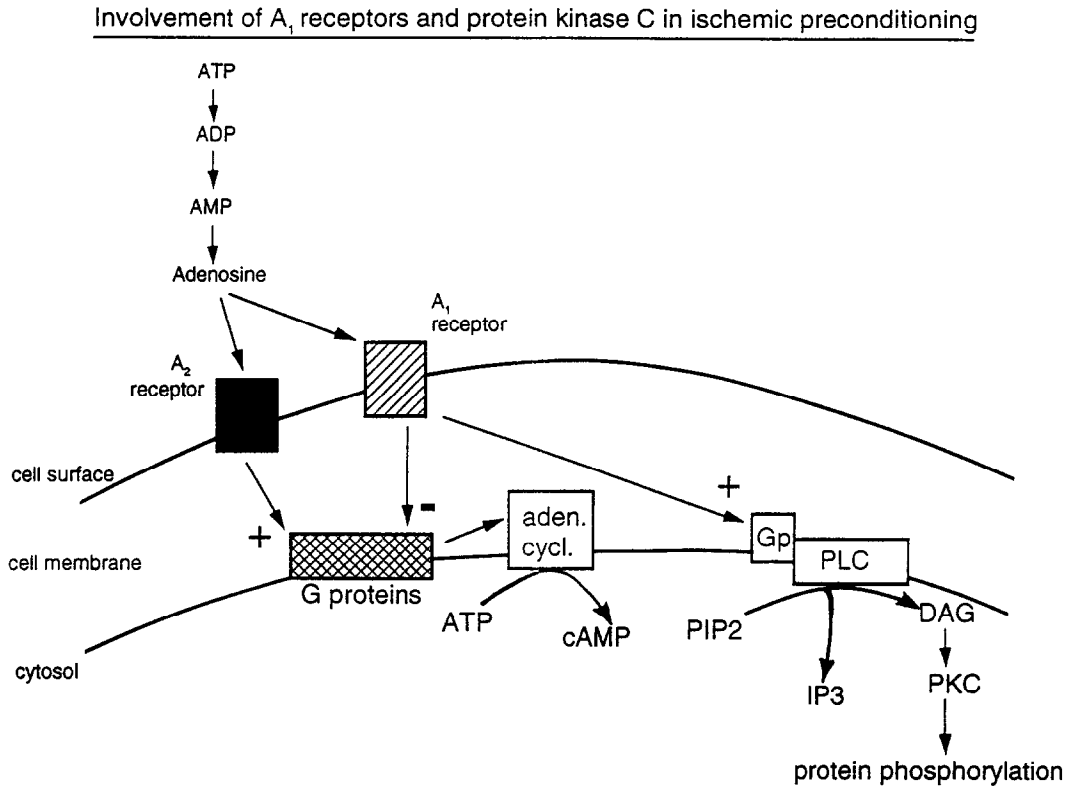

Fig. 4. Proposed protein kinase C (PKC) effector mechanism in ischemic preconditioning. PKC is activated through a cellular signaling pathway involving: (a) coupling of adenosine to A_1 receptors via pertussis toxin-sensitive protein (Gp); (b) activation of phospholipase C (PLC); (c) cleavage of phosphatidylinositol 4,5-biphosphate (PIP_2) by activated PLC to inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG); and (d) activation of PKC by DAG for protein phosphorylation.

of cellular catabolism, preserving ATP and reducing infarct size in guinea pig [98, 99] and dog [100, 101] myocardium. Furthermore, adenosine, which mimicked the cardioprotective effect of ischemic preconditioning, also lowered high-energy phosphate catabolism in dog myocardium [102].

It should be mentioned that the opinions on the involvement of K_{ATP} channels in ischemic preconditioning are not unanimous. There are reports indicating that K_{ATP} channel activation does not protect ischemic myocardium from infarction in dogs [103, 104] and rabbits [105], and a K_{ATP} channel antagonist did not block the cardioprotective effect of ischemic preconditioning in rabbit myocardium [105]. At the present time, it is unclear if these conflicting results could be attributed to differences in anesthetics, doses of K_{ATP} channel activators used, ischemic protocols, or laboratory technique [106, 107].

A_1 receptor-PKC-linked effector mechanism. There are several lines of evidence to indicate that the link of A_1 receptors and PKC is an effector mechanism of ischemic preconditioning in the rabbit myocardium. It has been observed that the PKC inhibitors staurosporine and polymyxin B block the cardioprotective effect in rabbits. Conversely, activation of PKC with 4β-phorbol 12-myristate 13-acetate or with 1-oleyl-2-acetyl glycerol mimic ischemic preconditioning [83]. As outlined in Fig. 4, these investigators hypothesized that the PKC effector mechanism involves coupling of A_1 receptors to PLC [108] via a pertussis toxin-sensitive G-protein [109]. The activated PLC cleaves PIP_2 to two second messengers, IP_3 and DAG. Activation of PKC by DAG [110–112] results in protein phosphorylation. A specific protein that is phosphorylated for protection against ischemic injury has yet to be identified.

There is probably a species difference in the mediator mechanism of ischemic preconditioning. There is evidence to indicate that ischemic preconditioning is mediated by α_1-adrenergic receptors instead of A_1 receptors in the rat [113, 114], and glybenclamide, a K_{ATP} channel antagonist, did not block ischemic preconditioning in rats [62, 115]. However, norepinephrine is also known to activate PKC. It is of interest to investigate if the effector mechanism in myocardial ischemic preconditioning in the rat may also involve activation of PKC.

EFFICACY OF ADENOSINE FOR AUGMENTATION OF SKELETAL MUSCLE ISCHEMIC TOLERANCE AND ITS CELLULAR MECHANISM OF ACTION

Experimental evidence is accumulating in our

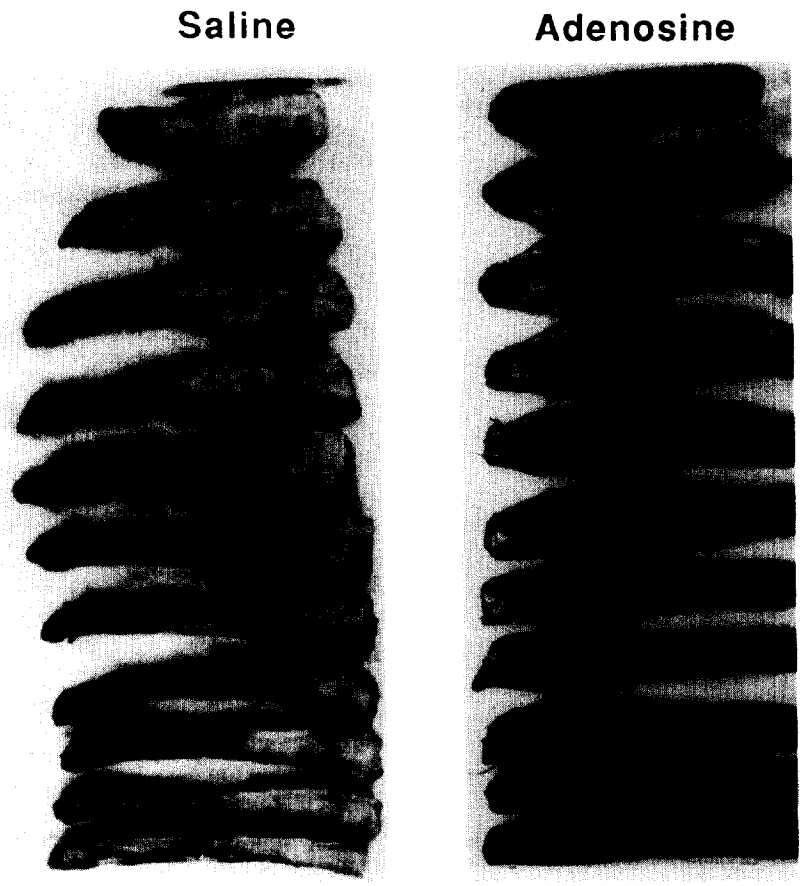

Fig. 5. Protective effect of adenosine against ischemic injury in the pig latissimus dorsi muscle subjected to 4 hr of warm global ischemia and 48 hr of reperfusion. Viable muscle was stained dark blue by nitroblue tetrazolium dye. Non-viable muscle (infarction) was stained red and is indicated by asterisks. Adenosine-reduced muscle infarction is compared with the saline-treated control.

laboratory to indicate that adenosine can also mimic ischemic preconditioning in skeletal muscles. Specifically, we observed that local intra-arterial infusion of adenosine at a dose of 0.5 mg/muscle flap over a period of 8–10 min significantly reduced the infarct size of pig latissimus dorsi and gracilis muscles when these muscles were subjected to 4 hr of warm global ischemia and 48 hr of reperfusion (Figs. 5 and 6). The infarct size of the latissimus dorsi and gracilis muscles was reduced by 50 and 63%, respectively [116]. This local administration of a low dose of adenosine did not cause any changes in systemic hemodynamics or local muscle blood flow. We also observed that 8-SPT, a non-selective adenosine receptor antagonist, and DPCPX, a selective A_1 receptor antagonist, blocked the protective effect of ischemic preconditioning and adenosine.* The mechanism of action of adenosine in the augmentation of ischemic tolerance in skeletal muscles is not known and is under investigation in our laboratory. However, we have demonstrated that ischemic preconditioning in pig skeletal muscles was associated with lower high-energy phosphate demand and lactate accumulation during sustained warm global ischemia.†

PHARMACOLOGIC PRECONDITIONING AS A POTENTIAL TREATMENT MODALITY FOR AUGMENTATION OF SKELETAL MUSCLE ISCHEMIC TOLERANCE.

There are several reasons that lead us to speculate that local intra-arterial adenosine infusion is a potential treatment modality for augmentation of skeletal muscle ischemic tolerance in vascular and musculoskeletal reconstructive surgery: (a) the treatment time is short because only 10 min of local intra-arterial infusion of adenosine is required; (b) a low dose of adenosine is required; (c) local infusion of adenosine will not affect systemic hemodynamics

* Pang CY, Zhong AG, Yang RZ and Forrest CR, Role of adenosine$_1$ (A_1) receptors in acute ischemic preconditioning against skeletal muscle infarction. *Proceedings XIIth International Congress of Pharmacology* **12**: P1-11-006, 1994.

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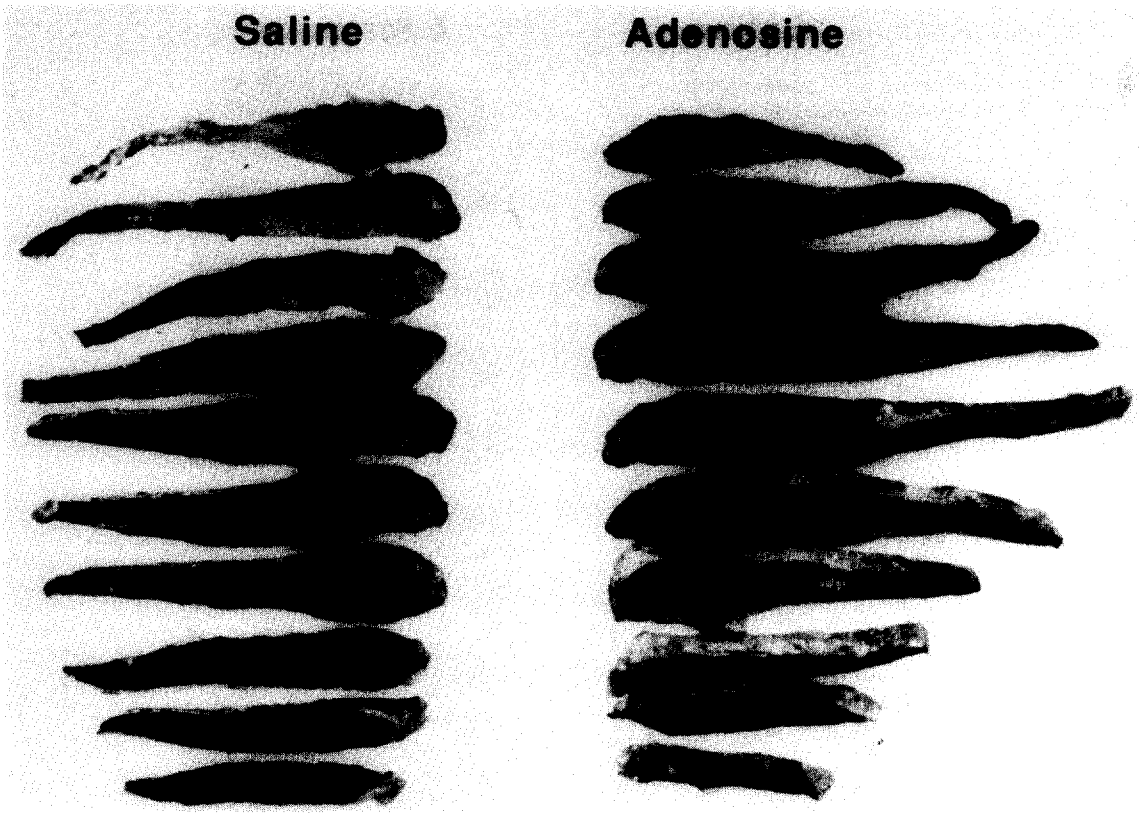

Fig. 6. Protective effect of adenosine against ischemic injury in the pig gracilis muscle subjected to 4 hr of warm global ischemia and 48 hr of reperfusion. Viable muscle was stained dark blue by nitroblue tetrazolium dye. Non-viable muscle (infarction) was stained red and is indicated by asterisks. Adenosine-reduced muscle infarction is compared with the saline-treated control.

because the biological half-life of adenosine is a matter of seconds [117]; (d) adenosine is not cytotoxic because it is an endogenous ATP catabolite; (e) optimal protection from I/R injury may be achieved by prevention of ischemic injury with adenosine and prevention of reperfusion injury with an anti-oxidant or scavenger; and (f) this treatment modality against I/R injury can be utilized for procurement of muscles and limbs for transplantation.

FUTURE STUDIES

There are several important areas of research that need to be addressed in future studies. The maximum ischemic time and the optimal dose of adenosine for protection of skeletal muscle from ischemic injury have yet to be determined. Furthermore, the potential combined therapeutic effect of adenosine with a scavenger, an antioxidant or an adenosine regulation agent [118, 119] on skeletal muscle ischemic tolerance is an important area of research, which may optimize protection against I/R injury. Last but not least, the efficacy and mechanism of action of adenosine for augmentation of human skeletal muscle ischemic tolerance have to be documented. To this end, we propose to use a cell culture system to study the cellular mechanism of adenosine in augmentation of human skeletal muscle cells ischemic tolerance. Specifically, the technique for long-term culture of human skeletal muscle cells has been published [120, 121], and the preliminary technique for ischemic preconditioning of cultured human cardiomyocytes has been reported recently [57]. These techniques are being modified for the study of cellular mechanism and protective effect of adenosine against ischemic injury in cultured human skeletal muscle cells.

SUMMARY

The phenomenon of ischemic preconditioning for augmentation of ischemic tolerance has been well documented in the myocardium of common laboratory animals and human cardiomyocytes. The cellular mechanism of ischemic preconditioning is unclear, but adenosine is most likely the mediator in the rabbit, dog, pig and human. We have demonstrated recently that the protective effect of ischemic preconditioning and adenosine against ischemic injury can also be induced in pig skeletal muscles [116]. We speculate that adenosine is a potential treatment modality for prevention of skeletal muscle ischemic injury in vascular and musculoskeletal reconstructive surgery and in muscle

and limb procurement for transplantation in the future. It is hoped that this review will stimulate workers at other laboratories to join the adventure in exploring the cellular mechanism and clinical application of adenosine for augmentation of skeletal muscle ischemic tolerance.

Acknowledgements—This research project was supported by the Medical Research Council of Canada (MA-12148) and the AO/ASIF Foundation. Christopher Forrest, M.D., is an MRC Scholar. The authors would like to thank Ms. Sonja Nainar for typing this manuscript.

REFERENCES

1. Tountas CP and Bergman RA, Tourniquet ischemia: Ultrastructural and histochemical observations of ischemic human muscle and of monkey muscle and nerve. *J Hand Surg [Am]* **2**: 31–37, 1977.
2. Blaipdell FW, Steel M and Allen RE, Management of acute lower extremity arterial ischemia due to embolism and thrombosis. *Surgery* **84**: 822–834, 1978.
3. Larsson J and Hultman E, The effect of long-term arterial occlusion on energy metabolism of the human quadriceps muscle. *Scand J Clin Lab Invest* **39**: 252–264, 1979.
4. Sjostrom M, Friden J and Eklof B, Human skeletal muscle metabolism and morphology after temporary incomplete ischemia. *Eur J Clin Invest* **12**: 69–79, 1982.
5. Rutherford RJ, Nutrient bed protection during lower extremity arterial reconstruction. *J Vasc Surg* **5**: 529–554, 1987.
6. Eckert P and Schnackerz K, Ischemic tolerance of human skeletal muscle. *Ann Plast Surg* **26**: 77–84, 1991.
7. Matsen FA and Krugmire RB, Compartment syndrome. *Surg Gynecol Obstet* **147**: 943–949, 1978.
8. Haimovici H, Metabolic complications of acute arterial occlusions. *J Cardiovasc Surg (Torino)* **20**: 349–357, 1979.
9. Hollier LH, Principles and techniques of surgical treatment of occlusive arterial disease of the lower extremities. In: *Clinical Vascular Disease* (Ed. Spittell JA Jr), Cardiovascular Clinics, Vol. **13**: pp. 37–48. F. A. Davis Co., Philadelphia, 1983.
10. Tawes RL Jr, Harris EJ, Brown WH, Shoor PM, Zimmerman JJ, Sydorak GR, Beare JP, Scribner RG and Fogarty TJ, Arterial thromboembolism. A 20-year perspective. *Arch Surg* **120**: 595–599, 1985.
11. Haimovici HJ, Muscular, renal and metabolic complication of acute arterial occlusions: Myonephropathic–metabolic syndrome. *Surgery* **85**: 461–468, 1979.
12. McCarron DA, Elliot WC and Rose JS, Severe mixed metabolic acidosis secondary to rhabdomyolysis. *Am J Med* **67**: 905–908, 1979.
13. Rakowski TA and Cerasaro TS, Myoglobinuria. *Am Fam Physician* **20**: 129–134, 1979.
14. Walker PM, Pathophysiology of acute arterial occlusion. *Can J Surg* **29**: 340–342, 1986.
15. Reimer KA, Jennings RB and Hill ML, Total ischemia in dog hearts *in vitro*. 2. High energy phosphate depletion and associated defects in energy metabolism, cell volume regulation and sarcolemmal integrity. *Circ Res* **49**: 901–911, 1981.
16. Murry CE, Richard VJ, Reimer KA and Jennings RB, Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode. *Circ Res* **66**: 913–931, 1990.
17. Rubin BB, Liauw S, Tittley J, Romaschin AD and Walker PM, Prolonged adenine nucleotide resynthesis and reperfusion injury in post-ischemic skeletal muscle. *Am J Physiol* **262**: H1538–H1547, 1992.
18. Strock PE and Majno GM, Microvascular changes in acutely ischemic rat muscle. *Surg Gynecol Obstet* **129**: 1213–1224, 1969.
19. Suval WD, Hobson RW, Mauricio PB, Ritter AB and Duran WN, Assessment of ischemia reperfusion injury in skeletal muscle by macromolecular clearance. *J Surg Res* **42**: 550–559, 1987.
20. Smith JK, Carden DL, Grisham MB, Granger DN and Korthuis RJ, Role of iron in post-ischemic microvascular injury. *Am J Physiol* **256**: H1472–H1477, 1989.
21. Smith JK, Carden DL and Korthuis RJ, Role of xanthine oxidase in post-ischemic microvascular injury in skeletal muscle. *Am J Physiol* **257**: H1782–H1789, 1989.
22. Sexton WL, Korthuis RJ and Laughlin MH, Ischemia–reperfusion injury in isolated rat hindquarters. *J Appl Physiol* **68**: 387–392, 1990.
23. McCutchan HJ, Schwappach JR, Enquist EG, Walden DL, Terada LS, Reiss OK, Jeff JA and Repine JE, Xanthine oxidase-derived H_2O_2 contributes to reperfusion injury of ischemic skeletal muscle. *Am J Physiol* **258**: H1415–1419, 1990.
24. Hickey MJ, Hurley JV, Angel MF and O'Brien B McC, The response of the rabbit rectus femoris muscle to ischemia and reperfusion. *J Surg Res* **53**: 369–377, 1992.
25. Diana JN and Laughlin H, Effect of ischemia on capillary pressure and equivalent pore radius in capillaries of the isolated dog hind limb. *Circ Res* **35**: 77–100, 1974.
26. Korthuis RJ, Granger DN, Townsley MG and Taylor AE, The role of oxygen-derived free radicals in ischemia-induced increase in canine muscle vascular permeability. *Circ Res* **57**: 599–609, 1986.
27. Harris K, Walker PM, Mickle DAG, Harding R, Gatley R, Wilson GJ, Kuzon W, McKee N and Romaschin AD, Metabolic response of skeletal muscle to ischemia. *Am J Physiol* **250**: 213–220, 1986.
28. Blebea J, Kerr JC, Skumko JZ, Feinberg KN and Hobson RW, Quantitative histochemical evaluation of skeletal muscle ischemic and reperfusion injury. *J Surg Res* **43**: 311–321, 1987.
29. Lee C, Kerrigan CL and Tellado JM, Altered neutrophil function following reperfusion of an ischemic myocutaneous flap. *Plast Reconstr Surg* **89**: 916–923, 1992.
30. Morris FS, Pang CY, Zhong A, Boyd B and Forrest CR, Assessment of ischemia-induced reperfusion injury in the pig latissimus dorsi myocutaneous flap model. *Plast Reconstr Surg* **92**: 1162–1172, 1993.
31. Dorion D, Zhong A, Pang CY, Chiu C, Boyd B and Forrest CR, Role of xanthine oxidase in reperfusion injury of ischemic skeletal muscles in the pig and human. *J Appl Physiol* **75**: 246–255, 1993.
32. Labbe R, Lindsay T and Walker PM, The extent and distribution of skeletal muscle necrosis after graded periods of complete ischemia. *J Vasc Surg* **6**: 152–157, 1987.
33. Walker PM, Lindsay TF, Labbe R, Mickle DAG and Romaschin AD, Salvage of skeletal muscle with free radical scavengers. *J Vasc Surg* **5**: 68–75, 1987.
34. Wright JG, Fox D, Kerr JC, Valeri CR and Robson RW, Rate of reperfusion blood flow modulates reperfusion injury in skeletal muscle. *J Surg Res* **44**: 754–763, 1988.
35. Korthuis RJ, Smith KJ and Carden DL, Hypoxic reperfusion attenuates post-ischemic microvascular injury. *Am J Physiol* **256**: H315–H319, 1989.
36. Lindsay T and Walker PM, Measurement of hydroxy-

conjugated dienes after ischemia reperfusion in canine skeletal muscle. *Am J Physiol* **254**: H578–H583, 1988.
37. Korthuis RJ, Grisham MB, Zimmerman BJ, Granger DN and Taylor AE, Vascular injury in dogs during ischemia–reperfusion: Improvement with ATP-$MgCl_2$ pretreatment. *Am J Physiol* **254**: H702–H708, 1988.
38. Carden DL, Smith JK and Korthuis RJ, Neutrophil-mediated microvascular dysfunction in post-ischemic canine skeletal muscle: Role of granulocyte adherence. *Circ Res* **66**: 1436–1444, 1990.
39. Carden DL, Smith JK and Korthuis RJ, Oxidant-mediated, CD18-dependent microvascular dysfunction induced by complement-activated granulocytes. *Am J Physiol* **260**: 1144–1152, 1991.
40. Morris SF, Pang CY, Lofchy NM, Davidson G, Lindsay WK, Zuker RM and Boyd B, Deferoxamine attenuates ischemia reperfusion injury in the skin and muscle of myocutaneous flaps in the pig. *Plast Reconstr Surg* **92**: 120–132, 1993.
41. Wright RM, Vaitaitis GM, Wilson CM, Repine TB, Terada LS and Repine JE, cDNA cloning, characterization, and tissue-specific expression of human xanthine dehydrogenase/xanthine oxidase. *Proc Natl Acad Sci USA* **90**: 10690–10694, 1993.
42. Downey JM, Free radicals and their involvement during long-term myocadial ischemia and reperfusion. *Annu Rev Physiol* **52**: 487–504, 1990.
43. Hallaway PE, Eaton JW, Panter S and Hedlund BE, Modulation of deferoxamine toxicity and clearance by covalent attachment to biological compatible polymers. *Proc Natl Acad Sci USA* **86**: 10108–10112, 1989.
44. Korthuis RJ, Grisham MB and Granger DN, Leukocyte depletion attenuates vascular injury in post-ischemic skeletal muscle. *Am J Physiol* **254**: H823–H827, 1988.
45. Lee C, Kerrigan CL and Picard-Ami LA, Cyclophosphamide-induced neutropenia: effect on post-ischemic skin flap survival. *Plast Reconstr Surg* **89**: 1092–1097, 1992.
46. Zimmerman BJ, Holt JW, Paulson JC, Anderson DC, Miyasaka M, Tamatani T, Todd RF III, Rusche JR and Granger DN, Molecular determinants of lipid mediator-induced leukocyte adherence and emigration in rat mesenteric venules. *Am J Physiol* **266**: H847–H853, 1994.
47. Homeistar JW and Lucchesi BR, Complement activation and inhibition in myocardial ischemia and reperfusion injury. *Annu Rev Pharmacol Toxicol* **34**: 17–40, 1994.
48. Rubin BB, Smith A, Liauw S, Isenman D, Romaschin AD and Walker PM, Complement activation and white cell sequestration in post-ischemic skeletal muscle. *Am J Physiol* **259**: H525–H531, 1990.
49. Murry CE, Jennings RB and Reimer KA, Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. *Circulation* **74**: 1124–1136, 1986.
50. Li GC, Vasquez BS, Gallagher KP and Lucchesi BR, Myocardial protection with preconditioning. *Circulation* **82**: 609–619, 1990.
51. Li Y and Kloner RA, Cardioprotective effects of ischemic "preconditioning" are not mediated by prostanoids. *Cardiovasc Res* **26**: 226–231, 1992.
52. Cohen MV, Liu GS and Downey JM, Preconditioning causes improved wall motion as well as smaller infarcts after transient coronary occlusion in rabbits. *Circulation* **84**: 341–349, 1991.
53. Cohen MV, Yang XM and Downey JM, Conscious rabbits become tolerant to multiple episodes of ischemic preconditioning. *Circ Res* **74**: 998–1004, 1994.
54. Martin BJ, McClanahan TB, Hamilton HW and Gallagher KP, PD 126,280 [endo(s) norbornyl-adenosine], a highly selective adenosine A_1-receptor agonist, reduces infarct size in swine. *Circulation* **88**: 1–430, 1993.
55. Cribier A, Korsatz L, Koning R, Rath P, Gamra H, Stix G, Merchant S, Chan C and Letac B, Improved myocardial ischemic response and enhanced collateral blood circulation with long repetitive coronary occlusion during angioplasty: A prospective study. *J Am Coll Cardiol* **20**: 578–586, 1992.
56. Deutsch E, Berger M, Kussmaul WG, Hirshfeld JW Jr, Herrmann HC and Laskey WR, Adaptation to ischemia during percutaneous transluminal coronary angioplasty: Clinical, hemodynamic and metabolic features. *Circulation* **82**: 2044–2051, 1990.
57. Ikonomidis JS, Tumiati LC, Mickle DAG and Weisel RD, Preconditioning protects human cardiac myocytes from ischemic injury. *Circulation* **88**: 1–570, 1993.
58. Reimer KA and Jennings RB, Preconditioning: Definitions, proposed mechanisms and implications for myocardial protection in ischemia and reperfusion. In: *Myocardial Protection: The Pathophysiology of Reperfusion and Reperfusion Injury* (Eds. Yellon DM and Jennings RB), pp. 165–183. Raven Press, New York, 1992.
59. Walker DM and Yellon DM, Ischemic preconditioning: From mechanisms to exploitation. *Cardiovasc Res* **26**: 734–739, 1992.
60. Lawson CS and Downey JM, Preconditioning: State of the art myocardial protection. *Cardiovasc Res* **27**: 542–550, 1993.
61. Rouslin W, Broge CW and Grupp IL, ATP depletion and mitochondrial functional loss during ischemia in slow and fast heart-rate hearts. *Am J Physiol* **259**: H1759–H1766, 1990.
62. Liu Y and Downey JM, Ischemic preconditioning protects against infarction in the rat heart. *Am J Physiol* **263**: H1107–H1112, 1992.
63. Yellon DM, Alkhulaifi AM, Browne EE and Pugsley WB, Ischaemic preconditioning limits infarct size in the rat heart. *Cardiovasc Res* **26**: 983–987, 1992.
64. Liu SL, Stanley AWH and Downey JM, Ischemic preconditioning is not dependent on neutrophils or glycolytic substrate at reperfusion in rabbit heart. *Cardiovasc Res* **26**: 1195–1198, 1992.
65. Miura T, Goto M, Urabe K, Endoh A, Shimamoto K and Limura O, Does myocardial stunning contribute to infarct size limitation by ischemic preconditioning? *Circulation* **84**: 2504–2512, 1991.
66. Matsuda M, Catena TG, Vander Heide RS, Jennings RB and Reimer KA, Cardiac protection by ischaemic preconditioning is not mediated by myocardial stunning. *Cardiovasc Res* **27**: 585–592, 1993.
67. Iwamoto T, Miura T, Adachi T, Noto T, Ogawa T, Tsuchida A and Iimura O, Myocardial infarct size-limiting effect of ischemic preconditioning was not attenuated by oxygen free-radical scavengers in the rabbit. *Circulation* **83**: 1015–1022, 1991.
68. Turrens JF, Thornton J, Barnard ML, Snyder S, Liu G and Downey JM, Protection from reperfusion injury by preconditioning hearts does not involve increased antioxidant defenses. *Am J Physiol* **262**: H585–H589, 1992.
69. Thornton J, Striplin S, Liu GS, Swafford A, Stanley AW, Van Winkle DM and Downey JM, Inhibition of protein synthesis does not block myocardial protection afforded by preconditioning. *Am J Physiol* **259**: H1822–H1825, 1990.
70. Patel V, Woolfson RG, Singh KJ, Neild GH and Yellon DM, Ischaemic preconditioning is not prevented by inhibition of endothelium-derived nitric oxide. *J Mol Cell Cardiol* **24** (Suppl I): S152, 1992.
71. Liu GS, Stanley AW and Downey JM, Cyclooxygenase products are not involved in the protection against

myocardial infarction afforded by preconditioning in rabbit. Cyclooxygenase pathway's involvement in preconditioning. *Am J Cardiovasc Pathol* **4**: 157–164, 1992.
72. Liu GS, Thornton J, Van Winkle DM, Stanley AWH, Olsson RA and Downey JM, Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. *Circulation* **84**: 350–356, 1991.
73. Thornton JD, Liu GS, Olsson RA and Downey JM, Intravenous pretreatment with A_1-selective adenosine analogues protects the heart against infarction. *Circulation* **85**: 659–665, 1992.
74. Tsuchida A, Liu GS, Wilborn WH and Downey JM, Pretreatment with the adenosine A_1 selective agonist, 2-chloro-N6-cyclopentyladenosine (CCPA), causes a sustained limitation of infarct size in rabbits. *Cardiavasc Res* **27**: 652–656, 1993.
75. Toombs CF, McGee DS, Johnston WE and Vinten-Johansen JV, Myocardial protective effects of adenosine: Infarct size reduction with pretreatment and continued receptor stimulation during ischemia. *Circulation* **86**: 986–994, 1992.
76. Thornton JD, Thornton CS and Downey JM, Effect of adenosine receptor blockade: Preventing protective preconditioning depends on time of initiation. *Am J Physiol* **265**: H504–H508, 1993.
77. Zhao Z-Q, Nakanishi K, McGee DS, Tan P and Vinten-Johansen J, A_1 receptor mediated myocardial infarct size reduction by endogenous adenosine is exerted primarily during ischaemia. *Cardiovasc Res* **28**: 270–279, 1994.
78. Grover GJ, Sleph PG and Dzwonczyk BB, Role of myocardial ATP-sensitive potassium channels in mediating preconditioning in the dog heart and their possible interaction with adenosine A_1-receptors. *Circulation* **86**: 1310–1316, 1992.
79. Yao Z and Gross GJ, A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs: Efficacy, time course, and role of K_{ATP} channels. *Circulation* **89**: 1229–1236, 1994.
80. Auchampach JA and Gross GJ, Adenosine A_1 receptors, K_{ATP} channels, and ischemic preconditioning in dogs. *Am J Physiol* **264**: H1327–H1336, 1993.
81. Van Winkle DM, Chien GL, Wolff RA, Soifer BE, Kuzume K and Davis RF, Cardioprotection provided by adenosine receptor activation is abolished by blockade of the K_{ATP} channel. *Am J Physiol* **266**: H829–H839, 1994.
82. Romano FD, MacDonald SG and Dobson JG, Adenosine receptor coupling to adenylate cyclase of rat ventricular myocyte membranes. *Am J Physiol* **257**: H1088–H1095, 1989.
83. Ytrehus K, Liu Y and Downey JM, Preconditioning rabbit heart by protein kinase C activation. *Am J Physiol* **266**: H1145–H1152, 1994.
84. MacDonald RL, Skerritt JH and Werz MA, Adenosine agonists reduce voltage-dependent calcium conductance of mouse sensory neurones in cell culture. *J Physiol (Lond)* **370**: 75–90, 1986.
85. Brechler V, Pavoine C, Lotersztajn S, Garbarz E and Pecker F, Activation of Na^+/Ca^{2+} exchange by adenosine in ewe heart sarcolemma is mediated by a pertussis toxin-sensitive G protein. *J Biol Chem* **265**: 16851–16855, 1990.
86. Kurachi Y, Nakajima T and Sugimoto T, On the mechanism of activation of muscarinic K^+ channels by adenosine in isolated atrial cells: Involvement of GTP-binding proteins. *Pflugers Arch* **407**: 264–274, 1986.
87. Ossar RA and Pearson JD, Cardiovascular purinoceptors. *Physiol Rev* **70**: 761–845, 1990.
88. Kida M, Fujiwara H, Ishida M, Kawai C, Ohura M, Miura I and Yabunchi Y, Ischemic preconditioning preserves creatine phosphate and intracellular pH. *Circ Res* **84**: 2595–2603, 1991.
89. Kirsch GE, Codina J, Birnbaumer L and Brown AM, Coupling of ATP-sensitive K^+ channels to A_1 receptors by G proteins in rat ventricular myocytes. *Am J Physiol* **259**: H820–H826, 1990.
90. Auchampach JA, Grover GJ and Gross GJ, Blockade of ischaemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. *Cardiovasc Res* **26**: 1054–1062, 1992.
91. Gross GJ and Auchampach JA, Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circ Res* **70**: 223–233, 1992.
92. Toombs CF, Moore TL and Shebuski RJ, Limitation of infarct size in the rabbit by ischemic preconditioning is reversible with glibenclamide. *Cardiovasc Res* **27**: 617–622, 1993.
93. Toombs CF, McGee DS, Johnston WE and Vinten-Johansen J, Protection from ischaemic-reperfusion injury with adenosine pretreatment is reversed by inhibition of ATP sensitive potassium channels. *Cardiovasc Res* **27**: 623–629, 1993.
94. Grover GJ, Sleph PG and Dzwonczyk S, Pharmacologic profile of cromaketine in the treatment of myocardial ischemia in isolated rat hearts and anesthetized dogs. *J Cardiovasc Pharmacol* **16**: 853–864, 1990.
95. Yao Z and Gross GJ, Effects of the K_{ATP} channel opener bimakalim on coronary blood flow, monophasic action potential duration, and infarct size in dogs. *Circulation* **89**: 1769–1775, 1994.
96. Toombs CF, Norman NR, Groppi VE, Lee KS, Gadwood RC and Shebuski RJ, Limitation of myocardial injury with the potassium channel opener cromakalim and the nonvasoactive analog U-89,232: Vascular vs. cardiac actions *in vitro* and *in vivo*. *J Pharmacol Exp Ther* **263**: 1261–1268, 1992.
97. Noma A, ATP-regulated K^+ channels in cardiac muscle. *Nature* **305**: 147–148, 1983.
98. Cole WC, McPherson CD and Sontag D, ATP-regulated K^+ channels protect the myocardium against ischemia–reperfusion damage. *Circ Res* **69**: 571–581, 1991.
99. McPherson CD, Pierce GN and Cole WC, Ischemic cardioprotection by ATP-sensitive K^+ channels involves high energy phosphate preservation. *Am J Physiol* **265**: H1809–H1818, 1993.
100. D'Alonzo AJ, Dabenzio RB, Parham CS and Grover GJ, Effects of intracoronary cromakalim on post-ischemic contractile function and action potential duration. *Cardiovasc Res* **26**: 1046–1053, 1992.
101. Yao Z, Cavero I and Gross GJ, Activation of cardiac K_{ATP} channels: An endogenous protective mechanism during repetitive ischemia. *Am J Physiol* **264**: H495–H504, 1993.
102. Vander Heide RS, Reimer KA and Jennings RB, Adenosine slows ischaemic metabolism in canine myocardium *in vitro*: Relationship to ischaemic preconditioning. *Cardiovasc Res* **27**: 669–673, 1993.
103. Kitzen JM, McCallum JD, Harvey C, Morin ME, Oshiro GT and Colatsky TJ, Potassium channel activators cromakalim and celikalim (WAY-120,491) fail to decrease myocardial infarct size in the anesthetized canine. *Pharmacology* **45**: 71–82, 1992.
104. Smallwood JK, Schelm JA, Bemis KG and Simpson PJ, Effect of activation of ATP-dependent potassium channels with (−)-pinacidil and (−)-3-pyridyl pinacidil on infarct size in a canine model of ischemia–reperfusion injury. *J Cardiovasc Pharmacol* **22**: 731–743, 1993.
105. Thornton JD, Thornton CS, Stering DL and Downey

JM, Blockade of ATP-sensitive potassium channels increases infarct size but does not prevent preconditioning in rabbit hearts. *Circ Res* **72**: 44–49, 1993.
106. Downey JM, An explanation for the reported observation that ATP dependent potassium channel openers mimic preconditioning. *Cardiovasc Res* **27**: 1565, 1993.
107. Grover GJ, An explanation for the reported observation that ATP dependent potassium channel openers fail to mimic preconditioning. *Cardiovasc Res* **27**: 1564, 1993.
108. Kohl C, Linck B, Schmitz W, Scholz H, Scholz J and Tóth M, Effects of carbachol and (−)-N^6-phenylisopropyladenosine on myocardial inositol phosphate content and force of contraction. *Br J Pharmacol* **101**: 829–834, 1990.
109. Thornton JD, Liu GS and Downey JM, Pretreatment with pertussis toxin blocks the protective effects of preconditioning. Evidence for a G-protein mechanism. *J Mol Cell Cardiol* **25**: 311–320, 1993.
110. Yuan S, Sunahara F and Sen A, Tumor-promoting phorbol esters inhibit cardiac functions and induce redistribution protein kinase C in perfused beating rat heart. *Circ Res* **61**: 372–378, 1987.
111. Kishimoto A, Takai Y, Mori T, Kikkawa U and Nishizuka Y, Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. *J Biol Chem* **255**: 2273–2276, 1980.
112. Capogrossi M, Kaku T, Filburn C, Pelto D, Hansford R, Spurgeon H and Zakata E, Phorbol ester and dioctanoylglycerol stimulate membrane association of protein kinase C and have a negative inotropic effect mediated by changes in cytosolic Ca^{2+} in adult rat cardiac myocytes. *Circ Res* **66**: 1143–1155, 1990.
113. Ganote CE, Armstrong S and Downey JM, Adenosine A_1 selective agonists offer minimal protection against ischemic injury to isolated rat cardiomyocytes. *Cardiovasc Res* **27**: 1670–1676, 1993.
114. Li Y and Kloner RA, The cardioprotective effects of ischemic preconditioning are not mediated by adenosine receptors in rat hearts. *Circulation* **87**: 1642–1648, 1993.
115. Grover GJ, Dzwoncyk S, Sleph PG and Sargent CA, The ATP-sensitive potassium channel blocker glibenclamide (glyburide) does not abolish preconditioning in isolated ischemic rat hearts. *J Pharmacol Exp Ther* **265**: 559–564, 1993.
116. Pang CY, Zhong AG, Xu N and Forrest CR, Protective effect of ischemic preconditioning (IPC) and adenosine in skeletal-muscle ischemia/reperfusion (I/R) injury. *FASEB-J* **7**: A494, 1993.
117. Belardinelli L, Linden J and Berne RM, The cardiac effects of adenosine. *Prog Cardiovasc Dis* **32**: 73–97, 1989.
118. Miura I, Ogawa T, Iwamoto T, Shimamoto K and Limura O, Dipyridamole potentiates the myocardial infarct size-limiting effect of ischemic preconditioning. *Circulation* **86**: 979–985, 1992.
119. Mullane K, Acadesine: The prototype adenosine regulating agent for reducing myocardial ischaemic injury. *Cardiovasc Res* **27**: 43–47, 1993.
120. Benders AAGM, van Kuppevelt THMSM, Oosterhof A and Veerkamp JH, The biochemical and structural maturation of human skeletal muscle cells in culture: The effect of the serum substitute Ultroser G. *Exp Cell Res* **195**: 284–294, 1991.
121. Larabia V, Lam L, Burdett E, Leiter LA and Klip A, Glucose transport in human skeletal muscle cells in culture. *J Clin Invest* **90**: 1386–1395, 1992.