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Remote preconditioning and delayed cardioprotection in skeletal muscle

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THE CONCEPT OF ISCHEMIC PRECONDITIONING (IPC) was introduced in 1986 by Murry, Jennings, and Reimer (10). These investigators showed that four brief 5-min periods of ischemia in canine hearts produced a marked reduction in myocardial infarct size in dogs subjected to 40 min of coronary artery occlusion and 72 h of reperfusion compared with a nonpreconditioned group. This finding has stimulated a tremendous amount of studies in an attempt to elucidate the mechanisms responsible for this powerful, protective effect in the heart. This phenomenon has been shown to occur in all species studied, including man, and has been extended to other organs, including the brain, skeletal muscle, kidney, liver, and intestine (24). IPC has also been mimicked by a number of pharmacological triggers and mediators, and it has been shown that IPC also has not only an acute phase that lasts 1–4 h but a delayed phase, which is usually observed 24 h after the ischemic or pharmacologic stimulus, a phase that has been shown to last up to 72 h before waning (2, 24). Many factors have been identified to be involved in triggering and mediating both acute and delayed preconditioning, and some of the main factors include adenosine, opioids, and bradykinin at the receptor level and intracellular kinases, such as PKC (23), mitogen-activated protein kinases (MAPKs), reactive oxygen species (ROS), nitric oxide (NO), and K_{ATP} channels in both sarcolemmal (sK_{ATP}) and mitochondria (mK_{ATP}) sites (24).

In 1993, Przyklenk et al. (16) produced an important paradigm shift (15) when they clearly demonstrated that regional IPC in the left circumflex bed of the canine heart protected the remote left anterior descending coronary artery bed from infarction during sustained ischemia in this region, intraorgan preconditioning. Although this could not be repeated by Nakano et al. (11) in isolated rabbit hearts in a Langendorff mode, possibly due to differences in species, experimental design, or in vivo vs. in vitro differences (6). Nevertheless, this finding stimulated other studies in which investigators were able to precondition organs distal to the site of the preconditioning stimulus, a phenomenon entitled "remote preconditioning." For example, renal preconditioning (18, 22) and intestinal preconditioning (4, 13) were both shown to protect the heart from a subsequent prolonged ischemic insult, and these effects appeared to be mediated, in part, via adenosine receptors, opioid receptors, and ROS (24). Recently, a role for mK_{ATP} channels has been implicated in the protection afforded by remote hindlimb IPC to explanted rat hearts removed and placed in the Langendorff mode (8). In these studies, the authors compared the effect of remote hindlimb IPC with that produced by local IPC directly applied to the isolated perfused heart without the previous hindlimb IPC stimulus. Both methods of IPC produced nearly equivalent degrees of protection against infarct size and myocardial stunning, an effect mimicked by the mK_{ATP} opener diazoxide. The effect of remote IPC was blocked by pretreatment with the nonselective K_{ATP} channel blocker glibenclamide and the selective mK_{ATP} channel blocker 5-hydroxydecanoic acid (5-HD) but not by the sarcolemmal-selective K_{ATP} inhibitor HMR 1098. These results suggested that remote IPC in explanted rat hearts is mediated by a mechanism linked to opening of the mK_{ATP} channel (8).

Two papers (1, 9) recently published by the group that has published a paper in the present issue of the *AJP–Regulatory, Integrative and Comparative Physiology* (9a) have addressed mechanisms and effects of remote IPC against infarction acutely in a pig model in which skeletal muscle flaps exposed to 4 h of ischemia and 48 h of reperfusion were used to investigate the protective effects of noninvasive remote IPC of skeletal muscle to protect distant skeletal muscle against infarction. Addison et al. (1) initially observed that three 10-min periods of occlusion and reperfusion of a hindlimb with a tourniquet reduced infarct size in the latissimus dorsi, gracilis, and rectus abdominus by ~55, 60, and 55%, respectively. Interestingly, these protective effects of remote IPC were blocked by both naloxone and 7-benzylidenenaltrexone, a nonselective and δ_1-selective opioid receptor antagonist, respectively. However, the effect was not blocked by a ganglionic blocker, hexamethonium, or the nonselective adenosine receptor antagonist 8-SPT as had been previously shown to occur following remote IPC using an occlusion of the anterior mesenteric artery to produce myocardial protection in rats (4). These results suggest an important role for endogenous opioid peptides in mediating these effects of remote IPC in pig skeletal muscle. In agreement with an important role for opioids in remote IPC, Patel et al. (13) and Weinbrenner et al. (21) have shown a role for opioids in remote IPC produced by renal or mesenteric occlusions. More recently, Moses et al. (9) found that remote IPC in pig skeletal muscle reduced infarct size in distant latissimus dorsi muscle flaps, and this effect was abolished by both glibenclamide and 5-HD but not HMR 1098. They also showed that the novel mK_{ATP} opener BMS-191095, mimicked the effect of remote IPC in this model and that the infarct-sparing effect of BMS-191095 was associated with a higher content of ATP in the muscle and a reduction of myeoperoxidase activity, an indication of neutrophil infiltration into the reperfused flap. These data agree with those previously observed in the explanted rat heart and suggest that opening mK_{ATP} channels produces an energy-sparing effect during sustained ischemia in heart and skeletal muscle. These data also suggest that mK_{ATP} channels are both a trigger and mediator of acute remote IPC in hindlimb skeletal muscle of rats and pigs.

Despite a number of papers suggesting that remote IPC performed in different organs results in acute protection of distant organs against infarction, only two previous papers (19, 20) suggested that remote IPC can result in delayed protection 24–72 h later, similar to that of delayed IPC because of a local IPC stimulus. Wang et al. (20) showed that intestinal preconditioning by ischemia resulted in a protective effect against myocardial infarction 24 h later and that this effect was

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mediated by inducible NO synthase (iNOS) based upon its blockade by two relatively selective iNOS inhibitors, aminoguanidine and S-methylthiourea. More recently, Tokuno et al. (19) showed that bilateral occlusion of the rat internal carotid arteries 24 h before studying their hearts in the Langedorff mode produced a reduction in infarct size and improved function, an effect also mediated by iNOS. Of course, this preconditioning protocol was not produced by brief occlusions of the carotid arteries, so these results are difficult to put in perspective with previous studies of remote IPC produced by brief periods of ischemia of a distant organ.

In this issue of *AJP–Regulatory, Integrative and Comparative Physiology*, Moses et al. (9a) have performed an elegant series of experiments in their pig flap model in which they clearly demonstrate that remote noninvasive IPC of the hindlimb results in a delayed protective effect at 24, 28, 36, 48, and 72 h after remote IPC. The authors also show that P-1075, a putative sK_{ATP} opener (17), also mimicked the effect of remote IPC to reduce infarct size. One major difference between the acute studies previously performed in the same model by these investigators is related to the role of the K_{ATP} channel subtypes in the trigger and mediator phases of the delayed PC response. That P-1075 triggered the protection seen and the observation that the trigger phase was blocked by pretreatment with HMR 1098 and not by 5-HD is different from the acute phase where 5-HD blocked both the trigger and mediator phase of remote IPC. Nevertheless, these results are in agreement with those of Patel et al. (14) who also found that the trigger phase of delayed IPC in rats was mediated by the sK_{ATP} channel. The reasons responsible for these differences in the trigger phase of acute vs. delayed IPC following local or remote IPC are not apparent but are worthy of further study. In addition, there is evidence that P-1075 is not a selective sK_{ATP} channel opener (5, 12). Therefore, studies in which P-1075 is given in the presence of 5-HD or HMR 1098 would help address this potential pitfall in the interpretation of these data. The downstream signaling pathways involved in mediating this delayed protected phenotype have not been addressed except in the two previous studies (19, 20) that suggested a role for iNOS, and this would be a fruitful area of further study. It would also be interesting to determine whether remote IPC of skeletal muscle or other organs can result in delayed cardioprotection or neural protection because such findings would have important clinical implications. A noninvasive method, such as that shown in the present study, would seem to be readily amenable to use in humans subjected to acute ischemic insults. In fact, this method has recently been used in several clinical studies to produce acute preconditioning (3, 7), so it seems possible that this methodology could be easily adapted to the clinical arena in the future.

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