Editorials

Preconditioning - A Paradigm of Yin and Yang

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The discovery of preconditioning (PC) was a major advance, as first described by Murry et al[1]. A practical definition of this phenomenon is that a relatively minor noxious stimulus protects the tissue subjected to it against a subsequent more severe, potentially lethal assault. This definition is expressly vague, for many reasons[2]:

– The noxious stimuli vary tremendously; heat shock, hypothermia, ischemia, local or at a distance, neurological or mental stress, chemical stressants and substances can all produce PC, as will be described later on.
– Any living tissue can undergo PC. Cardiac, skeletal muscular, vascular, cerebral, neuronal, hepatic, renal tissue are examples, as well as the mononuclear white blood cells. Indeed it can be termed a universal phenomenon.
– The influences can be cross-reacting. Thus, heat shock or stretch can protect against ischemia.

We shall confine ourselves to cardiac tissue. PC has been demonstrated in the isolated cell, either separated from its surrounding connective tissue or in culture, in the isolated heart, or in the intact human or animal organism.

A seeming paradox is the most striking element of PC, that a sublethal stimulus protects against a more severe one. However, the boundaries between the two are not always easy to define. In the case of "ischemic" PC, the duration of ischemia is often crucial. While ischemic periods as short as 2 minutes can precondition the heart, it is difficult to determine when the initial ischemia ceases to be protective and becomes harmful. Recently we investigated whether the varying intervals of initial ischemia have a different effect on a subsequently applied preconditioning stimulus.

We found that if an interval of global ischemia of 20 or 15 minutes is applied before a subsequent preconditioning stimulus, the latter is not effective. However, a shorter initial interval of ischemia i.e. a duration of 10 minutes, seems to precondition the heart[3]. In the same context Iliodromitis et al[4] have shown that after 6 cycles of PC, of 5 minutes duration each, the PC effect is attenuated. Both observations suggest that after a certain amount of ischemia is exerted, it is not protective any longer but harmful.

These data are pertinent when the influence of pre-infarction ischemia on a subsequently emerging myocardial infarction are considered. Thus, in most prospective studies, pre-existing ischemia is accompanied by a decrease of the size of the ensuing infarct and against out-of-hospital ventricular fibrillation in patients with acute occlusion of the left coronary artery[5]. However, this effect is not clearly seen in retrospective studies. These differences may be ascribed to the fact that it is much more difficult to accurately judge the duration and severity of previous ischemic episodes retrospectively. It is very important to assess whether additive physiologic stresses are protective or harmful. Hoshida et al showed very recently

that two sessions of sublethal stimuli extended the cardioprotective effect induced by only one of them[6]. We have also shown that «early» or «classical» ischemic preconditioning – induces cardioprotection to an additive degree above that produced by «late» preconditioning through a stress heat challenge exerted 24 hours earlier[7]. If these experimental interventions are applied in the clinical situation, it becomes apparent that the duration and intensity of both stimuli and the condition of the patient's heart will determine the final balance between benefit or detriment.

Of course these time limits are influenced by numerous factors. The experimental setting is a prime consideration. I mentioned that 15 minutes of continuous ischemia abrogate subsequent PC. However, if these same 15 minutes are applied as 3 cycles of 5 min ischemia separated by 5 minutes of reperfusion, they are a very potent PC technique. Similarly old age, heart failure, long standing diabetes mellitus blunt[8], chronic hypoxia, early diabetes and hyperthyroidism[9,10] enhance the PC effect, while left ventricular hypertrophy does not seem to affect it[11].

Equally intriguing is the situation seen with catecholamine administration. Isoproterenol, dobutamine and norepinephrine have all been found to protect the heart when given before a subsequent ischemic assault, ie to precondition the heart[12]. However, the oxygen increase in requirements produced by the two former drugs are well known. We have shown that when dobutamine is given after an ischemic period it is associated with functional deterioration of the isolated rat heart[13]. The clinical counterpart of this phenomenon is the finding by Tsoukas et al[14] that at dobutamine stress echo persistent dysfunction can be seen after the development of ischemia, tantamount to stunning. In the experimental animal du Toit and Opie have shown that isoproterenol exerts a pro-stunning effect on the rat heart subjected to ischemia[15]. Interestingly, Lameris et al[16] have shown that an opposite unfavorable situation can be encountered: with prolonged myocardial ischemia epinephrine is accumulated in the myocardial interstitial fluid. These considerations are important if one remembers that in experimental situations in the larger experimental animal, catecholamines are being used to counteract the myocardial dysfunction associated with post-ischemic myocardial stunning. The larger animal heart is better protected against ischemia than that of the smaller animal. However, one should consider the fact that in most situations accompanied by severe myocardial dysfunction dobutamine is the agent most frequently employed. Its net effect however is difficult to determine in view of the oxygen wasting effects of the catecholamines. Whether levosimendan, a calcium-sensitizing agent purported to manifest a potent anti-stunning action will prove more efficacious in this setting remains to be established. Another piece of this puzzle commands attention: catecholamines are at least partially thought to exert their inotropic effect by allowing more Ca^{2+} to enter the cardiac cell[17]. Calcium exerts a potent positive influence on myocardial contractility, but at the same time calcium overload is associated with stunning and even cell necrosis in the sequence of ischemia and reperfusion[18]. Thus, the observations by the group of Miyawake et al seem at first surprising[19]: they found that a transient initial entry of Ca^{2+} into the cell, either over a 5 minute interval or in 3 cycles of one minute each, resulted in cardiac PC. This effect was abolished by previous administration of the L-calcium channel antagonist, verapamil. Calcium antagonists are by themselves protective against ischemia[20]. Thus, like ischemia and catecholamine stimulation, Ca^{2+} can be a friend or a foe according to the circumstances. It should also not be forgotten that ischemia itself causes a high Ca^{2+} influx as well reviewed by Opie[21] and Marban[22]. This beneficial preconditioning action of Ca^{2+} entry seems very paradoxical in view of the fact that the function of KATP openers which mediate PC is to shorten the action potential duration, which results in decreased calcium entry[23].

Another interesting aspect is that just as catecholamines are used to boost the failing heart, often in the setting of an ischemic assault, intracardiac $CaCl^2$ administration was until a few years ago a mainstay of cardiopulmonary resuscitation. In an experiment now ten years old, Downing and Chen successfully used calcium to reverse the stunning effect of 2 hours of low flow ischemia in the piglet heart[24].

We are currently testing two hypotheses in trying to elucidate the Ca^{2+} PC:

- Does administration of KATP channel blockers abrogate Ca^{2+} PC?
- During Ca^{2+} PC, does APD shorten?

An even more puzzling phenomenon should round off this enumeration of paradoxes. At reperfusion, the generation of free radicals, the reactive oxygen species (ROS), is believed to kill more cells than the initial previous ischemia by itself[25]. This dreaded reperfusion

injury has not been adequately combated in the clinical arena, despite the initial promising results of ROS scavengers in the experimental setting. A theory that has emerged over the last few years however, is that the dreaded ROS can exert a PC mechanism on their own[26-28]. Thus, not only is ischemic preconditioning induced cardioprotection associated with an early increase in reactive oxygen species, but exogenous oxidants have also been found to induce preconditioning in the intact rabbit heart[29].

However, the story does not end here, since a reverse finding has emerged:

The aforementioned KATP channels, when opening before ischemia, generate free radicals that then activate the PKC and p38 MAP kinase cascade. This was shown 2 years ago by Pain et al by administering diazoxide, a KATP opener[30]. The authors' results are corroborated by more recent work by Forbes et al[31] and Carroll et al[32]. The paradoxes continue: under certain conditions, the hope of many investigators against ischemia –reperfusion injury, antioxidants[33,34], can be seen to inhibit ischemic PC. Chen et al used NAC, a glutathione precursor to this effect; this substance maintains glutathione levels[35]. Zhang et al[26] postulated that when superoxide (O_2^-) activates mitochondrial KATP channels, this effect may be associated with a direct action on the sulfhudryl groups of the channel protein.

These authors reconstituted mitochondrial KATP channels from bovine ventricular myocardium into planar lipid bilayers. They found that addition of xanthine/xanthine oxidase, a commonly used O_2^- generating system activated these channels within one minute.

However, it should not be forgotten that the human, rabbit and pig hearts have less xanthine oxidase activity than the rat heart, thus minimizing the potential production of free radicals[36]. This information stresses the difficulty in extrapolating findings in one species to another.

The whole picture becomes even more complex when it is considered that both norepinephrine and oxidative stimuli can affect the formation of heat shock proteins which may be involved in myocardial protection[37,38], and that oxidative stress enhances NO production, which induces late PC.

Thus in PC, as in other phenomena, the yin and yang holds full sway. The yin and yang, co-existing opposites, are described in the Dragon-Tiger Classic, which is a manual of external, sexual and internal Taoist alchemy. According to this manual, the essence of a substance is found in its complementary opposite[39].

This perusal of paradoxes is admittedly an oversimplification. But I hope that it may usher a train of thought towards harnessing the noxious elements and liberating the beneficial ones. As previously expressed, one should not only question but also actively search how to "bottle the genie".

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