Developments in Cardioprotection: “Polarized” Arrest as an Alternative to “Depolarized” Arrest

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During cardiac surgery or cardiac transplantation, the heart is subjected to varying periods of global ischemia. The heart must be protected during this ischemic period to avoid additional injury, and techniques have been developed that delay ischemic injury and minimize reperfusion injury. Almost universally, this involves using a hyperkalemic cardioplegic solution and these solutions have become the gold standard for myocardial protection for more than 20 years. Despite the extensive and continued research aimed at improving these basic hyperkalemic cardioplegic solutions, patients undergoing surgery almost invariably experience some degree of postoperative dysfunction. It is likely that this relates to the depolarizing nature of hyperkalemic solutions, which results in ionic imbalance caused by continuing transmembrane fluxes and the consequent maintenance of high energy phosphate metabolism, even during hypothermic ischemia. A potentially beneficial alternative to hyperkalemic cardioplegia is to arrest the heart in a “hyperpolarized” or “polarized” state, which maintains the membrane potential of the arrested myocardium at or near to the resting membrane potential. At these potentials, transmembrane fluxes will be minimized and there should be little metabolic demand, resulting in improved myocardial protection. Recent studies have explored these alternative concepts for myocardial protection. The use of compounds such as adenosine or potassium channel openers, which are thought to induce hyperpolarized arrest, have demonstrated improved protection after normothermic, or short periods of hypothermic, ischemia when compared to hyperkalemic (depolarized) arrest. Similarly, studies from our own laboratory, in which the sodium channel blocker, tetrodotoxin, was used to induce polarized arrest (demonstrated by direct measurement of membrane potential during ischemia) was also shown to provide better recovery of function after 5 hours of long-term hypothermic (7.5°C) storage. These promising initial studies need to be consolidated before experimental promise becomes clinical reality.

Myocardial ischemia can vary in duration from a few seconds, such as seen with transient vasospasm, or may persist for many years with an underlying and progressive evolution. In this form, it is likely that only a portion of the heart will be affected; this is known as regional ischemia. In contrast, global (or whole heart) ischemia occurs when it is electively induced during cardiac surgery or during heart storage prior to transplantation. The decision to induce global ischemia has arisen from the need of the cardiac surgeon to have a still, blood-free and flaccid field to correct the underlying lesion that required the surgery. This article will be restricted to a discussion of global ischemia, and to current and potentially new methods of myocardial protection which can be used to delay the damaging effects of global ischemia on the myocardium.

Myocardial ischemia initiates a continuum of progressively more severe cellular changes with time that will ultimately lead to irreversible myocardial injury, cell death, and tissue necrosis [1]. The only way in which this process can be completely prevented is by early reperfusion of the myocardium, at a stage where the injury to the cells is only reversible; however, even this can lead to problems as there is potential for “reperfusion-injury” to occur [2]. As well as the temporal aspects of myocardial ischemic injury, it has been demonstrated that injury also occurs in a spatial manner. The endocardium is more susceptible to ischemic injury than other regions of the heart [3], and this injury gradually progresses towards the epicardium as the ischemic duration increases; this has been likened to a “wavefront of cell necrosis.” Methods of protecting the myocardium against ischemic-reperfusion injury should take these factors into consideration.

The induction of elective ischemia during cardiac surgery on myocardium that is either diseased, or has been subjected to previous periods of ischemia (for variable periods of time), is likely to cause significantly deleterious effects on this myocardium. Techniques of cardioprotection have been developed that can be used to delay the myocardial injury during the relatively short period of global ischemia used during cardiac surgery, thereby maintaining the myocardium in a state of “reversible injury” for longer periods. The most widely used of these techniques is cardioplegic solutions; the underlying con-
cepts of cardioplegia have been designated by Hearse and colleagues [4]. These concepts are (i) chemical arrest, (ii) hypothermia, and (iii) additional protection. The first concept involves the use of agents to induce diastolic arrest; this is predominantly an elevated potassium concentration (hyperkalemia) but other agents (such as low [or zero] calcium, elevated magnesium, tetrodotoxin, procaine or calcium antagonists) have also been used. The second concept of hypothermia is generally recognized as additive to myocardial protection, although the recent use of continuous warm cardioplegic solutions have challenged this. The third concept is that of additional components or agents that are supposed to enhance the protection required to prevent ischemia-induced events. There have been many studies, investigating hundreds of different interventions, which have been suggested to slow the progression of myocardial ischemic injury [4, 5]. Thus, various approaches have examined (i) limiting the cellular acidosis and the accumulation of toxic metabolites during ischemia and reperfusion; (ii) modifying the provision of substrates and/or the rate of cellular energy depletion; (iii) manipulating ionic imbalance, in particular the sodium and calcium concentrations leading to overload; (iv) modifying the regulation of various enzymes and proteins; (v) preventing the loss of potassium and other essential ionic components, together with enzymatic cofactors and essential trace elements; (vi) limiting damage to nuclear material, particularly during reperfusion; (vii) reducing the activation of damaging lytic enzymes, particularly those in activated neutrophils and the inflammatory response; and (viii) countering cell swelling and the accompanying membrane disruption leading to severe ultrastructural changes. Many investigators have used a variety of animal species and experimental models (both in vivo and in vitro) to examine agents such as calcium antagonists, buffers, osmotic agents, substrates, extracellular high-energy phosphates, magnesium, blocking monoclonal antibodies, and anesthetic agents that stabilize cell membranes. These have all been used to demonstrate a reduction in the degree of ischemic and/or reperfusion injury and hence improve the recovery of function. Dose-response studies of many of these interventions have led to the realization that an optimal concentration of a component of, or an additive to, the cardioplegic solution can provide significant additional protection. These studies have also demonstrated that an excess of these factors may cause a detrimental effect which can attenuate any benefit. In addition to these interventions to slow the rate of ischemic injury, studies have shown that reperfusion injury can be reduced by modifying reperfusion solutions. Thus, agents which attenuate free radical-induced injury, manipulate vascular responses (such as coronary reflow or neutrophil infiltration), or control ionic or pH regulation and rapidly reestablish normal ionic homeostasis, have been shown to exert beneficial effects during reperfusion [6].

More recently, there have been a number of challenging concepts regarding the possibility of the heart to initiate endogenous adaptive effects which result in significant improvements in cardioprotection. These adaptive responses include the ability of the heart to induce “stress proteins” and the phenomenon known as “ischemic preconditioning”; these topics are covered in detail in other articles from this Symposium and will only be mentioned briefly here. The heart is now known to express a number of proteins, initially known as heat stress proteins, since they were first shown to be expressed after exposure of an animal to brief periods of hyperthermia. Studies demonstrated that expression of these proteins correlated with improved tolerance to ischemia resulting in reduced infarction and improved recovery of function [7]. Similarly, ischemic preconditioning has also been shown to be one of the most potent protective mechanisms known. Ischemic preconditioning occurs when brief periods of ischemia followed by brief periods of reperfusion precede a longer and potentially lethal period of ischemia [8]. Paradoxically, these cycles of ischemia–reperfusion appear to trigger a sequence of cellular events [9] that lead to pronounced protection of the ischemic myocardium, which has been shown to manifest as reduced infarct size following regional ischemia, reduced ventricular arrhythmias, and improved myocardial function after global ischemia. Interestingly, the phenomenon of ischemic preconditioning can be mimicked by appropriate pharmacological stimulation; thus, treatment of myocardium with various receptor agonists such as adenosine, norepinephrine, and bradykinin can also initiate a protective effect in the myocardium. These are areas of intense research and represents novel areas for cardioprotection.

Cardioplegic Protection: Depolarized Arrest

Hyperkalemic cardioplegic solutions have been the cornerstone and gold standard of myocardial protection during cardiac surgery for more than 20 years. However, the induction of a depolarization of the resting membrane potential by elevated potassium is not without problems. Hyperkalemic cardioplegic solutions typically have an elevated K+ concentration ranging between 12 and 25 mM [4] which leads to a depolarization of the membrane potential (E m) to around −50 mV from a resting E m of about −80 mV in cardiac tissue. At this depolarized potential, the fast Na+ channels are inactivated (since the threshold is −70 to −65 mV [10]), resulting in diastolic arrest. The reversal potential of the Na–Ca exchanger also occurs at −50 mV [11, 12], so no net movement of Na or Ca should be occurring through the exchanger; however, the Na “window” current [13] will, in turn, lead to increased intracellular [Ca2+] through the calcium “window” current and cause contraction even in the arrested condition. Higher potassium concentrations, which depolarize the membrane further (to around −40 mV), would tend to activate the slow calcium channel (which has a threshold of approximately −40 mV [10]) and cause calcium influx into the myocyte. At this membrane potential the Na–Ca exchanger may be reversed with a net influx of [Ca2+], particularly if intracellular [Na+] is high as a result of sodium pump inhibit-
tion, hypothermia, and ischemia. This will lead to contracture, calcium overload, and subsequent myocardial injury, particularly during reperfusion. Other ionic mechanisms also exist (such as Na–H exchange) which will cause an influx of Na\(^+\) and, because the sodium pump is inhibited during ischemia and by hypothermia, could lead to calcium overload during reperfusion [14].

An alternative to potassium-induced arrest is that used in the Bretschneider solution [15]; arrest is induced by zero calcium combined with low sodium and procaine (used as a membrane stabilizer). However, a solution containing zero calcium has the potential to induce the calcium paradox, although this is diminished by the low sodium concentration and the effect of hypothermia [16]. Nevertheless, zero calcium solutions appear to have an effect on the cell membrane such that there is a loosening of the gap junctions and the intercalated disc, which may have severe effects during the reperfusion phase [16]. These extreme perturbations are not well tolerated by the heart, and studies [17, 18] have shown that the Bretschneider solution is less efficacious than the St. Thomas’ solution.

**Cardioplegic Solution: Hyperpolarized Arrest**

An alternative to using elevated potassium concentrations to induce depolarized arrest is to induce electromechanical arrest of the heart in a “polarized” or “hyperpolarized” state, where the membrane potential is close to, or more negative than, the resting potential. This has a number of theoretical advantages; the transmembrane ion gradients are balanced at the resting membrane potential which should prevent ionic imbalance occurring during ischemia. As a consequence of this, few pumps or channels are activated [19] and metabolic demand should be negligible, thereby maintaining myocardial high-energy phosphate stores. Of primary importance, however, is that the influx of Na\(^+\) and Ca\(^{2+}\) should be abolished or minimized since the Na and Ca channels are both closed at these resting potentials; thus, the possibility of Na or Ca influx and overload is reduced. It is possible to achieve arrest at a polarized or hyperpolarized state by using a variety of drugs. Thus, adenosine and potassium channel openers have been proposed to induce hyperpolarization, whereas drugs such as tetrodotoxin, procaine, or lidocaine (which are Na-channel blockers) are thought to induce a polarized arrest. Recently, these compounds have been examined as cardioplegic agents and compared to the more conventional protective effects of hyperkalemic cardioplegic solutions.

**Adenosine**

In 1988, Belardinelli and coworkers [20] demonstrated that adenosine, at a concentration of 50 \(\mu\)M, induced complete arrest and a hyperpolarization of \(-12\) mV in isolated sino-atrial (SA) node pacemaker cells. This cardioplegic effect was examined by Schubert and colleagues [21] and compared to the effect of hyperkalemic cardioplegic solutions in isolated rat hearts. Using an adenosine concentration of 10 mM, they showed that adenosine cardioplegia, either alone or in combination with an elevated K\(^+\) concentration of 20 mM in Krebs Henseleit buffer, reduced the time to myocardial arrest significantly compared to hyperkalemic solution alone. In this study, the cardioplegia was washed out of the coronary vasculature before the hearts were subjected to global ischemia; on reperfusion, recovery of function was significantly better in the adenosine cardioplegia group of hearts compared to hearts arrested with either adenosine plus hyperkalemia or hyperkalemia alone. It was suggested that hyperpolarization of the conductive tissue during arrest was beneficial to the ischemic myocardium.

In a subsequent study [22], using the isolated rat heart preparation, a solution containing 1 mM adenosine in combination with 25 mM K\(^+\) was used as the protective cardioplegic solution. An improved rate of arrest combined with a better recovery of function was demonstrated when compared to a solution containing hyperkalemia alone. Electrophysiological measurements showed that the adenosine and hyperkalemic solution induced an initial transient hyperpolarization before depolarization; this initial hyperpolarization was thought to arrest SA node conduction before myocyte contractility arrest and thereby induce a more rapid arrest of the heart. In a clinical model of cardiopulmonary bypass in baboons, Boehm and colleagues [23] compared adenosine cardioplegia (10 mM adenosine added to Krebs Henseleit buffer) to St. Thomas’ Hospital cardioplegic solution No. 2 (STH2). It was shown that adenosine cardioplegia was as effective as STH2 in protecting the heart; interestingly, they observed that STH2 arrested the heart significantly more rapidly than adenosine cardioplegia, in contrast to previous findings [21, 22]. More recently, the electrophysiological effects of the addition of adenosine (1 mM) to a hyperkalemic cardioplegic solution were examined in isolated guinea pig myocytes [24]. Adenosine was shown to reduce the rate at which K\(^+\)-induced depolarization of the membrane but had no significant effect on the magnitude of the depolarization achieved. It was also demonstrated that adenosine was able to reduce K\(^+\)-induced intracellular Ca\(^{2+}\) loading, supporting the concept that adenosine may exert a cardioprotective effect.

**ATP-Sensitive Potassium Channel Openers**

In 1983, Noma described the presence of an adenosine triphosphate-(ATP) sensitive potassium channel (K\(_{ATP}\) channel) in cardiac muscle [25]. A number of drugs have subsequently been developed which act on this channel, either as openers or blockers of the channel, and they have been suggested as potential therapeutic agents for cardiovascular disease [26]. A number of studies have demonstrated that K\(_{ATP}\)-channel openers (KCOs) exert beneficial effects on the ischemic myocardium, providing a cardioprotective effect. This cardioprotective effect is thought to result from their properties of reducing the action potential duration, and inducing a hyperpolarization of the cell membrane by reequilibrating the resting membrane potential (\(E_{m}\)) closer to the equilibrium...
constant of potassium (E_K). This is associated with a reduction in myocardial contractility, attributed to a reduction in intracellular Ca^{2+} and preservation of high energy phosphate stores [27, 28].

KCOs have been used in combination with hyperkalemic cardioplegic solutions, but the results have been conflicting. Galíñanes and colleagues [29] showed, in isolated rat hearts, that lemakalim alone, given prior to ischemia, was cardioprotective but when added to a hyperkalemic cardioplegic solution, the cardioprotective effect was lost. In contrast, other studies utilizing a variety of experimental models have demonstrated that the addition of nicorandil or pinacidil to hyperkalemic solutions enhanced the protective effect compared to the cardioplegic solution alone [30–33].

At pharmacological concentrations, KCOs can also be used as cardioprotective agents; they are thought to induce a hyperpolarized arrest. The cardioprotective effect of these agents during global ischemia was first shown by Cohen and colleagues [34]; they compared the cardioprotective effect of aprikalim to a hyperkalemic solution (20 mM K^+ in Krebs Henseleit buffer; HiK) in an isolated, crystalloid-perfused Langendorff rabbit heart subjected to 20 min normothermic (37°C) global ischemia. The hearts arrested with aprikalim (at an optimal dose of 100 μM) recovered significantly better than those arrested with HiK. In additional studies, in which a fixed degree of ischemic injury was produced (defined as a rise in contracture development of 4 mmHg), 100 μM aprikalim was shown to not only extend the duration of ischemia necessary to produce this degree of injury (47 versus 36 minutes in the HiK group) but the recovery of function was also significantly greater (69% versus 44% in the HiK group) suggesting an additional component of protection. The authors suggested that the improved protection found with “hyperpolarized” arrest compared to the depolarized arrest induced by the HiK solution may be related to its ability to maintain minimal metabolic activity, thereby maintaining transmembrane ionic gradients. Despite these benefits, aprikalim was observed to induce a significantly increased incidence in postischemic arrhythmias; this proarrhythmic effect has been observed with other potassium channel openers [35].

Further studies from Masket and coworkers have extended these initial studies; thus, a more physiological preparation of an isolated, blood-perfused Langendorff rabbit heart and a parabiotic support animal was arrested with either aprikalim (100 μM) or a hyperkalemic (depolarizing) solution before 30 minutes of normothermic (37°C) ischemia [36]. The aprikalim treated hearts again recovered significantly better (90%) than hearts arrested with hyperkalemia (73%). In a similar study, again using a blood-perfused, parabiotic, isolated rabbit heart Langendorff preparation, Lawton and colleagues [37] demonstrated that hearts arrested with pinacidil (another widely-used K_ATP-channel opener), at an optimal concentration of 50 μM, were significantly better protected after 30 minutes of global normothermic ischemia than hearts arrested with KH containing 20 mM KCl. They concluded that KCOs represented a promising alternative to hyperkalemia as a means of arresting and protecting the heart during ischemia, which was assumed to be by a hyperpolarizing mechanism. The hyperkalemic solution used in the above studies was a Krebs Henseleit buffer containing added potassium (to a concentration of around 20 mM); this is a less than optimal cardioplegic solution. Consequently, further studies were conducted in which optimal concentrations of aprikalim (100 μM) or pinacidil (50 μM) were compared to the St. Thomas’ Hospital cardioplegic solution No. 2 (an optimal crystalloid hyperkalemic cardioplegic solution widely used clinically), in either normal rabbit hearts [38] or hearts that had been acutely injured before arrest and were then subjected to hypothermic (4°C) arrest and 50 minutes of hypothermic (15°C) global ischemia, to more closely simulate the clinical situation [39]. In these studies, hyperpolarizing arrest with the KCOs were shown to be equivalent to the depolarizing St. Thomas’ Hospital cardioplegic solution in their protective effect (Fig 1).

Hyperpolarized arrest should reduce the ionic imbalance associated with ischemia, which should lead to reduced metabolism and maintenance of high-energy phosphates. Recent studies by Lopez and colleagues [40, 41] have investigated the effect of KCOs and high potassium on intracellular Ca^{2+} concentrations ([Ca^{2+}]_i) in isolated guinea pig ventricular myocytes. Using Ca-sensitive fluorescent probes and confocal microscopy, these studies demonstrated that exposure to elevated potassium (16 mM, as used in St. Thomas’ cardioplegia)
resulted in a rapid increase in \([\text{Ca}^{2+}]\), which was not prevented by addition of 16 mM magnesium. However, supplementing hyperkalemic solutions with 40 \(\mu\)M aprikalim or 300 \(\mu\)M nicorandil prevented the rise in \([\text{Ca}^{2+}]\); addition of glybenclamide (a \(K_{\text{ATP}}\)-channel blocker) abolished the protective effect of the KCOs. These studies demonstrate that KCOs, albeit in combination with hyperkalemia, exert a protective effect on the myocardium by reducing the increase of \([\text{Ca}^{2+}]\); this might explain the results of studies in which KCOs, in combination with hyperkalemic cardioplegic solutions, were shown to improve posts ischemic recovery of function [30–33]. Lawton and coworkers [42] have examined the effects of arrest with pinacidil or hyperkalemia (St. Thomas' Hospital cardioplegia), in isolated parabiotic blood-perfused Langendorff rabbit hearts, on recovery of function and myocardial oxygen consumption (MVO\(_2\)) during reperfusion after 30 minutes of global normothermic (37°C) ischemia. There was no difference in recovery of function between the hyperpolarizing or depolarizing protective solutions, but the hearts arrested with pinacidil had significantly higher MVO\(_2\) during early reperfusion, representing an increased oxygen demand in this group. It was suggested that this may be related to reparative processes or to a higher oxygen debt generated during ischemia, and may represent a limitation to the potential protective effect of KCOs as alternative cardioprotective agents to hyperkalemia.

KCOs are thought to exert their protective effect by inducing a hyperpolarization of the myocardial cell membrane; in this context, it is assumed that hyperpolarization represents a resting membrane potential (E\(_m\)) that is more negative than E\(_m\) since opening of potassium channels should move the resting membrane potential towards the potassium equilibrium potential (E\(_K\)). However, there is little evidence for this, and resting membrane potential during the arrest induced by KCOs has not been measured in any of the above studies. The effect of nicorandil on membrane potential in isolated porcine coronary arteries at different potassium concentrations of the bathing solution has been reported in a review by Quast [43], demonstrating this shift of E\(_m\) towards E\(_K\); however, these measurements have not been made in myocardium from whole hearts subjected to global ischemia, and this is an area which needs further study, particularly in the context of hypothermic arrest and ischemia.

**Sodium Channel Blockers**

Sodium channel blockers (such as procaine) have been used as cardioplegic agents for many years [4]. The sodium channel blocker, tetrodotoxin (TTX), has been demonstrated by Tyers and colleagues [44] to exert cardioprotective effects in isolated rat hearts subjected to 60 minutes of normothermic (37°C) global ischemia; these authors suggested that the hearts were arrested in a “polarized” state which prevented activation of a calcium current. Studies by Sternbergh and coworkers [45] showed that reduced MVO\(_2\) and resting tension was obtained in hearts subjected to continuous low-flow infusion of a solution containing 25 \(\mu\)M TTX compared to a hyperkalemic (20 mM) solution.

In our laboratory, we have recently conducted studies in which we examined the concept of myocardial protection by polarized arrest. We used TTX as a tool to induce this polarized arrest, in the context of long-term (5-hour) myocardial preservation during profound hypothermic (7.5°C) storage. We demonstrated that isolated working rat hearts subjected to arrest with, and storage in, a Krebs Henseleit buffer containing TTX (at an optimal concentration of 22 \(\mu\)M) recovered significantly better than hearts arrested and stored in a hyperkalemic solution (KH containing 16 mM K\(^+\), or KH containing 22 \(\mu\)M tetrodotoxin (TTX). Values are shown as mean ± standard error of the mean; *p < 0.05 compared to the KH group, **p < 0.05 compared to the KH + 16 mM K\(^+\) group. Redrawn from Snabaitis and associates [46].

Fig 2. Posts ischemic recovery of aortic flow (expressed as a percent of preischemic control function) in hearts arrested and stored for 5 hours with Krebs Henseleit buffer (KH) alone, KH containing 16 mM K\(^+\), or KH containing 22 \(\mu\)M tetrodotoxin (TTX). Values are shown as mean ± standard error of the mean; *p < 0.05 compared to the KH group, **p < 0.05 compared to the KH + 16 mM K\(^+\) group. Redrawn from Snabaitis and associates [46].
During ischemia and increased maintenance of high myocardial protection, resulting in less ionic movement either hyperpolarization or depolarization should improve ion balance. The induction of arrest by a mechanism involving depolarization near to the resting potential should avoid or reduce metabolic processes despite the use of concomitant hypothermic solutions. Extracellular potassium accumulation during 5 hours of ischemia in hearts treated with TTX compared to hyperkalemic hearts [47]. We have also been able to show that drugs, which either prevent sodium influx (furosemide, a Na/K-Cl-cotransport inhibitor, and HOE694, a Na/H exchange inhibitor), or act as calcium-desensitizers (BDM), exert synergistic protective effects when added to the TTX solution [48]. In addition, combining these drugs in a polarizing arrest solution (TTX in KH buffer) provided significantly better recovery of function in rat hearts subjected to 8 hours of global hypothermic ischemia when compared to the St. Thomas’ Hospital cardioplegic solution (STH2).

In conclusion, myocardial protection during cardiac surgery or cardiac transplantation has relied on hyperkalemic solutions for many years. However, hyperkalemic solutions have a number of problems, predominantly related to their depolarizing mechanism of action which causes ionic imbalance and continuing metabolic processes despite the use of concomitant hypothermia. Maintenance of the myocardial membrane at or close to the resting potential should avoid or reduce some of the damaging effects associated with depolarization. The induction of arrest by a mechanism involving either hyperpolarization or polarization should improve myocardial protection, resulting in less ionic movement during ischemia and increased maintenance of high energy phosphate; this, in turn, should reduce myocardial reperfusion injury. Recent studies have demonstrated that these concepts may be correct. Despite these promising initial results, considerable additional studies are required before the experimental promise can be converted to clinical reality.

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