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Ouabain—The Key to Cardioprotection?

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Based on a wealth of mechanistic evidence supported by the fact that ouabain mimics the spleen–liver effect in this article, the hypothesis is established that the endogenous hormone ouabain not only mimics the effects of ischemic preconditioning but also may be an ideal drug for the prevention of ischemic diseases. Moreover, it is argued that the spleen–liver effect may represent a general protective mechanism for the protection of organisms against oxygen deficiency. Investigating the spleen–liver mechanism offers a new approach to decipher the secrets of ischemic conditioning. Preconditioning represents a basic mechanism to protect a wide variety of cells against stressful stimuli such as ischemia. The ability to undergo preconditioning is almost ubiquitous in tissues and is highly conserved across species. Reinvestigation of the ''spleen–liver mechanism'' will allow the study of metabolic inhibitors and hormone mimics that all could help to transform ischemic preconditioning into a cure of the epidemic ischemic heart disease. Ouabain mimics the effects of the spleen factor. Cardioprotection induced by ouabain is due to the activation of pathways that are also activated in ischemic preconditioning. Just like ischemic preconditioning, ouabain activates the reperfusion injury salvage kinase pathway. Activation of nuclear factor kappa B and other transcription factors contribute to the long lasting effects of ouabain. The endogenous hormone ouabain just like preconditioning offers multiorgan protection based on innate mechanisms, which warrants clinical investigation. Clinical studies with ouabain that correspond to current standards are warranted.

Keywords: ouabain, ischemic preconditioning, spleen–liver mechanism, cardioprotection

OUABAIN—THE KEY TO CARDIOPROTECTION?

Cardiovascular diseases are responsible for >50% of total mortality. Among them, ischemic heart disease is the number one cause of mortality and morbidity in all industrialized nations. Novel therapeutic strategies for protecting the heart against ischemia are urgently needed. Of special importance are efforts that mimic endogenous mechanisms to protect the heart from oxygen deficiency.

Because a constant, uninterrupted supply of oxygen is essential to sustain life, organisms possess innate defence mechanisms to increase tolerance to acute and chronic lack of oxygen. Many animal species that live in environments with variable oxygen supply exhibit a wide range of biochemical and physiological adaptations that allow them to withstand long periods of hypoxia.[1–3] In many species, the immature heart possesses a higher resistance to oxygen deprivation than the mature heart. Human newborns exhibit a hypometabolic response to hypoxia, in common with other infant mammals.[4] Populations residing at high altitude display lower incidences of hypertension[5] and mortality rates for coronary heart disease[6,7] and a reduced incidence of myocardial infarctions.[5] Obviously, exposure to chronically reduced oxygen levels induces protection against these disease states. Data generated by animal studies strongly support the hypoxia-induced cardioprotection paradigm. A novel concept emerged from these data: exposure to moderate lack of oxygen triggers defence mechanisms to deal with reduced oxygen supply and induces endogenous cardioprotective programs. During the past decades, intensive research on ''ischemic conditioning''—applying brief episodes of nonlethal ischemia and reperfusion to confer protection against a sustained episode of lethal ischemia and

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reperfusion injury—has shown that cardioprotection is indeed possible by conditioning of the heart with nonlethal ischemic episodes.[8] The phenomenon of ischemic preconditioning is not only observed in cardiac tissue but occurs in other organs as well. Neuroprotective responses against stroke and injury of the brain[9] and activation of intrinsic protective systems in patients undergoing liver surgery are well documented.[10] Thus, it would seem that preconditioning represents a generalized adaptation to protect a wide variety of cells against stressful stimuli such as ischemia.

An intriguing phenomenon is that myocardial adaptation to ischemia also can be provoked by short episodes of ischemia and reperfusion in other organs such as limbs, intestine, and kidney. The protection afforded by preconditioning can be transferred through giving whole blood from a preconditioned animal to a naive animal or by using perfusate from an isolated heart on a naive heart.[11,12]

The various forms of conditioning indicate that a universal protection is evoked.[13] The molecular mechanisms underlying cardiac protection by ischemic conditioning are the subject of intensive research. The current knowledge has been summarized in detail in excellent comprehensive reviews.[14–16] Conditioning of the heart has become an important approach in cardioprotection.[17,18] Ischemic preconditioning today is the most effective, reproducible form of protection against myocardial cell death yet described. Elucidation of the signal transduction pathways underlying ischemic conditioning has identified a variety of pharmacological agents that are capable of reproducing its cardioprotective actions. Despite a wealth of preclinical, experimental animal data demonstrating clear cardioprotective benefits with these treatment strategies, their translation into clinical therapy has been hugely disappointing.[19–21]

The ultimate goal still is to find drugs that mimic the effects of ischemic conditioning and allow a pharmacological preconditioning that protects the heart and other tissues from the deleterious effects of oxygen deficiency. Several classes of pharmacological agents that may be able to mimic the protection conferred by ischemic preconditioning have been tested.[22,23] However, most of the pharmacological compounds used to induce preconditioning were associated with side effects such as occurrence of hypotension (adenosine), arrhythmias (adenosine, KATP channel openers), or possible carcinogenic effects (protein kinase activators), which seriously limited their clinical potential.[24]

Based on a wealth of mechanistic evidence supported by the fact that ouabain mimics the spleen–liver effect in this article, the hypothesis is established that the endogenous hormone ouabain not only mimics the effects of ischemic preconditioning but also may be an ideal drug for the prevention of ischemic diseases. The endogenous hormone ouabain just like preconditioning offers multiorgan protection based on innate mechanisms that warrants clinical investigation. Moreover, it is argued that the spleen–liver effect may represent a general protective mechanism for the protection of organisms against oxygen deficiency. A recent review[25] concluded that the failure to develop clinical applications from ischemic preconditioning is due in part to the incomplete understanding of its mechanisms and that a new integrative scientific approach should be used to resolve the complex networks of preconditioning protection signaling. Investigating the spleen–liver mechanism offers a new approach to decipher the secrets of ischemic conditioning.

THE SPLEEN–LIVER MECHANISM

Murry et al[26] are credited to be the first to have demonstrated in 1986 that ischemic preconditioning induces cardioprotection. However, the German physiologist Hermann Rein reported hypoxia- and ischemia-induced cardioprotection as early as 1949. Exposure to air with low oxygen concentration[27,28] and supercritical coronary ligature[29] induces cardioprotection in dogs. In studying these effects, Rein discovered the ''spleen–liver mechanism.'' Splenectomized dogs tolerate cardiac anoxia and ischemia poorly. Splenic venous blood obtained from a normal dog under hypoxia/ischemia overcomes the functional deterioration of the heart produced by hypoxia and ischemia but only when administered transhepatically to the splenectomized dog. Obviously, in dogs, cardiac anoxia causes an unknown substance to be released from the spleen that activates the liver to produce a substance, which acts on the heart to improve myocardial function. Whether the spleen factor is a precursor or a messenger that initiates the production of the active compound in the liver is an open question. The spleen factor, which Rein named ''Hypoxie Lienin,'' in dogs only shows effects under hypoxia but no effects under normal conditions. In Rein's interpretation, his ''experiments indicate that the ''hypoxic'' spleen–liver reaction does not resolve O_2 deficiency as such but rather allows a subsistence despite persistent O_2 deficiency and eliminates some previously existing hypoxia disorders (myocardial insufficiency).'' Investigation of this mechanism may contribute significantly to understanding the phenomenon of remote ischemic preconditioning.

In the 1950s, Meesmann and Schmier[30,31] not only confirmed Rein's findings, but they also showed that ''Hypoxie-Lienin'' increases the efficiency of cardiac

energy utilization.[32] In the 1970s, Huckabee, too, confirmed the importance of the spleen. Acute hypoxemia produced by the inhalation of air with reduced oxygen content increased cardiac output in intact anesthetized dogs. Splenectomy abolished the increase in cardiac output produced by hypoxemia, suggesting that an intact spleen is required for the increased cardiac output that occurs during hypoxemia.[33] Electrical stimulation of splenic nerves or sympathetic stimulation of the spleen by infusion of norepinephrine into the splenic artery releases an unknown substance from the spleen that results in cardiostimulatory action.[34] Based on experiments in a cyanide hypoxia model in dogs, Liang and Huckabee[35] concluded that a humoral agent released from the spleen is necessary to achieve maximal cardiac output increase during both moderate and severe hypoxemia. The importance of the spleen is documented by the observation that splenectomized patients often suffer from fatigue, lack of physical endurance, dyspnea, and anginoid pain in the chest. A long-term follow-up of 740 American servicemen splenectomized because of trauma during the 1939–1945 war showed a significantly excessive mortality from ischemic heart disease.[36]

To test the obvious hypothesis that the spleen–liver effect is due to release of blood stored in the spleen into the general circulation when the spleen contracts, Dohrn and Rein[37] have performed a second set of experiments with sharks (Scyllium stellare). The spleen of this species does not store blood supply. The experiments not only confirmed that the spleen–liver effect is not caused by blood release from the spleen but also indicated that this species seems to be dependent on constant production of the spleen factor. In the sharks, a loss of the spleen resulted in disturbed organ functions even without induction of hypoxia, an effect not observed in dogs. More recently, it has been reported that the epaulette shark (*Hemiscyllium ocellatum*) living on shallow tropical coral reefs that repeatedly become cut off from the ocean during periods of low tides can withstand long periods of hypoxia.[3] Because the tides become lower and lower over a period of a few days, the hypoxic exposure during subsequent low tides will become progressively longer and more severe. Thus, this shark is under a natural hypoxic preconditioning regimen.

In a broader context, this leads to the hypothesis that animals that regularly undergo hibernation or withstand long periods of anoxia may be dependent on the spleen factor, too. Studying adaptations to anoxic/hypoxic survival in hypoxia-tolerant animals may offer fresh ideas for the treatment of hypoxia-related diseases and provide essential insights into metabolic changes in the ischemic heart that prevent myocardial injuries. The ''spleen–liver mechanism'' may be a universal mechanism of special importance for animals living under natural hypoxic conditions but obviously is used as well to protect human organisms from ischemia.

Rein did not isolate ''Hypoxie-Lienin.'' He characterized it as water soluble and dialyzable. This means that the spleen factor is filtered out from the blood of patients on hemodialysis during dialysis. High cardiovascular mortality is the major cause of reduced life expectancy of patients who are on hemodialysis.[38] Classical risk factors cannot fully explain the magnitude of the risk. Identification of a pharmacological agent that substitutes ''Hypoxie-Lienin'' might help to lower that risk.

OUABAIN MIMICS THE EFFECTS OF HYPOXIE LIENIN

When Rein performed his studies on the ''spleen-liver-mechanism,'' cardiotonic glycosides were the standard medication for treating heart diseases. In Germany, the Strophanthus glycosides g-Strophanthin (referred to as ouabain in English) and k-Strophanthin were widely used. Although k-Strophanthin was used exclusively for intravenous (iv) application, g-Strophanthin (ouabain) was used for oral and iv application. Edens[39] at the University of Dusseldorf had identified significant differences in the therapeutic profiles of cardiac glycosides. In particular, strophanthus glycosides, unlike digitalis glycosides, are well suited for the treatment of angina pectoris and exert an activating influence on cardiac metabolism. Although digitalis was likened to a ''whip to beat the starving horse,'' Strophanthin was known as ''oats for the starving myocardium.'' Thus, it is not surprising that Rein in his experiments compared the effects of Hypoxie-Lienin with those of ouabain. Ouabain exerts the same protective effects against hypoxia/ischemia as the spleen factor, except that the ouabain effects last longer. Although release of the spleen factor by electrical stimulation of the spleen nerves in dogs could be repeated after some time, after application of ouabain ''often this was not the case, because the animal simply became resistant against O_2 deficiency for hours.'' Although ouabain mimics the effects of the spleen factor, there is no evidence that ouabain is identical with Hypoxie-Lienin. Its chemical structure is still unknown.

Kuschinsky[40] observed that ouabain prevents hypertrophy of the heart and the adrenal cortex in rats exposed to hypoxia induced by extreme exercise. Sauer and Maehder[41] confirmed this effect of ouabain in splenectomized rats. More recent in vitro studies with

rat hearts[42] and rabbit hearts[43] confirm these in vivo findings. Short exposure to a low concentration of ouabain protects the heart against ischemia/reperfusion injury.

Remote ischemic preconditioning improves maximal performance in highly trained athletes,[44] suggesting the application of preconditioning for clinical syndromes in which exercise tolerance is limited by tissue hypoxemia or ischemia. Ouabain demonstrates similar effects. Orally administered ouabain improves physical endurance in guinea pigs[45] and in healthy volunteers.[46,47]

Today there is much evidence that ouabain is a mammalian hormone produced in the adrenal cortex and hypothalamus. Elevated levels of circulating ouabain have been suggested in chronic renal failure, hyperaldosteronism, congestive heart failure, and preeclampsia.[48] Ouabain dose dependently inhibits the activity of the Na^+/K^+-ATPase (NKA). In addition, at low concentrations, binding of ouabain to NKA activates multiple signal transduction pathways.[48,49] It has been demonstrated that cardioprotection induced by perfusion of isolated rat and rabbit hearts with ouabain is due to the activation of pathways that are also known to be activated in ischemic preconditioning.[42,43] ERK1/2, phosphoinositide 3-kinase (PI3 K)/Akt and protein kinase C are 3 well-known important prosurvival protein kinases in the heart. All of them have been reported to be activated upon treatment with oubain.[42,50] Just like ischemic preconditioning, ouabain activates the reperfusion injury salvage kinase (RISK) pathway.[51] Furthermore, there is evidence that ouabain-induced cardioprotection is triggered by NKA-mediated signaling pathways that induce reactive oxygen species production and require mitochondrial KATP channel opening.[52]

In addition, on binding to NKA, ouabain activates calcium oscillations generated by the NKA/IP3R complex. This activates the pleiotropic transcriptional factor nuclear factor kappa B (NF-κB) resulting in tissue-protective effects.[53] NF-κB also is involved in triggering cardioprotection through preconditioning.[54] Activation of NF-κB and other transcription factors results in increased transcription of cardioprotective genes and synthesis of multiple cardioprotective proteins that serve as comediators of protection in the late phase of ischemic preconditioning (''second window'' or ''delayed preconditioning'').[55] Increased NKA activity contributes to cardiac protection produced by hypoxia-induced preconditioning.[56] These findings indicate that modulation of NKA may play an important role in ischemic preconditioning.[57]

NKA is a major component in the cell's energy balance. It is responsible for a high fraction of total adenosine triphosphate (ATP) use in cells. High rates of ATP turnover caused by NKA are the single greatest source of heat in mammalian cells. Animals that live in environments with variable oxygen supply use hypometabolism when oxygen is unavailable; by suppressing the metabolic rate by >90%, they compensate for the interruption of oxidative phosphorylation and reduce their energy needs.[58,59] A critical universal mechanism of global metabolic suppression is reversible protein phosphorylation (RPP). RPP provides a fast, coordinated, and readily reversible mechanism for suppressing metabolic functions.[58,59] There is extensive evidence that RPP is the central mechanism that regulates the metabolic activities in hibernating animals over cycles of hibernation/arousal, just as it does in many other systems of natural hypometabolism. Phosphorylation of both NKA and sarcoplasmic reticulum Ca^{2+}-ATPase reduces the activities of these membrane ion pumps by about 50% in tissues from hibernating animals as compared with euthermic controls.[58,59] Thus, it is an intriguing hypothesis that ouabain on binding to NKA specifically regulates NKA by phosphorylation in control of the cells metabolic requirements. Regardless of the facts set forth herein, it has recently been speculated, based on phosphorylation studies, that ouabain might induce autoregulated phosphorylation of NKA.[60]

In mice exposed to chronic hypoxia, a specific modification in the bioenergetics of hypoxic hearts has been observed that compensates for decreased oxygen availability. Hearts exposed to chronic hypoxia keep the same control distribution among energy supply and demand under low oxygen availability as healthy hearts under normal oxygen conditions.[61] These results are in line with Rein's observation that ouabain does not resolve oxygen deficiency as such but due to changes of metabolism rather allows a subsistence despite persistent oxygen deficiency and eliminates myocardial insufficiency.[27,28]

Stability in composition of the internal milieu of cells is maintained by transporting epithelia that exchange substances with the environment in a highly specific and regulated manner. NKA is a vital component of such exchange, because it not only transports Na^+ and K^+ vectorially, but it is secondarily responsible for the exchange of essential nutrients across epithelia.[62] Hence, it has been suggested that the hormone ouabain may regulate the vectorial transport of Na^+ across epithelia, accompanied by the downregulation of glucose, amino acids, ions, and other biologically relevant substances.[63] This hypothesis underlines the general importance of ouabain for the regulation of the metabolism of cells.

Both animal and human findings indicate that reduced ATP utilization and, thus, metabolic downregulation is

a key factor for ''rapid'' ischemic preconditioning in the heart.[64] Ouabain has multiple effects on the cardiac metabolism[65] that result in cardioprotection. Ischemia leads to a progressive accumulation of protons and lactic acid, ultimately inhibiting synthesis of adenosine triphosphate.[24,66] Administration of ouabain in a myocardial infarct model in rats raises the pH of acidic cardiac tissue within a few minutes by up to 0.5 units.[67] The pH sensitivity of the myocardium is well documented. A drop in the pH <6.2 leads to irreversible damage. Therefore, in cardiac surgery, strict pH control is imperative. In the ''Strophanthin era,'' German surgeons routinely preconditioned the heart by applying 0.3 mg of ouabain preoperatively and thereby observed significantly fewer complications.[68]

Remote ischemic preconditioning is a powerful innate mechanism of multiorgan protection that can be induced by transient occlusion of blood flow to a limb with a blood-pressure cuff.[69] Protection by conditioning is well documented for the liver[10] and the brain.[9] There is evidence that preconditioning represents a basic mechanism to protect a wide variety of cells against stressful stimuli such as ischemia.[70] The ability to undergo preconditioning is almost ubiquitous in tissues and is highly conserved across species. The available data suggest that signaling cascades that are active in conditioning of the heart also trigger the protective effects in these organs. Experiments with mouse brain suggest that neuroprotective effects of ischemic preconditioning also may be due to an Src-kinase linked mechanism.[71] Ouabain activates Src-kinase[55] indicating potential application of this endogenous hormone in neuroprotection. This is supported by the observation that sublethal concentrations of ouabain provide neuroprotection against excitotoxicity.[72] Such mechanistic evidence again is supported by clinical experience that reports successful application of ouabain in treatment of stroke.[73] Metabolic regulation has become a major focus in research on neuroprotection induced by ischemic preconditioning.[74] Ouabain in addition to its well-known effects on myocardial metabolism modulates cerebral metabolism. In patients with the syndrome of cerebral malnutrition, ouabain therapy has proven very effective (''positive nutrition effect'').[75]

Cardiovascular and renal disease risks are entwined through hormonal mechanisms, and chronic kidney diseases are associated with increased risk for hypertension and death in cardiovascular disease. Fetal malnutrition endangers kidney development and results in an increased risk for renal disease and hypertension. Ouabain in vivo protects kidney development from adverse effects of malnutrition.[53,76] These data indicate that the endogenous hormone ouabain just like preconditioning offers multiorgan protection based on innate mechanisms that warrants clinical investigation.

CONCLUSIONS

Preconditioning represents a basic mechanism to protect a wide variety of cells against stressful stimuli such as ischemia. In addition to cardioprotection protection by conditioning is well documented for the liver and the brain. The ability to undergo preconditioning is almost ubiquitous in tissues and is highly conserved across species. The available data suggest that signaling cascades that are active in conditioning of the heart also trigger the protective effects in other organs. The ''spleen–liver mechanism'' that is mimicked by the endogenous hormone ouabain actually may be the common underlying molecular mechanism. Hence, it is suggested that this mechanism be reinvestigated. This will allow the study of metabolic inhibitors and hormone mimics that all could help to transform ischemic preconditioning into a cure for the ischemic heart disease epidemic.

Ouabain mimics the effects of the spleen factor. Unlike the short lasting effects of hypoxia and preconditioning with brief episodes of ischemia the cardioprotection induced by ouabain is maintained for hours. Cardioprotection induced by ouabain is due to the activation of pathways that are also activated in ischemic preconditioning. Just like ischemic preconditioning, ouabain activates the RISK pathway. Activation of NF-κB and other transcription factors contribute to the long lasting effects of ouabain. These observations clearly make a clinical reevaluation of ouabain necessary. In decades of clinical experience, the cardioprotective effects of ouabain and k-Strophanthin have been demonstrated. The therapeutic profile and the disease profiles for which the use of strophanthus glycosides is appropriate have been summarized in monographs and reviews.[39,65,68,77,78] Recent research has confirmed the uniqueness of ouabain, supported by the fact that ouabain has a different mechanism of action than digitalis glycosides.[79] Clinical studies with ouabain that correspond to current standards are warranted.

''Welcome to ouabain—a new steroid hormone'' was the title of the Lancet Editorial in June 1991 published in response to the identification of ouabain as an endogenous hormone. The therapeutic potential of this hormone is not limited to heart disease. Neuroprotection, organ transplantation, and renal diseases are untapped applications waiting to be exploited. Ernst Edens predicted: ''The time will come, in which failure to timely start ouabain therapy will be condemned as medical malpractice.'' Today, 153 years after

its discovery as a cardioprotective drug, once again it is time for a "Welcome back, ouabain!"

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