JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 1585–1589

Received November 8, 2005
Accepted January 16, 2006
DOI 10.1211/jpp.58.12.0004
ISSN 0022-3573

Ageing, gender and cardiac sarcolemmal K_{ATP} channels

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Abstract

Sarcolemmal ATP-sensitive K^+ (K_{ATP}) channels are abundant in cardiac myocytes where they couple the cellular metabolic state with membrane excitability. Structurally, these channels are composed of Kir6.2, a pore-forming subunit, SUR2A, a regulatory subunit, and at least four accessory proteins. The activation of K_{ATP} channels occurs during ischaemia to promote cardiac viability under this adverse condition. Age-dependent changes in the myocardial susceptibility to ischaemia have been reported in experimental animals as well as in humans. Recent research has demonstrated that ageing is associated with a decrease in the number of cardiac sarcolemmal K_{ATP} channels in hearts from females, but not males. This alteration is likely to be due to an age-dependent decrease in the concentration of circulating estrogens. In the heart, SUR2A is the least expressed protein of all K_{ATP} channel-forming proteins. The consequence of this phenomenon is that the level of SUR2A is the main factor controlling the number of sarcolemmal K_{ATP} channels. Estrogens specifically up-regulate SUR2A and govern the number of sarcolemmal K_{ATP} channels, and this may explain the effect of decreasing estrogen levels on the heart. An age-dependent decrease in the number of sarcolemmal K_{ATP} channels generates a cardiac phenotype more sensitive to ischaemia, which seems to be responsible for the ageing-associated decrease in myocardial tolerance to stress that occurs in elderly women.

Introduction

ATP-sensitive K^+ channels are highly expressed in the sarcolemma of cardiomyocytes (sarcolemmal K_{ATP} channels), where they link the metabolic status of a cell with membrane excitability. The openings of these channels have been found to decrease infarct size, mimic ischaemic preconditioning (a phenomenon when brief episodes of ischaemia/reperfusion protect against sustained ischaemia) and improve the functional and energetic recovery of cardiac muscle following ischaemic and hypoxic insults (reviewed by Jovanović & Jovanović 2004; Hanley & Daut 2005; Kane et al 2005). Recent research has suggested that ageing might regulate sarcolemmal K_{ATP} channels, which could affect cardiac resistance to ischaemia. The purpose of this review is to highlight research that addresses sarcolemmal K_{ATP} channels in ageing.

Sarcolemmal K_{ATP} channels

ATP-sensitive K^+ (K_{ATP}) channels were uncovered more than two decades ago, when Noma (1983) reported a novel K^+ channel in cardiac ventricular myocytes that was gated directly by intracellular ATP; in an ATP-free environment a K^+-selective conductance was activated and then inhibited by micromolar ATP applied on the intracellular face of an excised membrane patch. Later studies showed that similar types of K^+ channels are present in pancreatic β-cells, skeletal muscle, vascular and other smooth muscle cells, neuronal cells, endothelial cells and renal epithelial cells (reviewed by Seino & Miki 2003). An important step in understanding the function of K_{ATP} channels was determining their molecular cloning (Inagaki et al 1995, 1996; Isomoto et al 1996). It has been shown that the native K_{ATP} channels are a complex of regulatory protein(s) containing the sulfonylurea receptor (SUR subunit) and an inward-rectifying K^+ channel (Kir) serving as a pore-forming subunit. This and later research (Shyng & Nichols 1997) suggested that the molecular structure of K_{ATP} channels is a heteromultimeric

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Acknowledgment and funding: The work performed in the author's laboratory was supported through grants from the Anonymous Trust, British Heart Foundation, BBSRC, National Heart Research Fund, TENOVUS-Scotland and the Wellcome Trust.

assembly of Kir6.2 with SUR1 (SUR1/Kir6.2, pancreatic type), Kir6.2 with SUR2A (SUR2A/Kir6.2, cardiac type) and Kir6.1 with SUR2B (SUR2B/Kir6.1, vascular smooth muscle type). In the heart, the full composition of cardiac sarcolemmal K_{ATP} channels was vigorously studied. Research so far has suggested that sarcolemmal K_{ATP} channels are composed of Kir6.2/SUR2A plus four more accessory proteins, including adenylate kinase (AK), creatine kinase (CK), the muscle form of lactate dehydrogenase (m-LDH) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Elvir-Mairena et al 1996; Carrasco et al 2001; Crawford et al 2002a, b; Jovanović et al 2005). All these proteins are enzymes involved in energy production in the heart and they are believed to be important in the regulation of the K_{ATP} channel activity. The catalytic products of these enzymes, ATP, ADP, lactate and 1,3-bisphosphoglycerate, are regulators of K_{ATP} channel activity (Noma 1983; Carrasco et al 2001; Crawford et al 2002b; Jovanović & Jovanović 2005; Jovanović et al 2006). By virtue of their catalytic activities these enzymes control the concentration of ligands in the microenvironment surrounding the channel and regulate channel activity. In the early days of cardiac K_{ATP} channels, it was suggested that the opening of sarcolemmal K_{ATP} channels protected the heart against metabolic stress, including myocardial infarction (Noma 1983; Grover et al 1990; Gross & Auchampach 1992). However, K_{ATP} channels in the inner membrane of the mitochondria were described in 1991 (Inoue et al 1991). In 1997 Garlid et al suggested that the opening of these channels, rather then sarcolemmal K_{ATP} channels, may mediate cardioprotection. This idea was further developed when it was suggested that certain K_{ATP} channel openers may modulate the membrane potential of the mitochondria, which was ascribed to the effect on mitochondrial K_{ATP} channels (Liu et al 1998, 1999; Sato et al 2000). These studies were largely based on the use of diazoxide and 5-hydroxydecanoic acid as selective activator and antagonist of mitochondrial K_{ATP} channels, respectively (Garlid et al 1997; Liu et al 1998, 1999; Sato et al 2000). However, more recent reports have challenged the selectivity and specificity of these compounds and questioned the involvement of mitochondrial K_{ATP} channels in cardioprotection (Ovide-Bordeaux et al 2000; Ozcan et al 2002; Hanley et al 2002; Suzuki et al 2003; Das et al 2003). The fact that the structure of mitochondrial K_{ATP} channel has not yet been established and is controversial precludes further research that would potentially define the significance and role of these channels. On the other hand, more recent work using selective antagonists of sarcolemmal K_{ATP} channels, recombinant channel proteins and transgenic mice lacking sarcolemmal K_{ATP} channels in the heart has provided strong evidence that the activation of this ion channel is cardioprotective. Specifically, it has been shown that (i) coexpression of genes encoding K_{ATP} channels confers resistance against metabolic stress in otherwise stress-sensitive cells (Jovanović et al 1998, 1999; Crawford et al 2002b), (ii) in mice with a genetically disrupted sarcolemmal K_{ATP} channel the heart is more susceptible to physical stress (Zingman et al 2002), (iii) K_{ATP} channel opener-mediated protection against hypoxia/ischaemia is associated with an effect on cardiac membrane potential and sarcolemmal K_{ATP} channel opening (Jovanović & Jovanović 2001a, b; Suzuki et al 2002), (iv) an increase in the number of sarcolemmal K_{ATP} channels increases cardiac resistance to hypoxia/ischaemia (Ranki et al 2001, 2002a; Crawford et al 2003; Brown et al 2005) and (v) ischaemic preconditioning cannot be conferred in transgenic animals lacking sarcolemmal K_{ATP} channels and is associated with sarcolemmal K_{ATP} channel opening and trafficking (Suzuki et al 2002; Budas et al 2004). The mechanism underlying cardioprotection mediated by sarcolemmal K_{ATP} channels is still a matter of discussion. Intracellular Ca^{2+} overload and depletion of energy stores underlie the cell injury and death that occur during metabolic stress. The opening of K_{ATP} channels reduces action potential duration and decreases Ca^{2+} influx (preventing increase in cytosolic Ca^{2+}) and hypercontracture, conserving energy stores, protecting the heart against injury (Jovanović et al 1998; Jovanović & Jovanović 2001a, b; Crawford et al 2002b; Suzuki et al 2002). This is one possible explanation of the mechanisms underlying cardioprotection by K_{ATP} channels. However, it should be mentioned that these channels have exhibited cytoprotective properties even in cells that do not generate action potential. It is therefore quite possible that yet unknown mechanism(s) underlie K_{ATP} channel-mediated cardioprotection.

Ageing and cardiac resistance to ischaemia

It is a consensus view that the hearts of aged experimental animals are less tolerant to ischaemia and ischaemia–reperfusion injury than those of young-adult animals (Lesnefsky et al 1994; Starnes et al 1997; Tani et al 1997; Headrick 1998; Schulman et al 2001; Powers et al 2004). A similar ageing-associated increase in cardiac susceptibility has been also reported in humans. More specifically, elderly patients with coronary artery disease experience impaired recovery of myocardial function after cardiac surgery and other cardiac interventions when compared to younger patients (Reynen & Bachmann 1997; Hirose et al 2000). The prognosis of acute myocardial infarction is more serious and mortality higher in the elderly compared to their younger counterparts (Goldberg et al 1989; Mariani et al 2000). The underlying mechanism of the age-dependent increase in cardiac susceptibility to metabolic stress is yet to be fully understood. Several age-dependent alterations in cardiomyocytes have been reported so far and these may be involved with increased sensitivity to ischaemia. These include changes in proteasome and mitochondrial function (Jahangir et al 2001; Lesnefsky et al 2001; Bulteau et al 2002), antioxidant response (Boucher et al 1998) and Ca^{2+} handling (Cain et al 1998). Recently, it has been shown that ageing may be associated with changes in sarcolemmal ATP-sensitive K^+ (K_{ATP}) channels (see below).

Ageing, gender and sarcolemmal K_{ATP} channels

First reports on K_{ATP} channels and ageing suggested that the properties of sarcolemmal K_{ATP} channels change during

ageing and development. The single-channel conductance of sarcolemmal K_{ATP} channels in adult cardiomyocytes seems to be higher than in neonates (Chen et al 1992). It has been reported that the current density mediated by sarcolemmal K_{ATP} channels increases from the foetal period and reaches a maximal level around birth, after which it stays constant for a while then gradually decreases in adulthood. A change in K_{ATP} channel responsiveness towards channel ligands has been also suggested, i.e. ageing increases sarcolemmal K_{ATP} channel sensitivity towards ATP (Xie et al 1997). The most recent research suggests that ageing is associated with changes at the level of sarcolemmal K_{ATP} channel expression. The regulation of expression of sarcolemmal K_{ATP} channels as well as assembly, maturation and turnover of these channels are complex and as yet not fully understood processes (reviewed in detail by Zhuo et al 2005; Neagoe & Schwappach 2005). Although this channel is in vivo composed of at least Kir6.2, SUR2A and four accessory proteins (Inagaki et al 1996; Carrasco et al 2001; Crawford et al 2002a, b; Jovanović et al 2005), it is not yet known what regulates the expression/numbers of sarcolemmal K_{ATP} channels. The most recent hypothesis is that SUR2A is the least expressed of all K_{ATP} channel-forming proteins. Consequently, the intracellular level of SUR2A has been proposed to be a limiting factor that sets the level of sarcolemmal K_{ATP} channels (Du et al 2006). It has been shown that the levels of sarcolemmal K_{ATP} channels decrease with age in a gender-dependent manner. Specifically, it has been found that female guinea-pigs and rats express more sarcolemmal K_{ATP} channels than their male counterparts (Ranki et al 2001; Brown et al 2005). During ageing, the level of K_{ATP} channels in males remains unchanged while in females it drops significantly (Ranki et al 2002b). One of the hallmarks of normal ageing in women is a decrease in the circulating concentration of estrogens (reviewed by Lamberts 2003) and estrogens are known to regulate the expression of numerous genes (McDonnell & Norris 2002). In guinea-pigs, and also in humans (Ranki & Jovanović, unpublished observation), it has been shown that estrogens up-regulate K_{ATP} channels (Ranki et al 2002a). It is therefore logical to suppose that an age-dependent decrease in estrogens levels could be responsible for a decrease in sarcolemmal K_{ATP} channels. The gender-specific difference in K_{ATP} channels can be also explained by the effect of estrogens that stimulate expression of K_{ATP} channels. All changes in channel protein levels were associated with a sole change in the SUR2A mRNA level (Ranki et al 2001, 2002a, b; Crawford et al 2003). This confirms the hypothesis that the level of SUR2A is the main factor in the regulation of the number of sarcolemmal K_{ATP} channels and that age- and gender-dependent changes in factor(s) regulating the expression of the SUR2 gene may be responsible for age-dependent changes in sarcolemmal K_{ATP} channels. It should be mentioned that all of the ageing studies and K_{ATP} channels have been carried out on guinea-pigs. However, experiments with human SUR2 promoter have shown that the same principles of SUR2 gene regulation also apply to humans (Crawford et al 2003).

A link between age-dependent changes in sarcolemmal K_{ATP} channels and cardiac susceptibility to ischaemia

It has been suggested that age-induced changes of the cardiovascular system are more pronounced in females compared to males. While the risk of heart disease in men increases constantly with age, pre-menopausal women have a significantly lower risk, which increases rapidly after menopause to levels comparable to those of their male counterparts (Stampfer et al 1991; Rich-Edwards & Hennekens 1996). Traditionally, the major mechanism responsible for the protective effect of the female gender was believed to be the anti-atherogenic action of female sex hormones on the lipid profile (Rich-Edwards & Hennekens 1996). However, more recently, in addition to this traditional view, some evidence has been provided that gender difference in cardiac response to a metabolic stress may also be due to gender difference(s) in the efficiency of endogenous cardioprotective mechanism(s). In this regard it has been shown that (i) maintenance of cardiomyocytes in an estrogen-free environment increases their susceptibility to ischaemia/reperfusion (Jovanović et al 2000), (ii) an estradiol-mediated increase in resistance to ischaemia/reperfusion is mediated via sarcolemmal K_{ATP} channels as it can be blocked by HMR1098, a selective antagonist of these channels (Ranki et al 2002b) and (iii) women have an improved long-term outcome after acute coronary syndromes compared to men (Mueller et al 2002). Based on these reports it is probably fair to suggest that a link between estrogens, number of K_{ATP} channels and resistance to ischaemia in the heart exists. We and others have uncovered a range of different conditions/treatments that increase the number of sarcolemmal K_{ATP} channels. In each of these cases such a condition/treatment was associated with an increase in cardiac resistance to ischaemia (Ranki et al 2001, 2002a; Crawford et al 2003). Conversely, a reduced number of sarcolemmal K_{ATP} channels create an ischaemia/reperfusion-intolerant cardiac phenotype (Suzuki et al 2002; Zingman et al 2002). It is therefore logical to conclude that a decrease in K_{ATP} channel number due to a decrease in estrogens would increase cardiac sensitivity to metabolic stress, which is exactly what happens in elderly women. However, as the levels of K_{ATP} channels in men remain unchanged during ageing, changes in sarcolemmal K_{ATP} channels cannot explain age-mediated changes in the heart in men.

Conclusion

Ageing is associated with a decrease in the number of sarcolemmal K_{ATP} channels in females, but not in males. This age-dependent change could be responsible for a myocardial phenotype more sensitive to ischaemia. The underlying cause of this phenomenon is likely to be a decline in circulating levels of estrogens. It is probable that levels of the K_{ATP} channel regulatory subunit, SUR2A, control the number of sarcolemmal K_{ATP} channels, and estrogens seem to regulate the transcription of a gene that encodes the SUR2A protein, which may explain, at least in part, the underlying mechanism of the

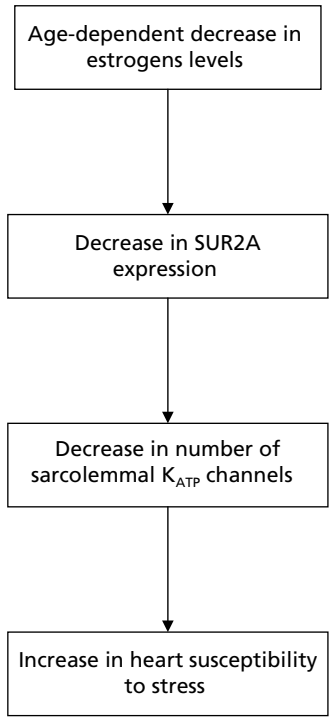

Figure 1 A possible chain of events in women during ageing is presented. This cartoon is based on data published in Ranki et al (2001, 2002a, b), Crawford et al (2003) and Du et al (2006).

age-associated increase in cardiac susceptibility to ischaemia in women (Figure 1).

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