Sarcolemmal ATP-sensitive K⁺ channels and cardiovascular function


Recent studies have demonstrated that ATP-sensitive K⁺ (K\textsubscript{ATP}) channel is a complex of the inwardly rectifying K⁺ channel subfamily Kir6.0 and the receptors for sulfonylureas (SUR). It is well established that K\textsubscript{ATP} channel in pancreatic β cell is composed of Kir6.2 and SUR1. However, functional molecules of K\textsubscript{ATP} channels in cardiovascular tissues have not been determined. Recent studies have suggested that cardioprotective effect of K⁺ channel openers or ischemic preconditioning is mediated by the activation of mitochondrial K\textsubscript{ATP} channels, another type of K\textsubscript{ATP} channel whose molecular structure is still undefined. Therefore, it would be important to determine the role of sarcolemmal K\textsubscript{ATP} channel in cardiac cells. Recently K\textsubscript{ATP} channel-deficient mice were generated by genetic disruption of Kir6.2. The Kir6.2-deficient mice may be useful for the evaluation of functional roles of sarcolemmal K\textsubscript{ATP} channels in cardiovascular tissues. In this review, we describe present understanding of molecular structure and physiological roles of sarcolemmal K\textsubscript{ATP} channels in cardiovascular tissues.

I. Introduction

Since the discovery of ATP-sensitive K⁺ (K\textsubscript{ATP}) channels in heart cells by Noma, similar types of K⁺ channels exist in a wide variety of tissues, including heart and vascular smooth muscle, and modulate the function by linking cell excitability with metabolic status. For instance, it has long been known that hypoxia or metabolic blockade produces a marked shortening of cardiac action potentials. In the mid 1980s it was demonstrated that sulfonylurea antidiabetic drugs inhibited K\textsubscript{ATP} channels not only in pancreatic β-cells but also in cardiac cells. In pancreatic β-cells, blockade of K\textsubscript{ATP} channels by antidiabetic sulfonylureas induces membrane depolarization, Ca⁺⁺ influx and insulin secretion. At the same period of time it was demonstrated that opening of K\textsubscript{ATP} channel was a mechanism of action shared by several vasodilating drugs such as cromakalim, pinacidil, nicorandil and diazoxide. Thereafter, the activators of K\textsubscript{ATP} channels are considered to be a new therapeutic class of drugs and called as K⁺ channel openers (KCOs). Recently the molecular structure of K\textsubscript{ATP} channels has been clarified by cloning members of the novel inwardly rectifying K⁺ channel subfamily Kir6.0 (Kir6.1 and Kir6.2) and the receptors for sulfonylureas (SUR1 and SUR2). Accumulating evidence suggests that the native K\textsubscript{ATP} channel is a complex of these two subunits and various combinations of Kir6.0 and SUR-subunits convey the heterogeneity in channels properties observed in native cells. Although the physiological significance of

1: Department of Pharmacology, Chiba University School of Medicine
2: Department of Molecular Medicine, Chiba University Graduate School of Medicine
pancreatic K\textsubscript{ATP} channel is well established, that of K\textsubscript{ATP} channels present in other tissues is less well understood. In terms of pathophysiological roles of cardiac K\textsubscript{ATP} channels, it has been assumed that administration of KCOs or myocardial ischemia produces the action potential shortening, reduction of Ca\textsuperscript{2+} influx through the L-type Ca\textsuperscript{2+} channel and resultant amelioration of intracellular Ca\textsuperscript{2+} overload. However, it has been recently postulated that activation of mitochondrial K\textsubscript{ATP} channel rather than sarcolemmal K\textsubscript{ATP} channel may be involved in cardioprotection induced by ischemic preconditioning and KCOs. In this review, we describe present understanding of molecular structures and physiological roles of sarcolemmal K\textsubscript{ATP} channels in cardiovascular tissues.

II. Molecular structures of sarcolemmal K\textsubscript{ATP} channels

Since the cloning of inwardly rectifying K\textsuperscript{+} (Kir) channel family members in 1993, several members of Kir channels have been identified and named as Kir subfamilies. In contrast to voltage-gated K\textsuperscript{+} (KV) channel subunits having six putative transmembrane segments, Kir channel subunits have a pore (H5) region and two flanking membrane-spanning segments, M1 and M2. Inwardly rectifying K\textsuperscript{+} channels are considered to function as tetramers like voltage-dependent K\textsuperscript{+} channels. In 1995 Inagaki et al. cloned Kir6.1 from a rat pancreatic islet cDNA library\textsuperscript{3}. Thereafter, they succeeded in cloning of Kir6.2, an isoform of Kir6.1, from a human genomic library\textsuperscript{4}. It was demonstrated that simultaneous expression of Kir6.2 with sulfonylurea receptor (SUR), a regulatory protein containing sulfonylurea binding site and two nucleotide binding folds (NBFs), exhibited K\textsubscript{ATP} channel activity\textsuperscript{4}. When Kir6.2 was coexpressed with SUR1 that was cloned from cDNA libraries of insulin-secreting cell lines, the expressed channel showed a conductance of 76 pS and was inhibited by ATP with an IC\textsubscript{50} value of about 10 µM. The reconstituted K\textsuperscript{+} channel was activated by diazoxide (EC\textsubscript{50} = \textasciitilde 60 µM) and inhibited by glibenclamide (IC\textsubscript{50} = \textasciitilde 18 µM) and tolbutamide (IC\textsubscript{50} = \textasciitilde 32 µM). These properties of the SUR1/Kir6.2 channel closely resemble those of pancreatic K\textsubscript{ATP} channels (Figure 1).

SUR2A, an isoform of SUR1 sharing 68 % amino acid identity with SUR1, was subsequently cloned by screening rat brain and heart cDNA libraries\textsuperscript{5}. Coexpression of SUR2A and Kir6.2 showed a K\textsubscript{ATP} channel of 79 pS, which was inhibited by ATP (IC\textsubscript{50} value of about 100 µM) and glibenclamide with less sensitivity compared with pancreatic-type K\textsubscript{ATP} channel (SUR1/Kir6.2). Such electrophysiological and pharmacological properties and tissue distributions suggest that cardiac K\textsubscript{ATP} channel appears to be composed of SUR2A and Kir 6.2 although direct evidence is still absent (Figure 1).

In terms of vascular K\textsubscript{ATP} channels SUR2B, a splice variant of SUR2A, was identified and coexpression of SUR2B and Kir6.1 reconstituted so called nucleotide diphosphate-dependent K\textsuperscript{+} (K\textsubscript{NDP}) channel observed in vascular smooth muscle cells\textsuperscript{6}. However, it has been suggested that K\textsubscript{ATP} channels in other smooth muscle cells such as colon and urinary bladder are composed of Kir6.2 and SUR2B. Therefore, it has not been determined with certainty whether the pore region of vascular K\textsubscript{ATP} channel is Kir6.1 or Kir6.2. (Figure 1)

Figure 1. K\textsubscript{ATP} channel as a complex of SUR and Kir channel subunits. NBF: Nucleotide binding fold.
III. Pathophysiological roles of sarcolemmal 
$K_{ATP}$ channels in cardiac cells

It has been originally hypothesized by Noma\(^1\) that activation of sarcolemmal $K_{ATP}$ channel may serve as an endogenous cardioprotective mechanism. Many studies have suggested that in hypoxic or ischemic myocardium sarcolemmal $K_{ATP}$ channels are activated and the action potential duration is shortened. In addition, KCOs accelerate the action potential shortening whereas sulfonylurea drugs partially attenuate the shortening under pathological conditions. The action potential shortening due to activation of $K_{ATP}$ channels is expected to reduce the time for Ca\(^{++}\) influx via L-type Ca\(^{++}\) channels and to increase the time for Ca\(^{++}\) extrusion through the Na\(^{+}\)-Ca\(^{++}\) exchange system. The resultant decrease in Ca\(^{++}\) influx would lead to reduction of mechanical contraction, amelioration of intracellular Ca\(^{++}\) overload and energy sparing. Indeed, a number of studies have demonstrated that KCOs such as pinacidil and cromakalim accelerate the action potential shortening during myocardial ischemia and the recovery of mechanical function during reperfusion\(^7\). On the other hand, the $K_{ATP}$ channel blockade by glibenclamide was reported to abolish the cardioprotective effect of KCOs or ischemic preconditioning\(^7\).

Recently alternative hypothesis has been proposed to explain the cardioprotective effect of KCOs or ischemic preconditioning\(^7\). Growing evidence indicates that the protective efficacy of KCOs does not always correlate with the degree of action potential shortening, implying additional cellular site(s) of drug action. The prime candidates include mitochondria, which may also harbour a type of $K_{ATP}$ channel. The opening of mitochondrial $K_{ATP}$ channel is supposed to depolarize the mitochondrial membrane thereby reducing Ca\(^{++}\) uptake into the mitochondrial matrix. Maintenance of mitochondrial Ca\(^{++}\) levels within a physiological range is considered to contribute to cardioprotection. It has been suggested that mitochondrial $K_{ATP}$ channels are activated by KCOs such as pinacidil and blocked by the sulfonylurea drug glibenclamide. In addition, it has been assumed that diazoxide and 5-hydroxydecanoate (5HD) are relatively selective activator and blocker of mitochondrial $K_{ATP}$ channels, respectively\(^7\).

Ischemic preconditioning is well-known phenomenon in which brief periods of ischemia paradoxically protect the heart against a more prolonged ischemic insult. It is now acknowledged that ischemic preconditioning is established by the opening of mitochondrial $K_{ATP}$ channels through the activation of protein kinase C\(^8\). Stimulation of various G protein-coupled receptors such as adenosine A\(_1\), bradykinin B\(_2\), opioid and $\alpha\_1$-adrenergic receptors have been shown to confer ischemic tolerance by activating protein kinase C (Figure 2). It has been recently reported that HMR1098, a selective blocker of sarcolemmal $K_{ATP}$ channel, failed to antagonize the cardioprotection afforded by diazoxide or ischemic preconditioning. Thus, it is now considered that activation of mitochondrial $K_{ATP}$ channels rather than sarcolemmal $K_{ATP}$ channels may be important for the cardioprotection produced by ischemic preconditioning or KCOs.

Two major contributions of sarcolemmal $K_{ATP}$ channel activation to electrophysiological...
disturbances during myocardial ischemia are action potential shortening and extracellular K⁺ accumulation. Such electrophysiological alterations can lead to shortened refractoriness and slowed conduction, both of which predispose to malignant ventricular arrhythmias. Is the only thing that sarcolemmal Kᵦₐₜp channel can do during myocardial ischemia to increase the susceptibility to ventricular arrhythmias? In order to answer the question further studies to evaluate functional roles of sarcolemmal Kᵦₐₜp channel in cardiac cells may be needed. In this context, it is noteworthy that delivery of two genes encoding the subunits of cardiac sarcolemmal Kᵦₐₜp channel, Kir6.2 and SUR2A, in conjunction with KCO prevented intracellular Ca²⁺ overload during hypoxia and reoxygenation⁹. 

IV. Functional analysis using Kir6.2-deficient mice

Miki et al. generated Kᵦₐₜp channel-deficient mice by disruption of Kir6.2 gene¹⁰. Kᵦₐₜp channel activity was absent in sarcolemmal membrane of β cells of the knockout mouse. The homozygous mice (Kir6.2⁻⁻) showed defects in both glucose-induced and sulfonylurea-induced insulin secretion¹⁰. In order to evaluate the functional roles of sarcolemmal Kᵦₐₜp channels in cardiovascular tissues we conducted several functional experiments using the Kir6.2-deficient mice. The Kir6.2-deficient mice appear to be useful model to define the molecular structure of Kᵦₐₜp channel on which various KCOs act. We will present some electrophysiological and functional data in cardiovascular tissues of Kir6.2-deficient mice. 

V. Conclusion and perspectives

Recent progress in molecular biology has provided useful information toward the better understanding of molecular structures of Kᵦₐₜp channel. In addition, much attention is focused on mitochondrial Kᵦₐₜp channel as an end effector of cardioprotection afforded by KCOs or ischemic preconditioning. However, the physiological significance of sarcolemmal Kᵦₐₜp channel in cardiovascular tissues remains to be established. Kir6.2-deficient mice may be useful for the evaluation of roles of sarcolemmal Kᵦₐₜp channels in cardiovascular tissues.

References