J. Life Science. (2002), 12(1), 33~42

JOURNAL OF
Life Science

Modulation of Cardiac ATP-Sensitive K^+ Channels Via Signal Transduction Mechanisms During Ischemic Preconditioning

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Received: June 7, 2002

Abstract In several species, a short period of ischemic preconditioning protects the heart by reducing the size of infarcts resulting from subsequent prolonged bouts of ischemia. The mechanism by which activation of ATP-sensitive K^+ (K_{ATP}) channels could provide the memory associated with ischemic preconditioning is still under debate. Several signal transduction pathways have been implicated in the mechanisms of protection induced by ischemic preconditioning. The exact receptor-coupled pathways involved in preconditioning remain to be identified. Likely extracellular agonists are those whose circulating levels increase under conditions that activate K_{ATP} channels; these conditions include ischemia and ischemic preconditioning. Potential physiological agonists include the following: (1) nitric oxide; (2) catecholamine; (3) adenosine; (4) acetylcholine; (5) bradykinin and (6) prostacycline.

The purpose of this review was to understand the mechanism by which biological signal transduction mechanism acts as a link in one or more known receptor-mediated pathways to increase K_{ATP} channel activity during ischemic preconditioning.

Key words: ischemic preconditioning, K_{ATP} channels, signal transduction mechanism

ATP-sensitive K^+ channels and ischemic preconditioning

ATP-sensitive K^+ (K_{ATP}) channels, which open when cytosolic ATP concentration ($[ATP]_i$) falls below a critical level (Fig. 1), are present at high density in the heart [15,45]. Activation of K_{ATP} channels shortens action potential duration, thus decreasing contractility and conserving energy during periods of ischemia [10,59]. A role has been also suggested for K_{ATP} channels in the phenomenon of ischemic preconditioning.

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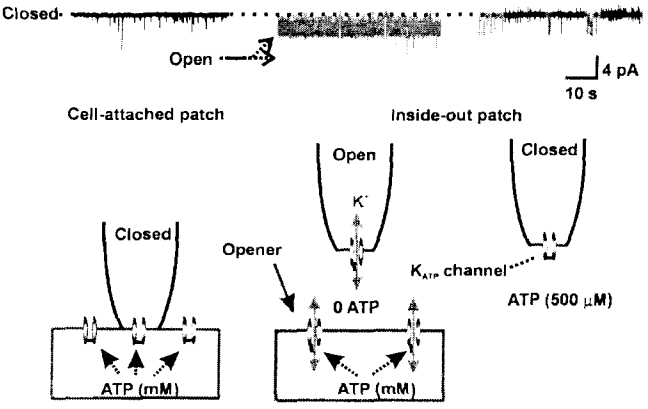

Fig. 1. Experiment illustrating the defining property of ATP-sensitive K^+ (K_{ATP}) channels. Intracellular ATP inhibits K_{ATP} channels. Under cell-attached configuration of patch-clamp technique (holding potential -50 mV), no activity of K_{ATP} channel can be recorded due to millimolar levels of ATP inside the cardiac cell. After patch excision in an ATP-free solution, K_{ATP} channels immediately open. Application of micromolar concentrations of ATP, to cytosolic side of inside-out patch, inhibits K_{ATP} channel openings. Dashed line represents the closed level.

In several species, a short period of ischemic preconditioning protects the heart by reducing the size of infarcts resulting from subsequent prolonged bouts of ischemia [13, 56,60,65]. The mechanism by which activation of K_{ATP} channels could provide the memory associated with ischemic preconditioning is still under debate [46].

Several signal transduction pathways have been implicated in the mechanisms of protection induced by ischemic preconditioning. Likely endogenous modulators are those circulating levels increase under conditions that activate K_{ATP} channels; these conditions include ischemia and ischemic preconditioning: (a) nitric oxide (NO) and bradykinin; the evidence is accumulating that K_{ATP} channel can be modulated by NO. In pancreatic -cells, NO activates K_{ATP} channels via reduction of ATP production [55], while in vascular smooth muscle, NO apparently activates K_{ATP} channels via

a cGMP-dependent mechanism [29, 43]. Bradykinin is also cardioprotective when released locally during ischemia [46, 46]. In addition, as observed before [40], preconditioning leads to a significant increase in tissue cGMP. It is, therefore, particularly important to know whether there is any interaction between protein kinase G (PKG) and K_{ATP} channel in the heart. (b) acetylcholine, whose binding to muscarinic receptors may activate cardiac K_{ATP} channels via second-messenger pathways coupled to protein kinase C (PKC); recently PKC has been implicated in ischemic preconditioning, and there is evidence that PKC's action involves activation of K_{ATP} channels. However, there have been little direct observations that PKC can activate single cardiac K_{ATP} channels at physiological levels of ATP, nor is there knowledge of a specific mechanism by which this may occur. (c) prostacycline and catecholamine; it has been proposed that preconditioning is dependent on the release of prostanoids. Vegh et al [58] suggest that prostacycline release may be important, as it is released from ischemic myocardium and it reduces arrhythmias during ischemia. The mechanism of action remains unclear, but which may involve cAMP-dependent processes [23]. It has been known to activate K_{ATP} channel in coronary vascular smooth muscle [24]. The levels of catecholamine are known to rise during periods of ischemia; It is, therefore, particularly important to know whether there is any interaction between protein kinase A (PKA) and K_{ATP} channel in the heart. (d) adenosine; perhaps the most likely candidate as an endogenous mediator of preconditioning is adenosine. It is released from many cells under stress including myocardial ischemia. It appears likely that adenosine released locally from ischemic myocytes plays role in attenuating ischemic damage (Fig. 2).

Therefore, the present review will mainly concentrate on the mechanism by which biological signal transduction mechanism acts as a link in one or more known receptor-mediated pathways to increase K_{ATP} channel activity during ischemic preconditioning.

K_{ATP} channels and protein kinase G activation

The activation of cardiac muscarinic receptors due to vagal stimulation, the release of myocardial NO and generation of bradykinin during ischemia play roles in ischemic preconditioning [42,46,47,64]. A common mechanism of these findings is a direct or indirect increase in tissue cGMP content. Furthermore, cGMP has also been shown to contribute to the cardioprotective effect against ischemia/reperfusion injury in various species [47]. NO has been known to activate guanylate cyclase and to generate cGMP [68]. It has recently shown that cardiac myocytes express NO synthase not only an inducible but also a constitutive isoform. In fact, recent studies have shown that NO level increases dramatically in the ischemic heart, to reduce both coronary vascular tone and the extent of the ischemia. Furthermore, NO can protect the heart against ischemia-induced reperfusion injury [46]. Thus, one would predict that NO modulates the K_{ATP} channel during ischemia and reperfusion injury. Thus, it seems reasonable that cGMP-mediated intracellular signal transduction plays an important role in the mechanism of ischemic preconditioning. cGMP is a second messenger that mediates a considerable part of its effects by PKG [16,34,35]. PKG is a serine/threonine protein kinase and has been shown to play a role in the mechanism of cardioprotection during ischemia [46,47]. Recently, it has been known that K_{ATP} channel is activated by phosphorylation of serine/threonine residue in rat cardiac myocytes. Indeed, there are potential phosphorylation sites including serine/threonine residues in the cloned K_{ATP} channels [16]. Previous findings raise the intriguing possibility that the myocardial protection afforded by K_{ATP} channel activation may involve phosphorylation of K_{ATP} channels by PKG during ischemia. Recently it was demonstrated that NO donors and PKG activators potentiated the pinacidil-induced K_{ATP} channel activity (Fig. 3), and that PKG inhibitors and protein phosphatase 2A (PP2A) inhibited the PKG-mediated K_{ATP} channel activity (Fig. 4). These results suggest that PKG is involved in the phosphorylation of K_{ATP} channel or an associated protein [16]. Such results may be important in understanding the mechanism by which PKG-signaling pathway acts as a link in receptor-mediated increase in K_{ATP} channel activity during ischemic preconditioning.

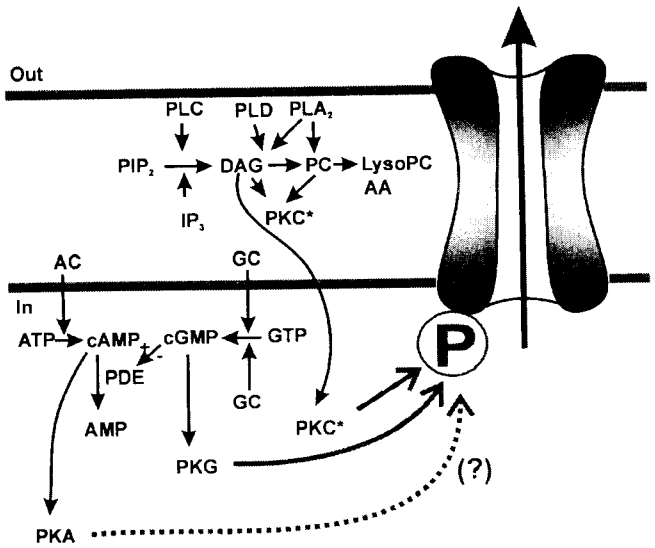

Fig. 2. Schematic diagram illustrating relationship between the stimulation of a variety of receptors and the modulation of K_{ATP} channel. K_{ATP} channel has been regulated by three kinds of signal transduction pathway: 1) that involves AC and PKA, 2) that involves PKC, 3) that involves GC and PKG. AC, adenylate cyclase; AC, adenyl cyclase; GC, guanyl cyclase; PDE, cGMP-activated (+) or inhibited (-) phosphodiesterase; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; AA, arachidonic acid; PC, phosphatidylcholine; LysoPC, lysophosphatidylcholine; IP_3, inositol 1, 4,5-triphosphate; PIP_2, phosphatidylinositol 4,5-biphosphate; DAG, diacylglycerol; PKA, protein kinase A; PKG, protein kinase G; PKC, protein kinase C.

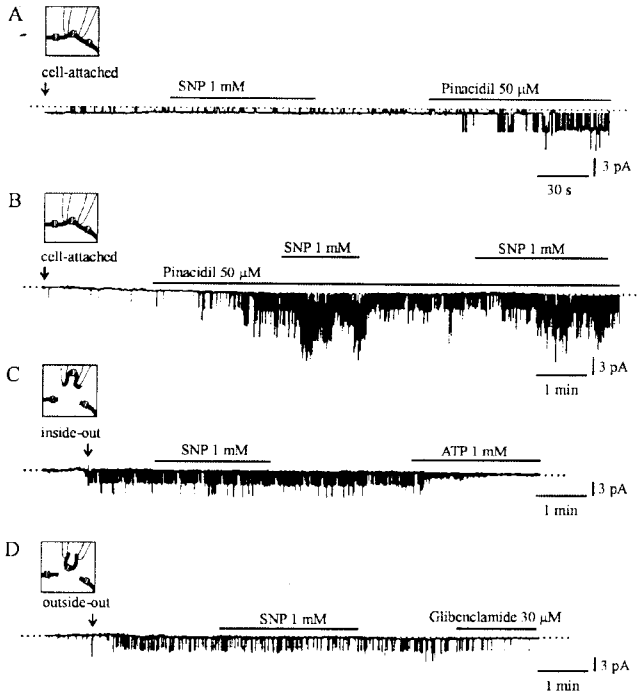

Fig. 3. The effect of SNP on the K_{ATP} channel activity in rabbit ventricular myocytes. Pinacidil, SNP, ATP, and glibenclamide were added to the bath solution for the periods indicated by the bars. A. Reversible activating effect of 1 mM SNP on the pinacidil-induced K_{ATP} channel activity. The pipette potential was held at 40 mV in cell-attached patches. B. The effect of SNP on the K_{ATP} channel activity in inside-out patches held at -40 mV. C. The effect of SNP on the K_{ATP} channel activity in outside-out patches held at -40 mV. Data were sampled at 20 kHz and filtered at 1 kHz. Dashed line indicates the zero current level.

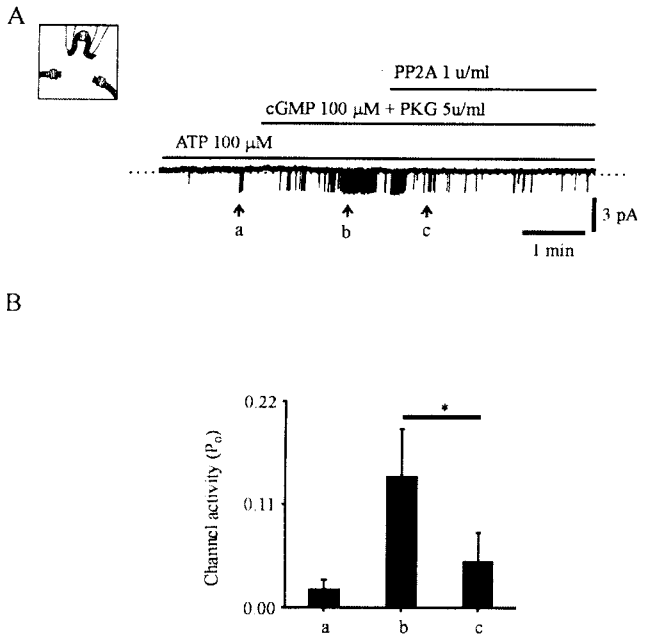

Fig. 4. Effect of exogenous protein phosphatase 2A (PP2A) on the K_{ATP} channel activity stimulated by PKG in rabbit ventricular myocytes. A. Current recording from an inside-out patch held at -40 mV. In the presence of ATP, cGMP and PKG caused an increase in the channel activity. In the same patch, PP2A was added to bath solution. PP2A caused an inhibition of PKG activation-induced channel activity. Data were sampled at 20 kHz and filtered at 1 kHz. Dashed line indicates the zero current level. Channel activities of the records at the times marked by are shown in B. B. Change of channel activity in response to PP2A in inside-out patches. Histogram showing the pooled data (mean ± S.E.) for P_o for the following conditions: ATP alone (a), PKG activation (b), and additional application of PP2A (c). *Significant ($P < 0.05$) difference from control (before application of PP2A) value.

It has been proposed that cAMP produced in response to activators of adenylate cyclase can cause cross-activation of PKG [34]. Consistent with this hypothesis, Jiang et al. [26] have found that forskolin and isoprenaline increased cAMP concentrations to levels which could activate PKG in pig coronary vascular smooth muscle. More recently, it has been suggested that PKA activation by low concentrations of cAMP, and PKG activation by higher concentrations of cAMP could account for the biphasic action of forskolin and membrane-permeable analogues of cAMP on L-type calcium current in canine colonic myocytes [28]. In addition, the nitrosovasodilator sodium nitroprusside (SNP) is known to activate PKG [35], and has been found to produce glibenclamide-sensitive membrane hyperpolarizations in rabbit mesenteric arteries [43]. However, in guinea-pig coronary arterial smooth muscle [63], SNP did not increase K_{ATP} currents but adenylate cyclase activators activated K_{ATP} current. In follicle-enclosed Xenopus oocytes, ANF potentiated glibenclamide-sensitive K^+ currents via the activation of receptor guanylate cyclase and consequent accumulation of cGMP [18]. Thus, it appears likely that the effects of cGMP and PKG on K_{ATP} channel function are tissue specific and depend on the signaling pathway to which PKG activation is linked.

PKG-signal pathway regulates conductive pathway in other cells. It appears to play an important role in the mediation of the actions of a number of pharmacological and endogenous, atrial natriuretic factor vasodilators by activating Ca^{2+}-activated K^+ channels [68]. In snail neurons it potentiates the serotonin-induced macroscopic calcium current and it increases the input resistance in neurons from the mammalian motor cortex. It also inhibits or activates L-type calcium current in cardiac myocytes.

K_{ATP} channels and protein kinase C activation

PKC is a family of at least 12 serine/threonine kinase, many of which are present in rabbit heart [48]. Studies in the rabbit by Ytrehus et al [67] and in the rat by Mitchell et al [41] simultaneously concluded that PKC activation is central to protection by ischemic preconditioning. They showed that PKC inhibitors block the protection of ischemic

preconditioning. Conversely, infusion of either phorbol 12-myristate 13-acetate (PMA) or the DAG (1-oleoyl-2-acetyl-sn-glycerol), two activators of PKC, in lieu of the brief ischemia, is just as protective as ischemic preconditioning. Preconditioning can be blocked by a variety of PKC inhibitors including polymyxin B [67] and chelerythrine [36]. Similar results were obtained in isolated human cardiac myocytes [21,51]. Therefore, preconditioning in human tissue also requires PKC activation. These data support a central role for PKC in the ischemic preconditioning signaling cascade and are consistent with observations that all PKC-coupled receptors are capable of triggering preconditioning.

Previous studies have associated activation of both K_{ATP} channels [46,47] and PKC [33,37,51,67,39] with the process of ischemic preconditioning. More specifically, several investigators have suggested recently that the K_{ATP} channels may be a link in a signaling pathway by which activation of PKC triggers ischemic preconditioning [25,51,57], even in human heart [51]. Most studies on this issue have been performed at the whole-heart level. Hu et al [20] and we showed that PKC directly activated single cardiac K_{ATP} channels at physiological levels of ATP (Fig. 5). However, Hu et al [20] reported that the PKC activator PDD did not activate K_{ATP} channels with ATP concentration of > 1 mM. It is possible that the twofold to fourfold increase over the K_{ATP} channel activity expected at millimolar ATP levels may not have been detected in their whole-cell recordings, because the expected change in whole-cell current is similar in magnitude (~100 pA) to the SEs in their collected data. Thus, we did not consider the data of Hu et al [20] to be in conflict with our own. In another recent study, Liu et al [38] demonstrated at the whole-cell level that PKC is capable of activating K_{ATP} channels. These two studies independently support our findings that PKC is capable of activating K_{ATP} channels from rabbit ventricular myocytes. We demonstrate the following points: (a) okadaic acid (OA) prevents the spontaneous reversal of PKC-induced activation of K_{ATP} channels; (b) Application of exogenous PP2A in the presence of PKC reverses the PKC-induced activation of K_{ATP} channels (Fig. 6). Taken together, these data suggest that an endogenous membrane-associated PP2A is responsible for the reversal of PKC-induced activation of K_{ATP} channels. This is accordance with our previous findings and implies that at physiological levels of ATP, ventricular K_{ATP} channels are under the control of both PKC and PP2A. Thus, these processes of phosphorylation and dephosphorylation could dynamically regulate the activity of K_{ATP} channels in the myocardium and provide a mechanism by which K_{ATP} channel activity and hence cellular excitability can be reversibly controlled.

It has been reported that PKC can either inhibit or activate K_{ATP} channels from insulin-secreting cell lines, depending on the time course of experiments [9]. It has also been demonstrated that PKC activates K_{ATP} channels from insulin-secreting cell lines via somatostatin receptor stimulation

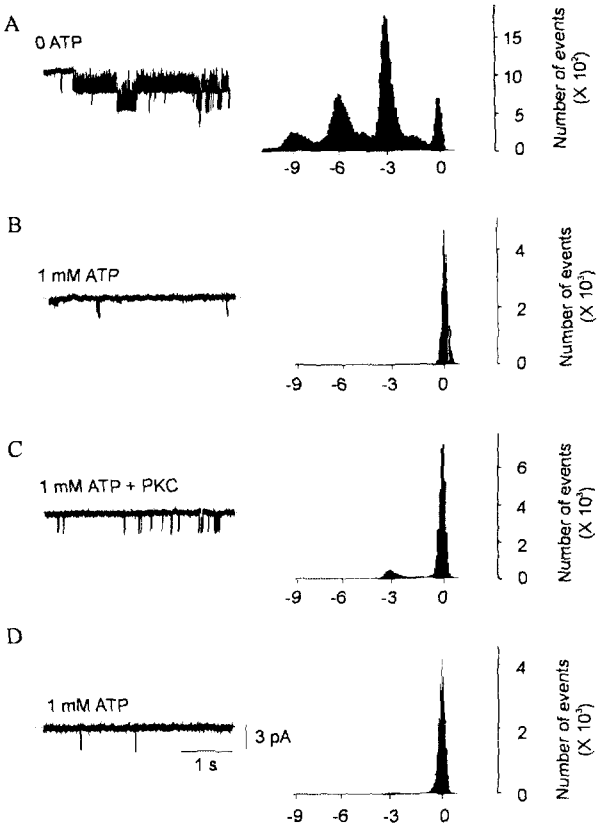

Fig. 5. Effects of PKC on the activity of K_{ATP} channel in the presence of 1 mM ATP. Single-channel current traces and accompanying all-points histograms obtained from an inside-out patch configuration are shown in control (ATP-free, A), in the presence of 1 mM ATP (B), in 20 nM PKC in the presence of 1 mM ATP (C) and after washing out the PKC (D). Membrane potential was held at 40 mV, and single-channel currents are indicated by downward deflections. Zero current levels are indicated by dashed lines. Histograms in A, B, C and D are obtained from a current recording duration of 45 sec.

coupled to G proteins [49]. In follicle-enclosed oocytes [19], smooth muscle [4], and kidney [61], activation of PKC, induced by acetylcholine [4,19] or bradykinin [61], leads to an inhibition of K_{ATP} channel activity. The results from our present study demonstrate that PKC is capable of activating ventricular K_{ATP} channel at near physiological levels of ATP. Thus, it appears that the effects of PKC on K_{ATP} channel function are tissue specific and depend on the signaling pathway to which PKC activation is linked.

In conclusion, it seems likely that K_{ATP} channels can be regulated by several intracellular signaling pathways, which act via PKC-dependent phosphorylation may provide a link in one or more of the signaling pathways that trigger ischemic preconditioning.

K_{ATP} channels and protein kinase A activation

Since both K_{ATP} channel activation and high circulating

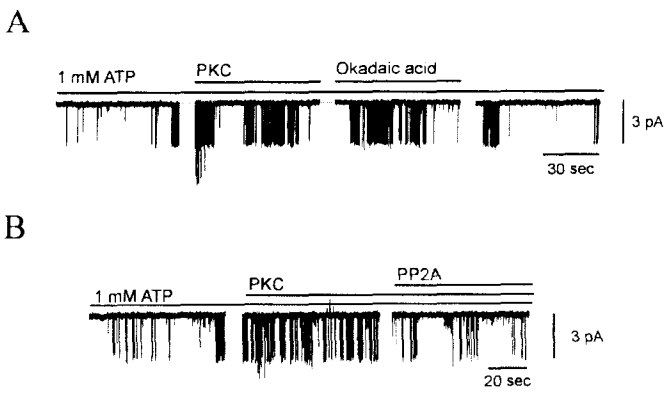

Fig. 6. A. Effect of okadaic acid (OA) on the spontaneous reversal of PKC-induced activation of K_{ATP} channel. Current recording from an inside-out patch configuration held at 40 mV. Addition of 1 mM ATP to the internal side of the patch reduced activity of K_{ATP} channels present. Application of PKC (20 nM) caused a significant increase in K_{ATP} channel activity. Upon removal of PKC and exposure to OA (5 nM), activation of K_{ATP} channel activity persisted. Removal of OA resulted in K_{ATP} channel activity returning to pretreatment levels. B. Effect of exogenous PP2A on the K_{ATP} channel activity stimulated by PKC in rabbit ventricular myocytes. A. Current recording from an inside-out patch configuration held at 40 mV. In the presence of 1 mM ATP, PKC (20 nM) caused an increase in the channel activity. In the same patch, PP2A (1U/ml) was added to bath solution in the presence of PKC. Note that PP2A reversed the stimulatory effects of PKC on K_{ATP} channel activity. Data were sampled at 400 Hz and filtered at 1 kHz. Dashed line indicates the zero current level.

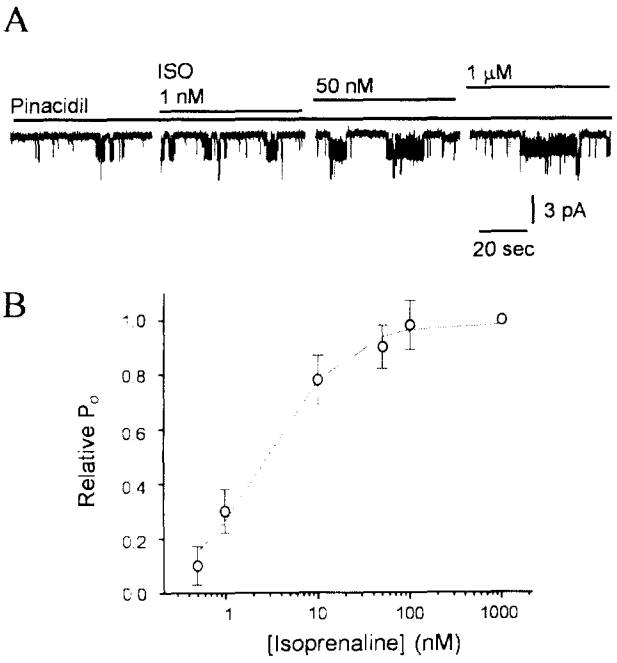

Fig. 7. Effect of varying concentrations of isoprenaline (ISO) on pinacidil-induced single-channel activity. Single-channel current trace obtained from a cell-attached patch configuration held at 40 mV. Application of pinacidil caused an increase in K_{ATP} channel activity. A. Representative records of the effects of varying concentrations of ISO (0.3-1000 nM) on pinacidil-induced single- channel activity. Data were sampled at 400 Hz and filtered at 1 kHz. Dashed line indicates the zero current level. B. Summarized data for the effects of varying concentrations of ISO on pinacidil-induced single channel activity plotted as a dose-response relationship on a semilogarithmic scale. Mean increases in pinacidil-induced single channel activity for groups of cells exposed to concentrations of ISO ranging from 0.3 to 1000 nM were plotted as symbols (o); error bars represent S.E.

levels of catecholamines might simultaneously occur during myocardial ischemia, it is possible to predict the proarrhythmic effects of K_{ATP} channels and the known arrhythmogenic effects of heightened sympathetic tone during ischemic injury [47]. A reported effect of β-agonists on modulation of K_{ATP} channels in heart is poorly understood and yet potentially of considerable importance. A previous study describing the effect of isoprenaline on stimulation of pinacidil-induced K_{ATP} channels in canine ventricular myocytes internally dialyzed with high levels of intracellular ATP has provided indirect evidence that a cAMP-dependent mechanism might be capable of mediating -stimulation of the K_{ATP} channels activated by K^+ channel opener [54]. We also found that pinacidil-induced single-channel activity can be stimulated by β-receptor agonists in rabbit ventricular myocytes (Fig. 7). The mechanism of this effect is apparently quite different from cAMP-dependent stimulation shown in a previous study (Tseng & Hoffman, 1990), although the difference between the two findings should not be surprising considering the markedly different conditions used to elicit K_{ATP} channel in their studies. Tseng & Hoffman [54] used high levels of ATP in the intracellular dialysate and K^+ channel opening agents, Some experiments in our study were also used K^+ channel opening agents in the cell-attached patches. This distinction is an important one, since it is known that the use of K^+ channel openers to elicit K_{ATP} channel while intracellular ATP levels are high may alter various basic channel properties. Two examples that illustrate this problem are (a) the ATP sensitivity of the channel is known to be decreased by K^+ channel opener [10]; and (b) a sensitivity to block by micromolar concentrations of extracellularly applied Cd^{2+} is conferred upon K_{ATP} channels by K^+ channel openers such as pinacidil, a property that is not found in native channels that are opened by low intracellular ATP [30]. Additionally, some experiments in the present study were performed using the cell-attached patch configuration of the patch-clamp technique [7,14] (Fig. 8) in order to avoid disruption of native K_{ATP} channels and any associated metabolic or regulatory apparatus that might possibly occur upon either (a) excision of membrane patches to achieve an inside-out recording configuration [52], or (b) permeabilization of large areas of sarcolemma with an agent such as saponin to achieve an open-cell attached recording configuration [44]. Under these conditions, K_{ATP} channel current developed gradually over the course

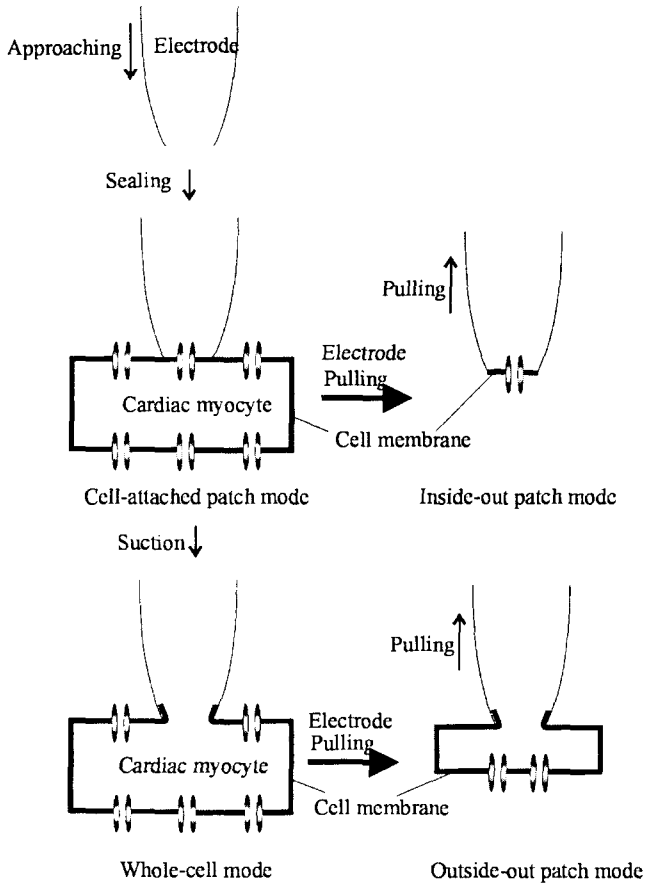

Fig. 8. Diagram illustrating the methods of making cell-attached, inside-out, whole-cell and outside-out patch configurations. Cell-attached recording is mainly used when the channel type in question requires unknown cytosolic factors for gating and these would be lost following patch excision. Inside-out patches were made by pulling the membrane patch off the cell into the bath solution. This configuration enables one easily to change the cytosolic side of the patch. Outside-out patch recording allows one easily to change the extracellular side of the patch. More steps are required to reach the outside-out configuration from cell-attached to whole-cell to patch excision.

of 1-2 min of application of pinacidil. The use of metabolic inhibitors such as 2-deoxyglucose of CN^- was avoided because an unpredictably rapid activation of K_{ATP} channel generally ensued.

There are thought to be general mechanisms by which K_{ATP} channel can be stimulated. The first involves a simple decrease in the intracellular ATP concentration ($[ATP]_i$); because the K_{ATP} channel is blocked by intracellular ATP, diminished $[ATP]_i$ will result in augmented K_{ATP} channel [45]. The second general mechanism that could be responsible for an increase in K_{ATP} channel activity involves an agonist-mediated decrease in the sensitivity of the channel to block by intracellular ATP (i.e. a rightward shift in the ATP sensitivity curve); here, assuming that $[ATP]_i$ remains constant, a decrease in ATP sensitivity would result in an enhancement of K_{ATP} channel activity. Examples of agents that are thought to act through such a mechanism include extracellularly applied adenosine (through the A_1-receptor coupled to G_i which is in turn directly coupled to the K_{ATP} channel itself and intracellularly applied nucleoside diphosphates such as ADP and GDP [32]. However, the precise molecular mechanism responsible for the decrease in K_{ATP} channel sensitivity to block by intracellular ATP in response to these experimental maneuvers is presently unknown. The third mechanism that may be responsible for enhancement of K_{ATP} channel activity involves reactivation (perhaps by phosphorylation) of K_{ATP} channels-usually by MgATP or MgADP-that had previously run down as a result of exposure to ATP-free solution; this restoration of channel activity (or increase in the number of available channels) generally occurs without any change in the ATP sensitivity of the channel [52]. Experiments performed in the present study indicate that β-agonists can be stimulated cardiac K_{ATP} channel activity predominantly via the first of the preceding three mechanisms.

The pathway responsible for mediating β-stimulation of K_{ATP} channel activity suggested by the present study is a conventional one that is consistent with known details of functioning second messenger systems in cardiac myocytes as well as in numerous other cell types. In contrast to previous study that suggested that intracellular cAMP (the product of adenylate cyclase activity) may mediate an increase in pinacidil-induced K_{ATP} channel activity in cells with high (5 ~ 10 mM) intracellular ATP levels [54], results presented here suggest that neither cAMP nor PKA is necessary for the increase in K_{ATP} channel activity subsequent to β-stimulation in the cell-attached and inside-out patch configurations. Extracellular application of a membrane-permeable cAMP analogue was by itself incapable of mimicking the isoprenaline response, while block of PKA by its selective inhibitor did not attenuate the β-mediated increase in the K_{ATP} channel activity. The activities of the membrane-permeable cAMP analogue and the PKA inhibitor were easily verified in L-type calcium current recordings obtained from separate groups of 5 mM ATP-dialyzed cells, further validating the interpretation of the results on K_{ATP} channel. It is perhaps not surprising that PKA did not play a role in mediating the isoprenaline-induced increase in K_{ATP} channel activity in our preparation. The validity of the idea that PKA cannot be an important contributor to β-stimulation of K_{ATP} channel activity under the experimental conditions in the present study appears to be borne out by findings showing that isoprenaline caused in an increase in pinacidil-induced single-channel activity despite the presence of Rp-cAMPS, a selective PKA inhibitor.

A possible mechanism for β-stimulation of K_{ATP} channel activity in rabbit ventricular myocytes can be proposed. Isoprenaline, acting through the β-receptor, activating G_s which in turn stimulates adenylate cyclase. Activated adenylate cyclase then depletes ATP (its substrate) near the subsarcolemmal surface, which results in increased K_{ATP}

channel activity as a consequence of relief of ATP-dependent channel block. No evidence was found for (a) PKA-mediated K_{ATP} channel stimulation; (b) a direct stimulatory effect of a cAMP analogue on K_{ATP} channel activity (Fig. 9).

K_{ATP} channels and adenosine A_1 receptor activation

Although the mechanism of ischemic preconditioning is unknown, recent results obtained in rabbits suggest that activation of adenosine A_1 receptors may trigger the protective effect. This hypothesis, first proposed by Liu et al [38], is based on the observations that nonselective adenosine receptor antagonists block the protective effect of ischemic preconditioning in anesthetized rabbits and selective adenosine A_1 receptor agonist mimic preconditioning in perfused rabbit hearts. These studies provide strong evidence that adenosine, which is released from myocytes during ischemia, activates adenosine A_1 receptors, which subsequently mediates preconditioning.

In an animal study, the extracellular adenosine concentration increased to above 10 μM during ischemia [53]. In addition, Driver et al [8] measured the extracellular adenosine concentrations in human undergoing open-heart surgery for ischemic heart disease. They demonstrated that the mean pleural fluid adenosine concentration is 9.45±2.88 μM and the mean adenosine concentration in pericardial fluid is 2.94±0.44 μM. The concentrations of extracellular adenosine reached during ischemia were higher than that (<0.55 μM) measured in nonischemic hearts [17,31]. Adenosine concentrations greater than 1 M have produced activating adenosine receptors *in vitro* [50]. Accordingly, the concentration of extracellular adenosine (20 μM) in our study would be capable of activating adenosine receptors.

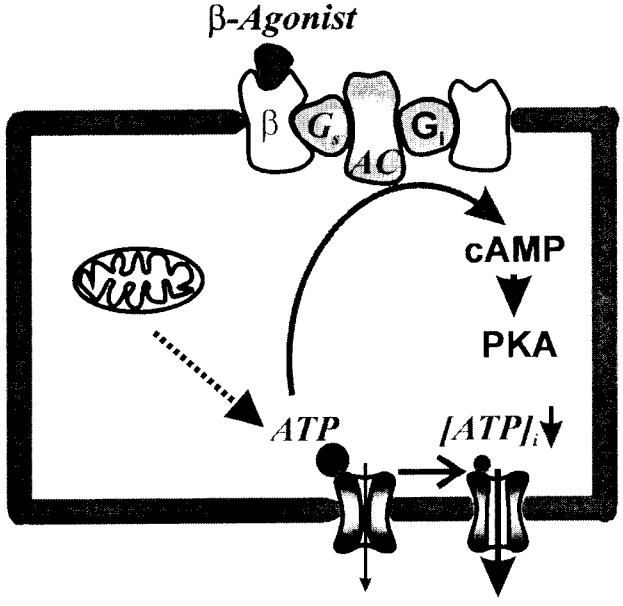

Fig. 9. Proposed mechanism for cAMP-mediated K_{ATP} channel modulation. β-adrenergic agonist, acting through the β- receptor, activating G_s which in turn stimulates adenylate cyclase. Activated adenylate cyclase then depletes ATP (its substrate) near the subsarcolemmal surface, which results in increased K_{ATP} channel activity as a consequence of relief of ATP-dependent channel block.

Activation of K_{ATP} channels in the ischemic myocardium has been associated with cardioprotection due to ischemic preconditioning [2,6,12]. Adenosine could be responsible for such myocardial protection during preconditioning by activating K_{ATP} channels [66]. In this context, it is important to characterize the role of adenosine in regulation of K_{ATP} channel activity.

The first study to suggest that adenosine may act via opening the channels in the myocardium was performed in rat neonatal ventricular myocytes. Kirsch et al [27] showed that K_{ATP} channels are opened by adenosine and the selective adenosine receptor agonist cyclohexylammonium and that the A_1 receptor is coupled to the K_{ATP} channel by a G_i protein. Furthermore, it has been proposed that A_1 adenosine receptors are coupled to the G protein-K_{ATP} channel system in guinea-pig ventricular myocytes [22]. We also demonstrated that pretreatment with the selective adenosine A_1 receptor antagonist 8-cyclopenyl-1,3-dipropylxanthine blocked the effect of adenosine (Fig. 10). Our results in rabbit ventricular myocytes agree with those previously reported by other investigators in different species [22,27]. These results, together with the earlier reports, suggest that the activation of K_{ATP} channels by adenosine is a result of adenosine A_1 receptor activation.

Considering the results whereby preconditioning was shown to be mediated via adenosine A_1 receptor activation, it seems reasonable to hypothesize that adenosine, which is formed during ischemia from the breakdown of ATP, acts on A_1 receptors, which serves to protect the myocardium from a subsequent ischemic insult by activating K_{ATP} channels via a G protein. Our results, at least in part, support the previous studies that implicate adenosine-mediated preconditioning by activating K_{ATP} channels in different species [1,3,38]. Theoretically, activation of K_{ATP} channels may result in favorable metabolic effects. K_{ATP} channel activation has been shown to shortening action potential duration and antagonize membrane depolarization [10,59]. These effects would be expected to reduce the open time of voltage-regulated calcium channels, which would be expected to ultimately lead to reduce free cytosolic calcium levels, a rapid loss of contractile activity, and preservation of ATP, which would be expected to delay cell death.

Activation of the K_{ATP} channel has been thought to be a major component of the increased potassium conductance during myocardial ischemia or hypoxia [10,59]. The mechanism by which activation of the K_{ATP} channel causes metabolic inhibition-induced action potential shortening despite only modest reductions in tissue ATP is not completely understood at the present time. Several possibilities

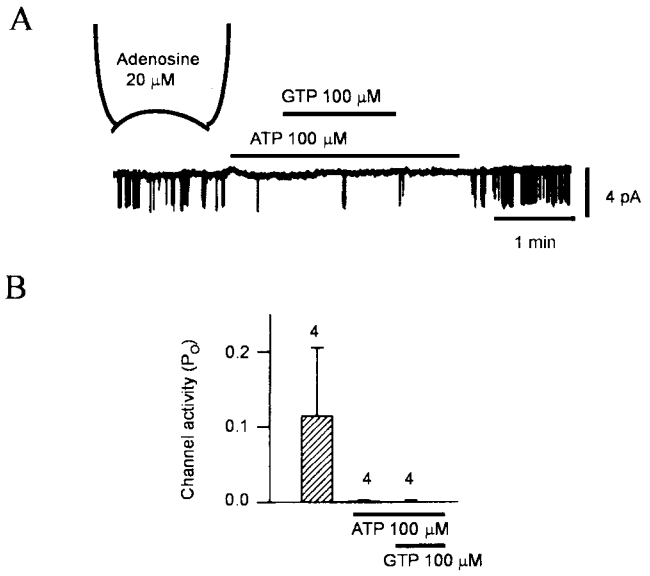

Fig. 10. Effect of adenosine on the K_{ATP} channel activity in the 8-cyclopentyl-1,3,-dipropylxanthine (DPCPX, 1mg/ml, > 7 h)-pretreated cells. Dashed line represents closed level for the K_{ATP} channels; the membrane potential was held at -50 mV; an inward current is downward. Low-pass filter, 1 kHz. Effect of extracellular adenosine on the K_{ATP} channel activity in an inside-out patch. A, continuous recording of single K_{ATP} channel currents in the presence of 20 μM adenosine at the pipette solution. Bath (intracellular) solution was changed sequentially to 100 μM ATP, 100 μM ATP + 100 μM GTP, and back to 100 μM ATP as indicated by horizontal bars. B, histogram showing the effect of extracellular adenosine on the K_{ATP} channel activity in the presence of extracellular adenosine. The numbers on top of each bar indicate the number of experiments.

have been suggested to account for the observed discrepancy. One of them is that some metabolic factors accumulated during ischemia and hypoxia decrease the sensitivity of K_{ATP} channel to ATP. Under the conditions of myocardial ischemia or hypoxia, the intracellular concentrations of H^+, ADP, and GDP all rise and may potentially influence channel activity [11,32,62]. Adenosine A_1 receptor activation caused increased burst duration and decreased the channel sensitivity to ATP. The result indicates that adenosine may also play a significant role in activating K_{ATP} channel even at moderate levels of intracellular ATP.

It is still early to conclude whether adenosine-induced channel activation during myocardial ischemia is physiologically beneficial or it is merely a pathological event. Some have suggested that the activation of K_{ATP} channel is of benefit since it comes into play to prevent irreversible cell damage in the early stage of myocardial ischemia [3,6], others have insisted on its disadvantages because of provoking malignant arrhythmias like ventricular tachycardia and fibrillation during myocardial ischemia [5]. Future studies are, therefore, needed to estimate the relevant effect of adenosine on K_{ATP} channel under myocardial ischemia or hypoxia in terms of physiological significance of cardiac muscle in vivo.

Summary

Following 15 years of research, parts of the signal transduction cascade of ischemic preconditioning have been identified, and there is good agreement on the major endogenous modulators (NO, bradykinin, acetylcholine, prostacycline, catecholamine, adenosine) involved in ischemic preconditioning, although, the importance of different triggers varies among different species. Agreement exists also on the involvement of certain signal transduction mechanisms, although the sequence of their activation appears to be once more species- and model-dependent. Furthermore, it is important to understand the mechanism by which endogenous biological signal transduction mechanism acts as a link in one or more known receptor-mediated pathways to increase K_{ATP} channel activity during ischemic preconditioning. If so, these information will open the way to develop tools for initiating its cardioprotection after ischemia or even prophylactically. If successful, such an intervention will lead to a salutary effect on morbidity and mortality from ischemic heart disease.

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