Brief Review

Roles of kappa opioid receptors in cardioprotection against ischemia —— the signaling mechanisms

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Abstract: There is evidence that the myocytes produce dynorphin and dynorphin-like peptides, which are kappa opioid receptor (κ-OR) agonists. Activation of κ-OR, a dominant opioid receptor in the heart, alters the cardiac function *in vivo* and *in vitro*. The observations suggest that the endogenous κ-opioid peptides may act as autocrines or paracrine in regulation of cardiac functions. Myocardial ischemia is a common cause of heart disorders, which is manifested in decreased myocardial performance, arrhythmia and infarct. When myocardial ischemia occurs, the sympathetic discharge increases, which in turn increases the work-load and oxygen consumption. This exacerbates the situation induced by ischemia. One of the mechanisms with which the body protects against ischemia-induced injury/arrhythmia is inhibition of stimulation of β-adrenoceptor (β-AR), the receptor mediating the actions of sympathetic stimulation. κ-Opioids inhibit the β-AR activation. The inhibition of the β-AR activation is due to inhibition of Gs-protein and to a lesser extent the adenylyl cyclase of the signaling pathway mediating β-AR stimulation by a pertussis sensitive G-protein that mediates κ-OR activation. Another mechanism against ischemia-induced injury is preconditioning, which is defined as prior exposures to ischemia or other insults make the heart more tolerant to subsequent and more severe insults. Protection occurs immediately or 1 - 3 days after preconditioning. κ-OR mediates protection of preconditioning with ischemia or metabolic inhibition, one of the consequences of ischemia, in the heart. Activation of κ-OR by U50488H, a selective κ-OR agonist (pharmacological preconditioning with U50488H, UP), activates protein kinase C (PKC), opens K_{ATP} channels and increases the production of heat shock proteins. Blockade of PKC, or closing of the K_{ATP} channels or inhibition of the synthesis of the heat shock protein abolishes the cardioprotection of UP. The findings indicate the important roles of PKC, the K_{ATP} channels and the heat shock protein in cardioprotection of UP. In addition, UP also attenuates the Ca^{2+} overload, a precipitating cause of cardiac injury, induced by ischemic insults, indicating that UP may confer cardioprotection via at least partly attenuating the Ca^{2+} overload. Most interestingly, blockade of the K_{ATP} channels with channel blockers, that abolishes the delayed cardioprotection of UP, also attenuates the inhibitory effect of UP on Ca^{2+} overload, suggesting that the cardioprotective effect of opening of the K_{ATP} channels may be due at least partly to the prevention/attenuation of Ca^{2+} overload.

Key words: kappa opioid receptor; myocardial ischemia; β-adrenoceptor; ischemic preconditioning

Kappa 阿片受体的抗缺血性心脏保护作用——信息机制

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摘　要： 有证据表明，心脏细胞产生强啡肽和强啡肽类多肽，它们是 kappa 阿片受体 (κ-OR) 的激动剂。κ-OR 是心脏一种优势的阿片受体，其激活可改变在体和离体心脏的功能。在正常和病理情况下，内源性 κ-阿片肽可能通过自分泌或旁分泌的方式调节心脏功能。心肌缺血是导致心脏功能紊乱的一个常见原因，主要表现为心肌功能减弱，心律失常及心肌梗塞等。心肌缺血时，交感神经发放增强，从而增加作功负荷及氧消耗量；而这又使缺血引发的状况更为恶化。机体抵抗缺血引发心肌损害/心律失常的保护机制之一是抑制 β-肾上腺素受体 (β-AR) 的兴奋。κ-OR 确实能抑制 β-AR 的激动。这种抑制主要是由于 GS 蛋白受到抑制，也在较小程度上由于信息通路的腺苷酸环化酶的抑制。因为该种酶能通过对百日咳毒素敏感的 G 蛋白转导 β-AR 的激动。另一保护心肌对抗缺血性损害的机制是预

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处理。预处理是指预先受到缺血等损伤使心脏对随后更严重的损伤产生较强的耐受能力。这种保护作用可以在预处理后即时产生,也可延至预处理后 1-3 天。在采用缺血或其产生的后果之一——代谢抑制作为预处理而致的心脏保护中,κ2OR 参与媒介预处理的作用。用 κ2OR 的特异性激动剂 U50488H 激活 κ2OR (U50488H 药理性预处理,UP) 可激活蛋白激酶 C (PKC),开放 ATP 敏感的钾通道 (K_{ATP} channels) 及增加热休克蛋白 (HSP) 的产生。阻断 PKC 的作用,关闭 K_{ATP} 通道或抑制 HSP 的合成,均可消除 UP 的心脏保护作用。这些发现表明,PKC、K_{ATP} 通道和 HSP 在 UP 的心脏保护中均具重要作用。此外,UP 也能减低缺血造成心肌损害的因素之一,即 Ca^{2+} 的超负荷。这个事实表明 UP 发挥心脏保护作用至少部分地是通过减低 Ca^{2+} 的超负荷。最有趣的是,以阻断剂阻塞 K_{ATP} 通道,在消除 UP 的延迟性心脏保护作用的同时也降低了 UP 对 Ca^{2+} 超负荷的抑制作用。这个事实揭示了 K_{ATP} 通道开放所致的心脏保护作用至少部分地可能是由于防止或减低了 Ca^{2+} 的超负荷。

关键词: kappa 阿片受体; 心肌缺血; β2肾上腺素受体; 缺血预处理
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Receptor binding studies showed that kappa2opioid receptor (κ2OR) is a predominant opioid receptor in the heart[1,2]. Functional studies also showed that activation of κ2OR with its selective agonists triggers cardiac responses, which is blocked by selective κ2OR antagonists *in vitro*[3-6]. The observations indicate that κ2OR may play an important role in the regulation of cardiac function. The presence of dynorphin and dynorphin2like peptides, which are selective κ2OR agonists, in the heart[7], and the expression of the mRNA of the precursor prodynorphin of these peptides in the cultured myocytes[8] indicate that the κ2opioid peptides are synthesized in the heart. The findings suggest that the opioid peptides may play an important role in the regulation of cardiac functions as autocrines and/or paracrines via the κ2OR.

Myocardial ischemia leads to anoxia/hypoxia, hyperkalaemia, acidosis and metabolic inhibition, which in turn triggers inflammatory responses and initiates apoptosis. Myocardial ischemia is a common cause of coronary heart diseases, which is manifested by decreased myocardial performance, arrhythmia and myocardial infarct. When myocardial ischemia occurs, the sympathetic activity is increased, a response to ischemia2induced stress. Increased sympathetic activity increases the beating rate and contractility of the heart, which increases the work2load and oxygen consumption. An increase in oxygen consumption at a time when oxygen supply is insufficient exacerbates the damage induced by ischemia. There are mechanisms in the body that counteract the detrimental effects of myocardial ischemia. Of these mechanisms one is inhibition of the sympathetic activity and another cardioprotection of ischemic preconditioning. There is evidence that the κ2opioid peptides and their receptors are involved in both mechanisms. In this article we review the evidence demonstrating the roles of the κ2OR, and the signaling mechanisms.

Inhibition of sympathetic stimulation

When an electrical stimulation is applied to a myocyte, like the arrival of an action potential, the sarcolemmal membrane is depolarized, which leads to opening of the voltage gated L2type Ca^{2+} channel, that allows influx of extracellular Ca^{2+} into the myocyte. The entry of Ca^{2+} triggers a massive release of Ca^{2+} from the sarcoplasmic reticulum (SR), the intracellular Ca^{2+} store, via a Ca^{2+}2induced2Ca^{2+}2release mechanism; the sudden increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) triggers contraction. The release of Ca^{2+} from SR and contraction is enhanced by stimulation of the β2drenoceptor (β2AR). The whole sequence of events could be demonstrated in a single isolated ventricular myocyte in laboratory conditions[9]. In addition it could be demonstrated that shortening of the myocyte in response to electrical stimulation is preceded by a $[Ca^{2+}]_i$ transient, which is enhanced by norepinephrine (NE), an effect abolished by blockade of β2AR with its antagonist, propranolol[9].

In keeping with the well established fact that the effect of β2AR stimulation is mediated via the Gs2protein/adenylyl cyclase (AC) pathway[10], we also demonstrated that β2AR stimulation increases the cAMP accumulation in the rat ventricular myocyte[11]. When NE was administered together with a κ2opioid receptor (κ2OR) agonist, U50488H, at 10^{-8} - 10^{-6} mol/L, a concentration range which itself has no effect, the stimulatory effects of NE on electrically stimulated $[Ca^{2+}]_i$ transient[9] and cAMP accumulation[11] were significantly reduced. The inhibitory effect of U50488H was abolished by blockade of κ2OR with a selective κ2OR antagonist, nor2BNI[9,11]. The observation indicates that β2AR stimulation is inhibited by κ2OR stimulation, cross2talk between β2AR and κ2

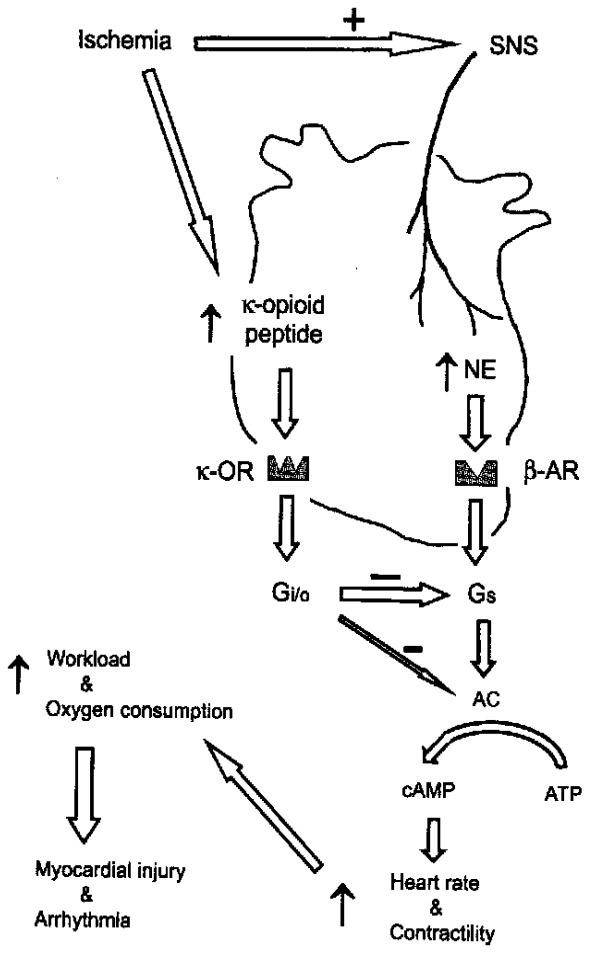

Fig11. Inhibition of β2drenoceptor by κ2opioids receptor during myocardial ischemia. SNS2sympathetic nervous system; NE2noradrenaline; β2AR2β2drenoceptor; κ2OR2kappa o2pioid receptor; AC2adenylyl cyclase; Gi/o2Gi/Go proteins; Gs2Gs protein.

OR (Fig11). A similar cross2talk between β2AR and κ2OR in rat heart has also been observed[12,13]. It was also shown that U50488H inhibited the effect of acti2vation of Gs2protein with cholera toxin[14]. In addi2tion, U50488H inhibited slightly, but significantly, the effect of activation of AC with forskolin[9]. The observations indicate that the cross2talk results mainly from inhibition of the Gs2protein and to a lesser extent is also due to inhibition of AC. The cross2talk was abolished by pertussis toxin (PTX)[9,14], an agent known to inhibit the inhibitory G2proteins. The find2ing indicates that the inhibition of Gs2protein and AC results from activation of a PTX2sensitive G2protein, known to mediate the action of κ2OR stimulation[6].

There is evidence suggesting that during myocardial ischemia there is an increased release of opioid peptides from the heart[3]. So we hypothesized that during myocardial ischemia the opioid peptide released may inhibit the effects of increased sympathetic activity, thus attenuating cardiac arrhythmia. To test the hy2pothesis, we induced arrhythmias with NE in the iso2lated rat heart perfused with a low flow, a situation, which mimics myocardial ischemia. We found that U50488H at 10^{-6} mol/L, which itself had no effect on cardiac rhythm, abolished the arrhythmias induced by NE[11]. The effects of U50488H were abolished by nor2BNI. The finding demonstrates that activation of κ2OR protects the heart against ischemia2induced ar2rhythmias. It is of interest to note that κ2OR ago2nists, U50488H or dynorphin at concentrations higher than 10^{-6} mol/L induces cardiac arrhythmias[3]. It is therefore likely that during myocardial ischemia there is an increased release of the κ2opioid peptides. These peptides may inhibit the β2AR, thus reducing arrhyth2mias. However, if ischemia is severe and prolonged, excessive amount of κ2opioid peptides may be released, which may induce arrhythmias.

Cardioprotection of ischemic preconditioning

In 1986 Murry[15] and co2workers first discovered that brief exposures of a heart to ischemia make the heart more tolerant to subsequent and more severe is2chemic insults. This phenomenon is termed cardiopro2tection of ischemic preconditioning. Subsequent stud2ies showed that preconditioning with one of the conse2quences of ischemia such as metabolic inhibition[16,17] or other insult such as heat[18,19] confers protection a2gainst ischemia and *vice versa*, a cross tolerance phe2nomenon. There are two windows of the protection, namely immediate (1 - 3 h after preconditioning)[15] and delayed (12 - 72 h after preconditioning)[20]. The clinical implication of protection by precondition2ing has aroused great enthusiasm in the research of the mechanisms involved. Up to now receptors to a num2ber of endogenous humoral substances such as adeno2sine, catecholamine, acetylcholine and δ2opioid have been shown to mediate the cardioprotection of precon2ditioning[21]. We found that the cardioprotection of preconditioning with ischemia[22] or metabolic in2hibiton[23] was mimicked by pretreatment with U50488H, a selective κ2OR agonist, but antagonized by administration of nor2BNI, a selective κ2OR antag2onist at the time of preconditioning. The observations indicate that κ2OR also mediates cardioprotection of preconditioning. We demonstrated that *in vivo* (Chen and Wong, unpublished result) and *in vitro*[23,24] that prior treatment with a κ2OR agonist, U50488H (UP), conferred the same cardioprotection as with is2chemia[22] or metabolic inhibition[23].

The signaling mechanism responsible for the immediate cardioprotection of preconditioning has been extensively studied. We found that the immediate cardioprotection of UP was abolished with blockade of either protein kinase C (PKC) or the mitochondrial (mito) K_{ATP} channel with selective blockers during preconditioning in the isolated perfused rat heart[22], indicating that both PKC and mito-K_{ATP} channel act to trigger the heart in a preconditioned state, leading to cardioprotection. The observation is in agreement with the well-established roles of these two messengers[25].

The duration of delayed cardioprotection is longer, which is clinically more useful. However the signaling mechanism of delayed cardioprotection has not been as well studied as that of immediate cardioprotection. We have delineated the signaling mechanisms of UP, hoping to provide more information on the signaling mechanism of delayed cardioprotection of preconditioning.

Similar to immediate protection, the delayed cardioprotection of preconditioning with metabolic inhibition (MIP) or with U50488H was abolished when an inhibitor of PKC was administered at the time of preconditioning[23]. The observation indicates that activation of PKC triggers the signaling mechanisms. In a subsequent study we found that both MIP and UP, that conferred delayed cardioprotection, induced an increased expression of PKC-ε and that blockade of the PKC isoform with a selective inhibitor, εV1-2, at the time of preconditioning, abolished not only the increased expression of PKC-ε, but more importantly delayed cardioprotection of preconditioning[26]. The observation indicates that PKC-ε is a trigger of delayed cardioprotection of MIP and UP. This is in agreement with the previous finding that PKC-ε triggers delayed cardioprotection of preconditioning with ischemia[27,28]. It seems that this PKC isoform is a common trigger of delayed cardioprotection of preconditioning of different kinds.

While the mito-K_{ATP} channel is widely believed to play an important role in cardioprotection of preconditioning[25,29-31], the role of sarcolemmal (sarc) K_{ATP} channel is controversial[32-34]. Recently we found that intravenous administration of U50488H to rats led to a reduction in infarct induced by ischemia 24 h later (Chen and Wong, unpublished result), confirming that UP confers delayed cardioprotection demonstrated in an *in vitro* isolated ventricular myocyte preparation[23]. The infarct sparing effect of UP was attenuated when either of the two channels was blocked

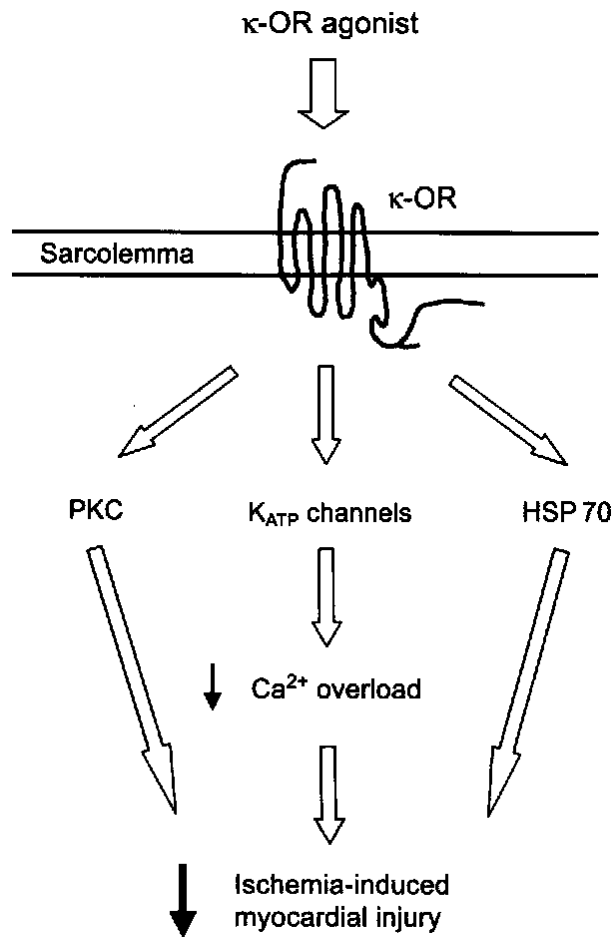

Fig12. Signaling mechanism of delayed cardioprotection of pharmacological preconditioning with U50488H against ischemic insult in cardiomyocytes. κ-OR-kappa opioid receptor; HSP 70-heat shock protein 70; IP-ischemic preconditioning; PKC-protein kinase C; K_{ATP} channels-ATP sensitive potassium channels.

by their selective blockers, namely 5-HD, a selective blocker of mito-K_{ATP} channel or HRM-1098, a selective blocker of sarc-K_{ATP} channel, at the time of preconditioning (Chen and Wong, unpublished result), indicating that both channels act as a trigger of delayed cardioprotection of UP. On the other hand, blockade of mito-K_{ATP} channel, but not sarc-K_{ATP} channel, before ischemia abolishes the delayed cardioprotection of UP (Chen and Wong, unpublished result), indicating that mito-K_{ATP} channel, but not sarc-K_{ATP} channel, is also a mediator/end-effector of cardioprotection of UP. This is in agreement with the previous observation[35].

Heat shock proteins are known to play an important role in cardioprotection[20]. We also found that both UP and MIP increased the expression of an inducible heat shock protein 70 in the heart. More importantly, we found that blockade of synthesis of the protein

with a selective antisense also blocked the delayed pro2 tection[36]. The observations indicate a mediating role of this protein.

It has been shown that cardiac injury is preceded by an intracellular Ca^{2+} ($[Ca^{2+}]_i$) overload upon is2 chemia[37,38]. We also found an increased $[Ca^{2+}]_i$ fol2 lowing metabolic inhibition[24]. More importantly we found that pretreatment with an intraperitoneal injec2 tion of BAPTA2AM, a Ca^{2+} chelate, attenuated the infarct size induced by subsequent more sever myocar2 dial ischemia and reperfusion in the rat (Yan and Wong, unpublished observation). The observations confirmed the belief that $[Ca^{2+}]_i$ overload is a precipi2 tating cause of injury. Recently we observed that in2 travenous administration of U50488H into the rat con2 ferred delayed cardioprotection against ischemic insults and that the delayed cardioprotection was accompanied by attenuation of $[Ca^{2+}]_i$ overload induced by is2 chemic insults (Chen and Wong, unpublished re2 sults). The observation suggests the delayed cardio2 protection of UP may result, at least partly, from at2 tenuation of Ca^{2+} overload induced by ischemic in2 sults. Similar observations have also been reported in immediate cardioprotection of UP against ischemic in2 sults[24].

Interestingly, blockade of mito2 or sarc2K_{ATP} chan2 nels during UP or mito2K_{ATP} channel before ischemic insults, that abolished the delayed cardioprotection of UP, also suppressed the attenuating effect of UP on $[Ca^{2+}]_i$ overload (Chen and Wong, unpublished re2 sults). The observation suggests that the cardiopro2 tective effect of the K_{ATP} channels may result at least partly from attenuation of Ca^{2+} overload (Fig12).

Conclusion

When myocardial ischemia occurs there may be an increased release of κ2opioid peptides in the heart, that would inhibit the harmful action of increased sympa2 thetic activity via activating the κ2OR. Ischemia may also trigger another protective mechanism, cardiopro2 tection of preconditioning, that confers protection when the heart is exposed to a more severe ischemic insult again. κ2OR is one of the receptors that mediate the protection of preconditioning. PKC, K_{ATP} chan2 nels, heat shock protein and intracellular Ca^{2+} are all involved.

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