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Review

The cardioprotection of calcitonin gene-related peptide-mediated preconditioning

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Abstract

Preconditioning induced by brief ischemia or hyperthermia or some drugs shows two phases, early and delayed protection. The cardioprotection afforded by preconditioning is related to stimulation of endogenous mediators release. Calcitonin gene-related peptide (CGRP), a major transmitter of capsaicin-sensitive sensory nerves, has recently been shown to play an important role in mediation of the preconditioning induced by brief ischemia or hyperthermia or by some drugs, and α-CGRP seems to play a major role in the mediation of delayed preconditioning. It has been shown that the cardioprotection afforded by CGRP-mediated preconditioning is due to inhibition of cardiac tumor necrosis factor-α (TNF-α) production, but not to the activation of the K_{ATP} channel. © 2002 Published by Elsevier Science B.V.

Keywords: Preconditioning; CGRP (calcitonin gene-related peptide); Capsaicin; Reperfusion injury; TNF-α (tumor necrosis factor-α)

1. Introduction

Since the term ischemic preconditioning was originally recognized by Murry et al. (1986), considerable progress has been made in understanding this phenomenon. Ischemic preconditioning shows two phases: an early phase of protection termed ''classic preconditioning'', which occurs within minutes and disappears within 2 to 4 h. Subsequently, a delayed phase of protection termed ''second window of protection'' appears 24 h after initial ischemic preconditioning and lasts for 48 to 72 h. Preconditioning of the heart has been exploited from ischemic stimulus to heat stress and some drugs (Pagliaro et al., 2001; Bolli, 2000).

The mechanisms underlying preconditioning have not yet been elucidated but one suggestion is that the protective effect is mediated by endogenous active substances, including neurotransmitters and autocoids (Parratt, 1994). We and others have shown that endogenous calcitonin gene-related peptide (CGRP) plays an important role in mediation of preconditioning (Li et al., 2000).

2. The biology characteristic of CGRP

CGRP is a major transmitter in capsaicin-sensitive sensory nerves and has two isoforms named $CGRP_{\alpha}$ and $CGRP_{\beta}$. $CGRP_{\alpha}$ was cloned in the early 1980s from the gene encoding calcitonin (Amara et al., 1982). In the thyroid, calcitonin is the main product of this gene, whereas, in neural tissues, a novel neuropeptide, $CGRP_{\alpha}$, is generated. A second CGRP homologue, $CGRP_{\beta}$, was later characterized, bearing high sequence homologies with the α-form but that was not derived from the calcitonin gene (Amara et al., 1985). Subsequently, CGRP has been identified and characterized in several mammalian species, including the human (Morris et al., 1984). CGRP is a polypeptide that contains 37 amino acids, with a molecular weight of 3787 for human $CGRP_{\alpha}$, and with marked homology of amino acid sequence between species (Collyear et al., 1991). $CGRP_{\alpha}$ and $CGRP_{\beta}$ differ from each other by only one amino acid in rats, and by three amino acids in humans. In the human, both genes are located on the short arm of chromosome 11 between the catalase and parathyroid hormone genes (Kittur et al., 1985; Hoppener et al., 1985; Alevizaki et al., 1986). The α gene, consisting of six exons, stretches over approximately 6.5 kb and is transcribed in full. The organization of the β gene is similar to that of the α gene, but both 3′ and 5′ non-coding regions of the two genes diverge significantly.

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CGRP interacts with its receptor to produce physiopharmacological effects, such as positive inotropic actions, vasorelaxation, and protective effects of myocytes and endothelial cells (Kallner, 1998). There exist at least two classes of CGRP receptors, $CGRP_1$ and $CGRP_2$ (Dennis et al., 1990; Gardiner et al., 1990). The receptors belong to the rhodopsin-like superfamily of G-protein-coupled receptors (Aiyar et al., 1996). Cardiovascular effects of CGRP are mediated by the $CGRP_1$ receptor, which can be blocked by the CGRP receptor antagonist, CGRP-(8–37).

3. CGRP and ischemic preconditioning

The release of CGRP is regulated by multiple factors, such as transient ischemia, hyperthermia or autocoids, and the elevated level of CGRP during ischemia probably constitutes a compensatory response (Kallner, 1998; Franco-Cereceda, 1988; Kress and Zeilhofer, 1999). Administration of exogenous CGRP has been shown to alleviate the incidence of reperfusion-induced ventricular arrhythmia and to protect the cultured myocyte against hypoxygen injury (Zhang et al., 1994; Ren et al., 1993). It has been reported that pretreatment with high-dose capsaicin to deplete endogenous CGRP exacerbates the ischemia–reperfusion injury in the porcine heart (Kallner and Franco-Cereceda, 1998). Clinical studies have shown that the CGRP concentration in plasma was significantly elevated in patients with myocardial infarction (Lechleitner et al., 1992). These findings suggest that CGRP may be an endogenous myocardium protective substance.

Recently, we and others have shown that CGRP plays an important role in the mediation of ischemic preconditioning (Li et al., 1996; Ferdinandy et al., 1997; Lu et al., 1999). In the isolated perfused rat heart, the CGRP concentration in the coronary effluent was elevated during the preconditioning period. Ischemic preconditioning significantly improved the recovery of cardiac function during reperfusion after 30 min of global ischemia, and this cardioprotection by ischemic preconditioning was abolished by CGRP-(8–37), a selective $CGRP_1$ receptor antagonist. Exogenous CGRP or pretreatment with capsaicin to evoke the release of endogenous CGRP produced a preconditioning-like cardioprotection (Zhou et al., 1999). In rats pretreated with high-dose capsaicin to desensitize sensory nerves, the cardioprotection by pacing-induced preconditioning was abolished (Ferdinandy et al., 1997). The protective effect of ischemic preconditioning in vivo was also abolished by CGRP antibody (Ou-Yang et al., 1999). Clinical studies have shown that the cardioprotection by ischemic preconditioning is associated with the release of CGRP (Lu et al., 1996; Kallner et al., 1999; Li et al., 1999, 2001). Furthermore, delayed cardioprotection or gastroprotection by intestinal or gastric preconditioning is mediated by CGRP (Tang et al., 1999; Xiao et al., 2001; Pajdo et al., 2001).

4. CGRP and heat stress-induced cardioprotection

It has been documented that sublethal hyperthermia is also capable of inducing preconditioning-like protection, including classical or early preconditioning and delayed preconditioning or "second window" protection (Gowda et al., 1998; Yamashita et al., 1998). The mechanism responsible for the beneficial effect of heat stress is still not fully understood. Early studies have found that a stress, cold or heat, is also capable of activating capsaicin-sensitive sensory nerves and stimulating the release of neurotransmitters from their peripheral terminals (Tsuchiya et al., 1996; Kress and Zeilhofer, 1999). Based on the involvement of CGRP in the mediation of ischemic preconditioning, it is likely that the cardioprotection afforded by heat stress is mediated by endogenous CGRP. Recent studies have shown that retrograde hyperthermia reperfusion (42 °C) in the isolated rat heart for 5 min significantly improved the recovery of cardiac function, and decreased the release of creatine kinase concomitantly with an increase in the level of CGRP in coronary effluent. Whole-body hyperthermia for 15 min also induced early and delayed cardioprotection or improved the preservation with cardioplegia and increased the plasma concentration of CGRP, which was abolished by pretreatment with capsaicin to deplete CGRP in sensory nerves (Song et al., 1999a,b).

5. CGRP and pharmacological preconditioning

Substitution of some drugs for ischemic stimulus is also capable of inducing a protection similar to that with ischemic preconditioning and this is described as pharmacological preconditioning. The protective effects of some drug-induced preconditioning have been suggested to be related to stimulation of endogenous active substances. For example, angiotensin-converting enzyme inhibitors or monophospheryl lipid A-induced preconditioning protects the myocardium against damages due to ischemia–reperfusion through stimulation of nitric oxide generation (Jin and Chen, 1998; Zhao et al., 1997; Tosaki et al., 1998; Xi and Kukreja, 2000). Previous investigations have shown that endogenous nitric oxide can regulate the release of CGRP, and one can postulates that the protective effects of some drugs, which evoke the release of nitric oxide or induce the production of nitric oxide, may involve endogenous CGRP. There is evidence that, in isolated rat hearts, pretreatment with nitroglycerin, a nitric oxide donor, for 5 min significantly attenuates ischemia–reperfusion injury and increases the release of CGRP in coronary effluent. The effects of nitroglycerin are abolished by CGRP-(8–37) or pretreatment with capsaicin (Hu et al., 1999). Studies in vivo have shown that nitroglycerin-induced delayed preconditioning is also mediated by CGRP (Zhou et al., 2001). A similar effect has been seen in the rat small intestine (Dun et al., 2001).

Involvement of endogenous nitric oxide in monophospheryl lipid A-induced delayed protection has been demonstrated in different animal species (Tosaki et al., 1998; Yoshida et al., 2000). Our recent study has shown that monophospheryl lipid A-induced delayed preconditioning enhances preservation with cardioplegia and that the protective effects of monophospheryl lipid A are related to stimulation of CGRP release via the nitric oxide (NO) pathway (He et al., 2001). In order to test whether the increase of CGRP with monophospheryl lipid A is secondary to stimulation of CGRP synthesis, the expression of CGRP in lumbar dorsal root ganglia was measured. Monophospheryl lipid A-induced delayed preconditioning reduced infarct size and creatine kinase release concomitantly with an increase in the release and synthesis of CGRP, which was abolished by pretreatment with the nitric oxide synthase inhibitor, L-nitroarginine methyl ester, or the heme oxygenase-1 inhibitor, Zinc protoporphyrin IX (Peng et al., 2002b). These findings further support the conclusion that preconditioning mediated by nitric oxide, endogenous or exogenous, is related to the stimulation of the release and synthesis of CGRP.

6. Mechanisms of CGRP-mediated cardioprotection

The exact mechanisms responsible for the protective effects of CGRP remain unclear. Endogenous mediators including neurotransmitters bind to specific receptors and then activate the endogenous protective mechanisms via complex signal pathways, which are related to the activation of protein kinase C or ATP-sensitive K+ (K_{ATP}) channels. There is evidence to suggest that CGRP enhances the activity of protein kinase C and activates K_{ATP} channels in vascular smooth muscle (Bell et al., 1995; Wellman et al., 1998). It is probable that CGRP-mediated preconditioning activates protein kinase C and/or K_{ATP} channels. It has been shown that exogenous CGRP or pretreatment with capsaicin to evoke the release of endogenous CGRP produces preconditioning-like cardioprotection, an effect which can be abolished by the protein kinase C inhibitor, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (Peng et al., 1996). However, the cardioprotection afforded by CGRP-mediated preconditioning is not affected by glibenclamide, a blocker of K_{ATP} channels. Recently, it has been shown that ischemic preconditioning or exogenous CGRP-induced preconditioning dramatically reduces cardiac tumor necrosis factor-α (TNF-α) production, an ultimate effector in signal transduction pathways of ischemic preconditioning (Meldrum et al., 1998; Peng et al., 2000; He et al., 2001). These findings suggest that the cardioprotection afforded by CGRP-mediated preconditioning is due to the inhibition of cardiac TNF-α production via protein kinase C signal transduction pathways.

It is noteworthy that, of the two isoforms, α-CGRP seems to play a major role in the mediation of delayed preconditioning. Previous studies suggested that there were no distinguishable differences in biological activity between α- and β-CGRP (McLatchie et al., 1998). However, recent investigations have shown that only α-CGRP elicits effects on axonal transport in sensory neurons (Hiruma et al., 2000), and only α-CGRP mRNA has been detected in the enteric nervous system of rat small intestine (Doi et al., 2000), and only β-CGRP mRNA, but not α-CGRP mRNA, has been detected in rat T lymphocytes (Xing et al., 2000). These findings suggest that there are some unknown differences in biological actions between α- and β-CGRP. Our recent studies have also shown that monophospheryl lipid A or heat stress induces only α-CGRP but not β-CGRP mRNA expression (Peng et al., 2002a,c), suggesting that the cardioprotection afforded by monophospheryl lipid A or heat stress is mainly mediated by the α-CGRP isoform in the rat.

7. Summary

The results from animal experiments and clinical studies have shown that CGRP plays an important role in the mediation of ischemic preconditioning. Endogenous CGRP is also involved in mediation of the preconditioning of the heart induced by heat stress or some drugs. It is likely that CGRP-mediated early preconditioning only involves the release of CGRP, while the development of CGRP-mediated delayed preconditioning is related to the upregulation of α- but not β-CGRP gene expression via NO and/or CO pathways. Up to now, multiple endogenous chemical substances have been reported to participate in mediation of preconditioning. CGRP is one of the endogenous chemical substances participating in the mediation of preconditioning. It is most possible that interaction of peptide neurotransmitters with various autocoids mediates the protective effects of preconditioning.

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