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Review

New insights into nitroglycerin effects and tolerance: Role of calcitonin gene-related peptide

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Abstract

It has been shown that calcitonin gene-relate peptide plays an extensive role in cardiovascular system. CGRP is a potent vasodilator and plays an important role in mediation of nitroglycerin-induced vascular relaxation. Recently, calcitonin gene-relate peptide is emerging as a potential player in nitroglycerin tolerance. There is increasing evidence that the decreased depressor effect of nitroglycerin in tolerant states is closely related to a decrease in calcitonin gene-relate peptide release. The reduced release of calcitonin gene-relate peptide in nitroglycerin tolerance is associated with the decreased nitroglycerin biotransformation due to the mitochondrial dysfunction. Recent work has been shown that the inhibited activity of mitochondrial isoform of aldehyde dehydrogenase and the upregulation of phosphodiesterase 1A1 are the key factors that lead to the decreased nitroglycerin biotransformation in nitroglycerin tolerance, with a subsequently reduced release of calcitonin gene-relate peptide.

Keywords: Nitroglycerin tolerance; CGRP (calcitonin gene-relate peptide); ALDH-2 (aldehyde dehydrogenase); PDE A1 (phosphodiesterase 1A1)

Contents

1. Introduction

Nitroglycerin remains one of the foremost drugs in the treatment of angina pectoris. When given in the short term, nitroglycerin has clear benefits for the treatment of angina pectoris, congestive heart failure and myocardial infarction due to its potent vasodilator capacities on arteries, veins, and coronary collateral vessels. Nitroglycerin induces vasorelaxation by releasing NO, which activates soluble guanylyl cyclase and subsequently increases cGMP. cGMP in turn activates a cGMP-dependent protein kinase that has been shown to mediate vasorelaxation via phosphorylation of proteins that regulate intracellular calcium (Munzel et al., 2005). The chronic efficacy of nitrates, however, is blunted because of the

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development of nitrate tolerance. The mechanisms underlying this phenomenon are still poorly defined. Several mechanisms have been proposed to account for this phenomenon including neurohormonal counterregulatory mechanisms, increases in activity of the phosphodiesterase 1A1, desensitization of the sGC, increases in production of reactive oxygen species, and impairment of nitroglycerin biotransformation, which have been intensively discussed by others (Csont and Ferdinandy, 2005; Gori and Parker, 2002; Kim et al., 2001; Munzel et al., 1996; Rutherford, 1995). Calcitonin gene-related peptide (CGRP), a very potent vasodilator, which plays an extensive role in cardiovascular system, is recently emerging as a potential player in nitroglycerin tolerance (Ghatta and O'Rourke, 2006; Munzel et al., 2005; Oroszi et al., 1999; Zhou et al., 2001b; Zhou et al., 2002). The present review mainly focused on the evidence for the role of CGRP involved in nitroglycerin tolerance and its possible mechanisms.

2. The biological properties of CGRP

CGRP is a 37-amino acid residue vasoactive neuropeptide. There are two isoforms named α-CGRP and β-CGRP. Alpha-CGRP was cloned in the early 1980s from the gene encoding calcitonin. The second CGRP homologue, β-CGRP, was later characterized, bearing high sequence homologies with α-CGRP but it derived from a different gene. The two CGRP isoforms, α and β in rats and I and II in humans, differ in their peptide sequences by one and three amino acids, respectively, and the biological activities of the two peptides are quite similar in most vascular beds (Bell and McDermott, 1996; Li and Peng, 2002).

CGRP is widely distributed in the nervous and cardiovascular systems. In the peripheral nervous system, the prominent site of CGRP synthesis is the dorsal root ganglion (DRG), which contains the cell bodies of capsaicin-sensitive sensory neurons. The peripheral processes of these sensory neurons terminate on blood vessels and transport CGRP to the nerve endings. Most blood vessels are surrounded by a dense perivascular CGRP neural network, which plays an important role in modulation the tension of resistance vessels (Bell and McDermott, 1996). By interaction with its receptors, CGRP produces physiological functions, such as positive inotropic actions, vasorelaxation. There are two CGRP receptors, CGRP1 and CGRP2, have been identified so far. The receptors belong to the rhodopsin-like superfamily of G-protein-coupled receptors. Cardiovascular effects of CGRP are mediated by the CGRP1 receptor, which can be blocked by the CGRP receptor antagonist, CGRP-(8–37). Circulating CGRP is thought to be from the perivascular nerve terminals via a spillover manner to promote vasodilation or other functions. The half-life of CGRP in mammalian plasma is approximately 10 min, where it is cleaved and then inactivated by the neutral endopeptidase, tryptase and chymotrypsin (Bell and McDermott, 1996; Deng and Li, 2005; Dennis et al., 1990).

3. The role of CGRP in modulation of vascular tone

It is well known that resistance vessels play a key role in regulation of blood pressure and local organ blood flow. The blood vessels are widely innervated both by sympathetic and capsaicin-sensitive sensory nerves, which play significant roles in controlling the resistance vascular tone through the release of two classes of vasoactive neurotransmitters, vasoconstrictors and vasodilators (Bevan and Brayden, 1987). CGRP is the most potent vasodilator to date. It is approximately 100–1000 times more potent than other vasodilators such as adenosine, substance P or acetylcholine (Brain et al., 1985; DiPette et al., 1989). There is plenty of evidence suggest that CGRP plays an important role in regulating vascular resistance and regional organ blood flow under both physiological and pathophysiological conditions (Maggi and Meli, 1988). Intracerebroventricular administration of CGRP evokes a transient elevation of mean arterial pressure and sustained tachycardia in rats (Bell and McDermott, 1996). In contrast, systemic administration of CGRP decreases blood pressure in a dose-dependent manner, coupled with a more sustained tachycardia, in normotensive animals and humans or hypertensives (Wimalawansa, 1996). The primary mechanisms underlying the depressor effect of CGRP are peripheral arterial dilation through: (1) reducing intracellular Ca^{2+} via inhibition of Ca^{2+} influx and Ca^{2+} release from sarcoplasmic reticulum; (2) reducing the sensitivity of contractile unit response to Ca^{2+}; (3) increasing intracellular cyclic adenosine monophosphate (cAMP); (4) activating ATP-activated potassium channels; and (5) releasing nitric oxide (NO). The vasodilator response mediated by NO is endothelium dependent, whereas the other mechanisms directly affect the vascular smooth muscle (Bell and McDermott, 1996; Popescu et al., 1985; Wimalawansa, 1996). The mechanisms by which CGRP dilates blood vessels through endothelium-dependent and/or -independent manners are varied in different vascular beds (Bell and McDermott, 1996).

4. The role of CGRP in the mediation of nitroglycerin-induced vascular relaxation

It is thought that nitroglycerin induces vasorelaxation by generating NO. NO activates soluble guanylyl cyclase and subsequently increases cGMP. cGMP in turn activates a cGMP-dependent protein kinase that has been shown to mediate vasorelaxation via phosphorylation of proteins that regulate intracellular Ca^{2+} levels (Munzel et al., 2005). It has been shown that capsaicin-sensitive sensory nerve terminals can release CGRP in response to multiple autocoids including NO (Garry et al., 2000; Hughes and Brain, 1994). There is evidence that nitroglycerin activates sensory nerves fibers to release CGRP in both central and periphery vascular tissues (Booth et al., 2000; Wei et al., 1992). Vasodilator responses to nitroglycerin in feline cerebral arterioles or in rat aortas were associated with endogenous CGRP (Booth et al., 1997, 2000; Wei et al., 1992). Others have reported that L-arginine, a substrate of NO synthase (NOS), enhances the release of CGRP evoked by endotoxin in the isolated mesenteric arterial bed, an effect which is attenuated by N^G-nitro-L-arginine, a selective inhibitor of NOS (Wang et al., 1996). The capsaicin or sodium nitroprusside (NO donor) induced the release of CGRP from the spinal cord was markedly reduced by inhibitors of NOS or hemoglobin, an extracellular scavenger of

NO (Garry et al., 1994, 2000). Clinical studies have shown that in nitroglycerin-induced cluster headache, plasma concentrations of CGRP in the extracerebral circulation were markedly increased (Fanciullacci et al., 1995). During spontaneous migraine attacks, early activation of the L-arginine/NO pathway which accompanies the release of vasoactive peptides (including CGRP) from trigeminal ending may intervene in maintaining the headache phase (Sarchielli et al., 2000).

The aforementioned role of NO in regulating CGRP release is consistent with our recent studies. In the isolated rat aorta, preincubation with nitroglycerin induced significantly release of CGRP and led to vascular relaxation in a concentration-dependent manner, and the effect was attenuated by pretreatment with CGRP-(8–37), a selective CGRP receptor antagonist, or by capsaicin, which specifically depletes the transmitter content of sensory nerves (Booth et al., 2000; Zhou et al., 2002). In agreement with the studies in vitro, studies in vivo have also shown that nitroglycerin caused a significant decrease in blood pressure concomitantly with an increase in the plasma concentration of CGRP. However, desensitizing sensory nerves with capsaicin significantly impaired the depressor effect of nitroglycerin (Zhou et al., 2001a). Collectively, these results support the conclusion that CGRP mediates, at least in part, the nitroglycerin-induced vascular relaxation.

5. The role of CGRP in nitroglycerin tolerance

As mentioned above, nitroglycerin can evoke the release of CGRP from the capsaicin-sensitive sensory nerves through which to exert the depressor effect and vasodilation. It is reasonable to speculate that CGRP may also involve in nitroglycerin tolerance. There is increasing evidence to support the role of endogenous CGRP in nitroglycerin-induced tolerance (Ghatta and O'Rourke, 2006; Oroszi et al., 1999; Zhou et al., 2001b, 2002). It has been shown that tolerance developed after repeated administration of nitroglycerin characterized by a diminished depressor effect concomintantly with a decrease in plasma concentrations of NO and CGRP in rats. The nitroglycerin-induced depressor effect in the tolerant rat was restored after removal of the drug accompanying the elevation of NO and CGRP content, suggesting that the diminished depressor effect of nitroglycerin may be secondary to a reduction of CGRP release (Zhou et al., 2001b). In isolated rat aortic rings, nitroglycerin caused a greater than twofold increase over basal levels in CGRP release in nontolerant rings, which was abolished in rings treated with capsaicin and in nitroglycerin tolerant rings. These results suggest that nitroglycerin releases CGRP from sensory nerves in response to vascular relaxation, and that interference with either the release or action of endogenous CGRP may enhance the extent to which nitroglycerin tolerance occurs (Ghatta and O'Rourke, 2006).

Both the sulfhydryl donor *N*-acetylcysteine and thiol-containing angiotensin converting enzyme inhibitor captopril have been shown to partially reverse the development of tolerance to nitroglycerin in vivo and in vitro (Boesgaard et al., 1994; Kukovetz and Holzmann, 1990; Salvemini et al., 1993). The mechanism for thiols reversing tolerance to nitroglycerin may be associated with the increased formation of NO and in turn the increased release of CGRP. Our recent results have shown that the thiol-containing compounds *N*-acetylcysteine or captopril can partially restore nitroglycerin tolerance and enhanced the plasma content of NO and CGRP (Zhou et al., 2002), suggesting that reversal of tolerance to nitroglycerin with *N*-acetylcysteine or captopril is related to the increased release of CGRP, and further supporting the conclusion that CGRP plays an important role in vasorelaxation of nitroglycerin.

6. Mechanisms underlying the reduced CGRP release in nitroglycerin tolerance

It is well recognized that the mechanisms underlying the nitrate tolerance are multifactorial and the impairment of nitroglycerin biotransformation is responsible for the so called mechanism-based or classical tolerance. There is evidence that the mitochondrial isoform of aldehyde dehydrogenase (ALDH-2) plays a central role in nitroglycerin biotransformation (Chen and Stamler, 2006). In mitochondrial, ALDH-2 catalyzes the conversion of nitroglycerin to 1,2-glyceryl dinitrate and nitrite (Chen et al., 2002; Daiber et al., 2004). Inhibitors of ALDH-2 blocked the vasorelaxation by nitroglycerin that is dependent on cGMP both in vitro and in vivo. Recently, Sydow et al. reported that development of nitroglycerin tolerance was due to the inhibition of ALDH-2 activity concomitantly with the impaired nitroglycerin biotransformation and the increased production of reactive oxygen species by mitochondria (Sydow et al., 2004). In addition, this study has demonstrated that increases in cGMP in response to nitroglycerin were severely blunted in cultured endothelial cells deficient in mitochondria. These findings provide a new paradigm for understanding nitrate tolerance and suggest that mitochondrial dysfunction may be a central step in evolution of nitroglycerin tolerance.

We and others have shown that the decreased depressor effect of nitroglycerin in tolerant states is related to a decrease in CGRP release. Since ALDH-2 plays a key role in nitroglycerin biotransformation, it is likely that the decreased release of CGRP in nitroglycerin tolerance is associated with the reduced ALDH-2 activity. In our recent study, pretreatment with ALDH-2 inhibitors and nitroglycerin significantly attenuated vasodilator responses to nitroglycerin concomitantly with a decrease in the release of CGRP from the isolated thoracic aorta (Chen et al., 2007). In vivo, pretreatment with an inhibitor of ALDH-2 or nitroglycerin for 8 days produced the similar impaired depressor effect accompanying a decrease in plasma concentrations of CGRP (Chen et al., 2007), indicating there is a positive correlation between ALDH-2 activity and CGRP release.

There is plenty evidence that nitroglycerin induces vasorelaxation by activating the target enzyme soluble guanylyl cyclase (sGC) and in turn increasing tissue levels of the second messenger cGMP, and the cGMP-dependent vasodilator effects are significantly attenuated in nitroglycerin tolerance. In the study of Sydow et al., they found that inhibition of ALDH-2 significantly reduced vascular cGMP levels in response to acute nitroglycerin challenge (Sydow et al., 2004). Another study reported that the activity of phosphodiesterase 1A1 (PDE 1A1), which preferentially hydrolyzes cGMP, was significantly increased in nitrate tolerance, and that inhibition of PDE1A1 with vinpocetine, a

selective inhibitor of PDE 1A1, in tolerant vessels restored vasorelaxation and cGMP response to subsequent nitroglycerin exposure (Kim et al., 2001). Our previous work showed that the depressor effect and elevated concentrations of CGRP with nitroglycerin were significantly reduced by methylene blue, an inhibitor of the soluble guanylate cyclase (Zhou et al., 2001a). Interestingly, in our recent study, we have found that decreased depressor and vasodilator effects and the attenuated release of CGRP after nitroglycerin tolerance were restored in the presence of vinpocetine (Nie et al., in press). Taken together, these findings suggest that long-term nitroglycerin treatment decreases the release of CGRP either through the decreased production (reduced ALDH-2 activity leads to reduced biotransformation of nitroglycerin) or increased hydrolyzation (upregulation of PDE 1A1) of cGMP, resulting in attenuation of the hypotensive effect.

Considerable evidence shows that nitrate tolerance is closely related to oxidative stress induced by an increased production of reactive oxygen species. It is probable that nitroglycerin induces the production of reactive oxygen species, with a subsequent decrease in mitochondrial ALDH-2 activity and CGRP release, resulting in nitrate tolerance. In a recent study with cultured endothelial cells, *N*-acetylcysteine or captopril, which has antioxidant activity, reversed the tolerance to nitroglycerin concomitantly with an increase in the release of CGRP and an increase in the activity of mitochondrial ALDH-2 by decreasing reactive oxygen species production (Chen et al., 2007), supporting a direct contribution of oxidative stress to nitroglycerin tolerance via oxidative inhibition of ALDH-2, with a subsequently reduced CGRP release. However, the mechanisms underlying the increased activity of PDE 1A1 in nitrate tolerance is not clear. Most recently, it has been shown that nicotine and TNF-alpha upregulate phosphodiesterase expression in vascular smooth muscle cells through formation of superoxide (Hotston et al., 2007). In both studies mentioned above (Kim et al., 2001; Nie et al., in press), nitroglycerin-induced tolerance was restored by administration of vinpocetine, the selective inhibitor of PDE 1A1. There are reports that vinpocetine can exert neuroprotection by its antioxidant property through preventing the formation of reactive oxygen species and lipid peroxidation (Pereira et al., 2000; Santos et al., 2000). It is likely that inhibition of PDE 1A1 by vinpocetine to reverse the nitrate tolerance may be at least partially due to its antioxidant activity. Taken together, we hypothesize that the increased production of reactive oxygen species in nitrate tolerance may account for, at least in part, the increased activity of PDE 1A1 (Fig. 1). However, further studies are needed before drawing a firm conclusion. It is worth to mention that reactive oxygen species have been reported a direct inhibitory effect on sGC level and function in rat aortic smooth muscle cells (Gerassimou et al., 2007). Therefore, it could not rule out the possibility that the impairment of nitroglycerin biotransformation in nitrate tolerance is related to the decrease in sGC activity due to the overproduction of reactive oxygen species.

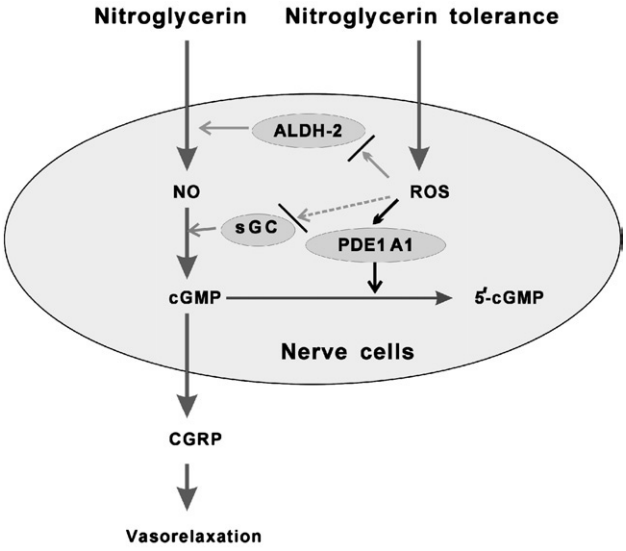

Fig. 1. Schematic showing the role of the calcitonin gene-related peptide (CGRP) in nitroglycerin-induced tolerance. In the state of nitrate tolerance, the production of reactive oxygen species is increased. On one hand, the increased production of reactive oxygen species inhibits the activity of the mitochondrial isoform of aldehyde dehydrogenase (ALDH-2), which in turn leads to the impaired biotransformation of nitroglycerin. On another hand, the increased production of reactive oxygen species may activate phosphodiesterase 1A1 (PDE 1A1), which accelerates the degradation of cGMP. Both the reduced biotransformation of nitroglycerin and the accelerated degradation of cGMP lead to the impairment of NO/cGMP signal, which results in the decrease in CGRP release and in turn the attenuation of vasorelaxation. However, the reduction of nitroglycerin biotransformation through directly inhibiting soluble guanylyl cyclase (sGC) by reactive oxygen species could not be ruled out.

7. Summary

Due to its vasodilator property and wide distribution in cardiovascular system, it is not surprising that CGRP is a major player in regulation of vascular tension under physiological and pathophysiological conditions. It not only participates in the mediation of nitroglycerin-induced vascular relaxation but also plays an important role in nitroglycerin tolerance. The reduced release of CGRP in nitroglycerin tolerance is closely associated with the decreased nitroglycerin biotransformation because of mitochondrial dysfunction. Two key factors here are proposed to contribute to the reduced nitroglycerin biotransformation and CGRP release (Fig. 1). First, the increased production of reactive oxygen species by mitochondrial during nitroglycerin tolerance may have a decisive role in inhibition of vascular ALDH-2, which in turn leads to the decrease in nitroglycerin biotransformation in mitochondrial, with a consequently reduced release of CGRP. Secondly, the increased production of reactive oxygen species may also increase the hydrolyzation of cGMP via upregulation of PDE1A1, which in turn indirectly attenuates nitroglycerin biotransformation. We believe that the new insights into the role of CGRP in nitroglycerin tolerance may provide a clue in searching the novel approach to the prevention of nitroglycerin tolerance.

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