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Stimulated release of calcitonin gene-related peptide from the human right atrium in patients with and without diabetes mellitus

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ABSTRACT

Calcitonin gene-related peptide (CGRP), a potent vasodilator released during activation from a subset of sensory Aδ- and C-fiber afferents, has been suggested to play a beneficial role in myocardial ischemia. Variations in CGRP release can possibly be correlated with diseases that involve changes in activity or degeneration of cardiac afferent fibers. The aim of the present study was to examine basal and stimulated CGRP release from cardiac tissue in patients who underwent cardiopulmonary bypass surgery and to compare patients with and without known history of diabetes mellitus. Small pieces of the right atrium routinely excised during the bypass operations were passed through series of oxygenated solutions. The TRPV1 receptor agonist capsaicin and the nitric oxide donor NONOate were added for stimulation of cardiac afferent fibers. The eluates were processed using an enzyme immuno-assay (EIA) for measurement of CGRP concentrations. Both capsaicin and NONOate caused significant increases in CGRP release. No significant differences in CGRP release between patients with and without diabetes mellitus were examined. The present study evaluates a simple and reproducible model for measuring stimulated CGRP release from the human right atrium.

1. Introduction

In patients suffering from diabetes mellitus, the development of sensory polyneuropathy is frequently associated with symptoms that may result from functional and structural alterations of nociceptive neurons [24]. These symptoms include loss of pain, temperature sensations as well as burning and cutaneous hyperesthesia [18]. The etiology of hypo- and hyperalgesia in diabetes mellitus is complex and still uncertain, but previous studies of diabetic rats have shown that all types of nerve fibers are involved in the process [8,22].

The neuropeptide calcitonin gene-related peptide (CGRP) consisting of 37 amino acids is found in the afferent nervous system [2,46], where it is generated in a subset of mainly nociceptive neurons by an alternative RNA splicing process during calcitonin gene expression [1,32]. Activation of these primary afferent Aδ- and C-fibers causes release of CGRP, which acts as a powerful vasodilator [4].

In cardiac tissues CGRP containing sensory nerves were found at high densities in the right atria, the sinoatrial and atrioventricular nodes, in the pericardium and in the adventitia of the coronary arteries, mostly located in sub-epicardial and subendocardial tissues [34,40]. Several lines of

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evidence suggest that CGRP may participate in the regulation of cardiovascular hemodynamics [28,36] and to be involved in several pathophysiological conditions such as shock, hypertension and myocardial ischemia [13,17]. In the cardiovascular system, CGRP seems to primarily exert a protective function [26,44]. In patients with acute myocardial infarction, an almost two-fold increase in plasma CGRP was observed within 24 h of hospital admission [30]. In those pathological states, CGRP may also play a deleterious role, facilitating norepinephrine release from cardiac sympathic nerve fibers, which may contribute to dysrhythmias and sudden cardiac death [35].

It has been suggested that the vasodilatory effects of CGRP involve an endothelium-dependent mechanism mediated by the release of nitric oxide (NO) [14,31], an endothelium-independent mechanism causing adenylate cyclase activation in vascular smooth muscle cells [11,15] and the activation of adenosine triphosphate (ATP) sensitive potassium channels (K^+_{ATP} channels) [23,25].

Most CGRP containing afferent neurons are characterized by their sensitivity to capsaicin, the pungent agent of hot peppers. In a previous study, we demonstrated the release of CGRP by capsaicin and low pH in different genetically mutant mice lacking either the capsaicin receptor (TRPV1), acid-sensing ion channels (ASIC3) or bradykinin (B2) receptors [37]. In these experiments, TRPV1 was a major player in proton-induced CGRP release. In myocardial ischemia and during experimental perfusion of pig and guinea pig hearts with low pH solutions, which mimicks an important aspect of ischemia, CGRP has been found to be released from activated capsaicin-sensitive afferents [21]. These afferents respond not only to capsaicin and low pH but also to noxious heat, whereby the heat-threshold can drop below body temperature during tissue acidosis or inflammation [20].

Nitric oxide (NO) is also a powerful mediator of vasodilatation in many tissues including the cardiovascular system [27] and may thereby interact with CGRP released from primary afferents. The vasodilatory effect of NO donors on feline cerebral arterioles has been shown to be reduced after chronic trigeminal denervation (trigeminal ganglionectomy) or after application of the CGRP receptor antagonist $CGRP_{8–37}$ [45].

The aim of the present study was to examine if patients with and without known history of diabetes mellitus show differences in CGRP release from human cardiac tissue stimulated either with capsaicin or with an NO donor.

2. Materials and methods

2.1. Tissue sampling

The tissue samples were collected from patients undergoing coronary artery bypass graft (CABG) surgery under a cardiopulmonary bypass installed via aorto–right atrium cannulation. During the cannulation a small piece of right atrium was excised and immediately stored in cold synthetic interstitial fluid (SIF). These tissue flaps with weights between 160 and 380 mg (285 ± 68 mg; mean ± S.E.M.) are routinely removed to insert the backflow tube of the bypass. Table 1 summarizes data on patients' demographic and cardiovascular risk factors. The European System for Cardiac Operative Risk Evaluation (EuroSCORE) is a score for the risk of early mortality in cardiac surgical candidates [29].

2.2. Preparation

All tissue samples were rinsed for 30 min of equilibration at room temperature in synthetic interstitial fluid (SIF) containing (mmol/l): 108 NaCl, 3.48 KCl, 3.5 $MgSO_4$, 26 $NaHCO_3$, 1.67 NaH_2PO_4, 1.5 $CaCl_2$, 9.6 sodium gluconate, 5.55 glucose, 7.6 sucrose [5], buffered at pH 7.4. The SIF was continuously gassed with 95% oxygen and 5% carbon dioxide (carbogen gas mixture). Capsaicin (Sigma) was dissolved in 98% ethanol as a stock solution and diluted with SIF to a final concentration of 10^{-6} and 10^{-5} mmol/l. Diethylamine-NONOate (NONOate, Calbiochem, Bad Soden, Germany) was applied at concentrations of 10^{-5} and 10^{-4} mmol/l (diluted in SIF).

The specimens were passed through a series of solutions that were provided in 1 ml Eppendorf micro test tubes arranged in a thermostatic shaking bath at 36.5 °C. With a thread fixed to the tissue the samples could be moved from one test tube to the other without direct contact.

2.3. Drug application and sampling

The tissue was transferred to the first Eppendorf tube filled with 250 μl SIF. After 5 min of immersion, each specimen was forwarded to the second tube for another 5 min and thus passed throughout the whole series of tubes. In all cases the stimulation fluid, either capsaicin (10^{-6} or 10^{-5} mmol/l) or NONOate (10^{-5} or 10^{-4} mmol/l), was applied in the third

Table 1 – Patients' characteristics

	Median (range) or percent	
	Without diabetes	With diabetes
Gender	Six males, six females	Six males, six females
Age (years)	63.8 (52–81)	67.1 (50–80)
Body mass index	29.9 (24–36)	28.6 (22–38)
Patients with hypertension (%)	85.7	100
Smokers (%)	25	8.3
Patients with myocardial infarction (%)	28.6	41.6
Serum creatinine (mg/dl)	1.0 (0.8–1.6)	1.0 (0.8–1.3)
Patients with hyperlipoproteinemia (%)	78.6	100
Left ventricular function (EF %)	60.8 (40–70)	54.4 (40–70)
EuroSCORE [29]	3.4 (0–13)	5.4 (2–9)