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Upregulation of the kappa opioidergic system in left ventricular rat myocardium in response to volume overload
Adaptive changes of the cardiac kappa opioid system in heart failure

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ABSTRACT

Opioids have long been known for their analgesic effects and are therefore widely used in anesthesia and intensive care medicine. However, in the last decade research has focused on the opioidergic influence on cardiovascular function. This project thus aimed to detect the precise cellular localization of kappa opioid receptors (KOR) in left ventricular cardiomyocytes and to investigate putative changes in KOR and its endogenous ligand precursor peptide prodynorphin (PDYN) in response to heart failure. After IRB approval, heart failure was induced using a modified infrarenal aortocaval fistula (ACF) in male Wistar rats. All rats of the control and ACF group were characterized by their morphometrics and hemodynamics. In addition, the existence and localization as well as adaptive changes of KOR and PDYN were investigated using radioligand binding, double immunofluorescence confocal analysis, RT-PCR and Western blot. Similar to the brain and spinal cord, [^{3}H]U-69593 KOR selective binding sites were detected the left ventricle (LV). KOR colocalized with $Ca_v1.2$ of the outer plasma membrane and invaginated T-tubules and intracellular with the ryanodine receptor of the sarcoplasmatic reticulum. Interestingly, KOR could also be detected in mitochondria of rat LV cardiomyocytes. As a consequence of heart failure, KOR and PDYN were up-regulated on the mRNA and protein level in the LV. These findings suggest that the cardiac kappa opioidergic system might modulate rat cardiomyocyte function during heart failure.

1. Introduction

Opioids have long been known for their analgesic effects and are therefore widely used in anesthesia and intensive care medicine. However, in the last decade research has focused on the opioidergic influence on cardiovascular function. Beside their analgesic properties opioids induce arterial hypotension and bradycardia, which seems to be transmitted via the dorsal vagal complex in the CNS [1]. Moreover, activation of cardiac opioid receptors has been shown to be cardioprotective against myocardial infarction and may trigger processes similar to ischemic preconditioning [2]. Stimulation of cardiac KOR induces a subcellular kinase pathway in which opening of sarcolemmal and mitochondrial ATP-sensitive K^+-channels seem to protect the heart against ischemia induced calcium overload [3,4]. KOR may also play a role within the complex network for the integration of the heartís neuronal in- and output as they were identified in parasympathetic, sympathetic, and sensory neurons within intracardiac ganglia and myocardium of healthy rats [5]. Finally, KOR ligands inhibit an isoprenaline-induced cardiac hypertrophy [6] and seem to reduce oxidative stress by preventing an increase in lipid peroxidation and depletion of myocardial antioxidants [7].

On the contrary, the role of the cardiac opioid system in congestive heart failure (CHF) is less clear. Bolte et al. found an augmented negative inotropic and lusitropic response to administration of KOR agonists in the failing heart that was mediated by a pertus-

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sis toxin-sensitive G-protein [8]. These acute effects were seen in the absence of an altered expression of KOR and a change in opioid peptides levels in plasma and cardiac tissue. In addition, the systemic opioid antagonist naloxone improved myocardial contractile function in dogs with rapid pacing induced heart failure [9]. Especially the selective kappa-opioid receptor antagonist nor-binaltorphimine (nor-BNI) improved left ventricular function during recovery from chronic myocardial stunning [10]. In this context it is interesting that KOR were detected by Polymerase Chain Reaction (PCR), Western blot and immunohistochemistry in healthy porcine myocardium [11].

This project thus aimed to detect the precise cellular localization of KOR in left ventricular cardiomyocytes, and to investigate putative changes in KOR and its endogenous ligand precursor peptide prodynorphin (PDYN) in response to chronic volume overload in rats.

2. Material and methods

Male Wistar rats 280–300 g (Harlan Winkelmann, Borchen, Germany) were used for this study in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. They were maintained on standard laboratory rat chow and water ad libitum and kept on a 12-h light–dark cycle. The experimental protocol was approved by the local Ethics Committee on the Use of Animal Experiments (Landesamt für Gesundheit und Soziales, Berlin, Germany).

2.1. ACF induction

Heart failure was induced in Wistar rats ($n = 8$) using a modified infrarenal aortocaval fistula (ACF) as previously described [12]. After induction of Isoflurane anesthesia, the abdominal cave was opened via median laparotomy. After temporal manually compressing the aorta and venous vessels proximally and distally, the aorta was punctured with a 16G disposable needle (Braun, Melsungen, Germany) distal to the renal arteries. By further advancing the needle through the aortic wall into the adjacent inferior vena cava ACF was created. The aortic puncture site was sealed with a drop of cyanoacrylate glue and ACF patency could be judged by the pulsatile flow of oxygenated blood into the vena cava inferior [13]. Vessels of Sham-operated control animals were treated identically except that no puncture of the aorta was performed ($n = 8$). Post surgical analgesia was provided by subcutaneous metamizole (40 mg/kg).

2.2. Hemodynamic evaluation

Hemodynamic evaluation was carried out using the "closed chest" method as described previously ($n = 6$) [14]. Measurements were performed under tiletamine/zolazepam anesthesia (Zoletil®, 10 mg/kg s.c. followed by 50 mg/kg i.m.) 28 days after fistula induction in spontaneously breathing rats [15]. Rat body temperature was maintained using a heating pad and rats received a tracheostomy. Central venous pressure (CVP) was assessed with a plastic catheter (PE-50) that was inserted via the left jugular vein into the superior vena cava. The right carotid artery was cannulated with a micro-tip pressure-volume conductance catheter (Millar®, SPR-838 NR), which was then advanced into the left ventricle in order to measure arterial and intraventricular pressures as well as volumes. Parallel conductance volume was registered by injecting 100 μl of 15% saline into the central venous line. All signals were recorded and analyzed by the PowerLab®-system and software (AD Instruments, Dunedin, New Zealand). After completion of the hemodynamic measurements rats were killed by exsanguination and organs were eviscerated.

2.3. Radioligand binding assays

KOR specific binding sites in membrane preparations of healthy rat brain, spinal cord (SC) and left ventricle (LV) ($n = 6$) were identified according to previous studies [16]. Tissues were immediately placed on ice in cold assay buffer (50 mM Tris–HCl, 1 mM EGTA, 5 mM $MgCl_2$, pH 7.4), homogenized and centrifuged at 48,000 × *g* at 4 °C for 20 min, then resuspended in assay buffer followed by 10 min incubation at 37 °C to remove endogenous ligands. The homogenates were centrifuged again and resuspended in assay buffer. Membranes were aliquoted and stored at −80 °C. Saturation binding experiments were performed by using the specific KOR antagonist [^{3}H]U-69593 (specific activity 50 ci/mmol, Hartmann Analytic, Braunschweig, Germany). Membrane protein (70–100 μg) was incubated with various concentrations of 0.25–16 nM [^{3}H]U-69593 for 1 hour at 22 °C in a total volume of 1 ml of binding buffer (50 mM Tris–HCl, 5 mM EDTA, 5 mM $MgCl_2$, 100 mM NaCl, 0.2% bovine serum albumin [BSA]). At the end of the incubation period, bound and free ligands were separated by rapid filtration over GF/C filters under vacuum by using a Brandel cell harvester (Gaithersburg, MD). Filters were washed three times with 4 ml of cold buffer (50 mM Tris–HCl, pH 7.4). Bound radioactivity was determined by liquid scintillation spectrophotometry after overnight extraction of the filters in 3 ml of scintillation fluid. Nonspecific binding was defined as radioactivity remaining bound in the presence of 10 μM unlabeled U-69593 and subtracted from the total binding. Six independent experiments were performed in duplicate and the means and S.E.M. were calculated. B_{max} and K_d values were determined by nonlinear regression analysis of concentration-effect curves using GraphPad Prism (GraphPad® Software, San Diego, CA).

2.4. Immunohistochemistry

To perform double immunofluorescence staining deeply anesthetized rat hearts of two additional animals of each group were transcardially perfused with 100 ml warm saline, followed by 300 ml 4% (w/v) paraformaldehyde in 0.16 M phosphate buffer solution (pH 7.4) ("fixative solution"). The hearts were removed after perfusion, postfixed in fixative solution for 90 min, and cryoprotected overnight at 4 °C in PBS containing 10% sucrose. The tissues were then embedded in tissue-Tek compound (OCT, Miles Inc. Elkhart, IN), frozen, and cut into 8 μm thick sections using a cryostat and the sections were thaw-mounted onto chrome alum gelatin-coated glass sildes. Double immunofluorescence staining was processed as described previously [17,18]. The sections mounted in gelatin coated slides were incubated overnight with the following primary antibodies: rabbit polyclonal anti-KOR (1:1000) (gift from S.J. Watson, Michigan, USA) [19] alone, or in combination with mouse monoclonal anti-dihydropyridine receptor (α2 subunit) (1:1000) to identify the voltage-gated L-type Ca2+ channel (*anti-$Ca_v1.2$*) (SIGMA®, Missouri, USA) [20,21], or in combination with mouse monoclonal anti-Inositol-1, 4, 5-trisphosphate receptor type III (1:600) to identify ryanodine receptors (*RyR*) of the sarcoplasmatic reticulum (BD Biosciences®, Europe) [22]. After incubation with primary antibodies, the tissue sections were washed with PBS and then incubated with Texas Red conjugated goat anti-rabbit antibody (Vector Laboratories) and FITC conjugated donkey anti-mouse secondary antibodies (Vector Laboratories, Inc. Burlingame, CA). Thereafter, sections were washed with PBS and the nuclei stained bright blue with 4′-6-Diamidino-2-phenylindole (DAPI) (0.1 μg/ml in PBS) (SIGMA®, Missouri, USA). Finally, the tissue sections were washed in PBS, mounted on vectashield (Vector Laboratories) and viewed under a Zeiss LSM 510 laser-scanning microscope (Carl Zeiss, Göttingen, Germany). The integrated opti-