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Distribution of mRNA and binding sites of adrenoceptors and muscarinic receptors in the rat heart

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Abstract

Since there exist some obscurities in the expression of mRNAs and their receptors in the heart, we have investigated the gene expression (mRNA levels) of adrenoceptors (α_{1A}-, α_{1B}-, β_1-, β_2-, β_3-) and muscarinic receptors (M_2) and the density of receptor binding sites (α_{1A}-, α_{1B}-, β_1-, β_2-adrenoceptors, muscarinic receptors). Moreover, the heart regions consist of tissue rich in ganglion cells (that are of importance in heart neural circuits) and those virtually free of them (myocytes). Therefore, we have examined the differences in the distribution of mRNAs/receptor binding sites in the atrial samples of the heart rich in ganglion cells vs. those are virtually free of them. Binding sites and mRNAs of muscarinic receptors and α_{1B}-adrenoceptors differ in their distribution in different heart regions. The mRNAs for β_1- and β_2-adrenoceptors were almost equally distributed herein, while the amount of β-adrenoceptors significantly differs in the heart regions. The α_{1A}- and β_3-adrenoceptors mRNAs were also found in all investigated heart regions, but at significantly lower level and have not shown region differences. This is a new finding, especially to β_3-adrenoceptors, as they were not regularly found in each heart regions. α_{1B}-adrenoceptors have similar distribution of their mRNAs and binding sites in some heart parts. Thus, we can conclude that there are noticeable differences in the presence of receptors in heart regions that contain ganglion cells in comparison to those are virtually free of them.

Keywords: Adrenoceptors; Muscarinic receptors; Heart; Binding; mRNA

Introduction

The force and rate of cardiac contractions adapt continually to the changing needs of the organism. Sympathetic nerves (releasing noradrenaline) and vagal nerves (releasing acetylcholine) are mainly responsible for the short-term regulation of the heart activity in mammals, together with the changing concentrations of adrenomedullary hormones (adrenaline and noradrenaline) in the blood plasma. Noradrenaline and adrenaline act via the adrenoceptors, while the action of acetylcholine is mediated by the muscarinic receptors (for review see Brodde and Michel, 1999). Myocardial cells express a broad spectrum of muscarinic receptors and adrenoceptors, which belong to a large family of seven transmembrane-spanning proteins, namely G-protein coupled receptors (GPCRs). M_2 muscarinic and β_1-adrenoceptors are the most important receptor subtypes expressed in cardiomyocytes (Brodde and Michel, 1999), but the heart cells express also the other subtypes, i.e. β_2, and α_1-adrenoceptors probably also other non-M_2 subtype of muscarinic receptors (Wang et al., 2004) and β_3 adrenoceptors (Gauthier et al., 2000). The mRNAs for M_2 muscarinic receptors and β_1- and β_2-adrenoceptors have been repeatedly found in the heart (for review see Brodde and Michel, 1999). The expression of minor subtypes is now under investigation and has been discussed in

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recent reviews (Brodde and Michel, 1999; Granneman, 2001; Zimmer, 1997; Mysliveček and Trojan, 2003; Wang et al., 2004) or original papers (Kaumann and Molenaar, 1997; Gautier et al., 1996; Hamilton et al., 2001; Colecraft et al., 1998; Brixius et al., 2004). Wang et al. (2001) have identified the muscarinic receptor subtypes and mRNAs in the human heart. mRNAs for all muscarinic receptor subtypes have been recently quantitated in the rat heart (Krejčí and Tuček, 2002). Similarly, the α_1-adrenoceptor subtype expression in the heart is still a matter of debate and the differences in species expression could exist (see Brodde and Michel, 1999; Michel et al., 1994; Autelitano and Woodcock, 1998). Some discrepancies still exist about the expression of β_3 adrenoceptor subtype in the heart. While Gautier et al. (1996), Gauthier et al. (2000) repeatedly described their role in the heart, the others (Kaumann and Molenaar, 1997) have challenged these findings (for review see Gauthier et al., 2000). Very recently, the data about the function of β_3-adrenoceptors in human heart dramatically increased (Brixius et al., 2004; Tavernier et al., 2003; Pott et al., 2003) but the data on rat myocardium are still controversial. Moreover, the data about the gene expression of this receptor subtype in cells that are almost of neuronal origin (neuronal ganglia) and that of mainly of myocyte origin (non-ganglionic heart parts) are still missing.

Cardiac ganglia are groups of cells that possess heterogenous population of preganglionic, ganglionic and interconnecting local circuit neurons. The neurotransmitter systems including receptor to these molecules are still the subject of continuing research. In the mammalian heart the intrinsic cardiac ganglia are situated within the subepicardial plexus. A majority of them were found in the left subepicardial plexus adjacent to the pulmonary veins. Complex interactions between cardiac and extracardiac ganglion cells play a crucial role in the regulation of cardiac function. According to our knowledge, the gene expression of receptor mRNA and the receptor binding in the heart regions rich in ganglion cells and that virtually free of them were not followed yet. Therefore, we have divided the atrial heart parts into regions rich in these cells and those virtually free of neuronal cells in order to discriminate the possible differences in the gene expression (using the RT-PCR identification of mRNA) and receptor binding site density (using ligand binding studies).

We tried to identify mRNAs of adrenoceptor (α_{1A}, α_{1B}, β_1, β_2, β_3) and muscarinic receptors (M_2) and their binding sites (α_{1A}, α_{1B}, β_1, β_2, muscarinic receptors) in ten different heart parts. We divided the rat heart into left and right atria rich in ganglion cells or virtually free of ganglion cells, left and right ventricles and septum. As there is no suitable binding technique for β_3-adrenoceptor detection, these receptors were identified on the mRNA level only.

Materials and methods

Tissue preparation

Adult male Sprague–Dawley rats (300–350 g b.w.) were used. Animals were treated in accordance with the Guide for Care and Use of Laboratory Animals and the experimental protocol was approved by the Ethic Committee of the Institute of Experimental Endocrinology. Animals were kept on standard laboratory conditions with 12/12 light/dark cycle (light on at 6.00 a.m.) and supplied by dry rat food and drinking water ad libitum. After decapitation, hearts were quickly removed and immediately separated into parts as stated thereinafter. Seven heart parts were dissected for radioligand binding experiments: right and left heart atrial part virtually free of ganglion cells, right and left atrial tissue with the majority of heart ganglia, left and right ventricles and septum. Three more parts were dissected for the RT-PCR experiments: atrial septum, apex and the ventricular septum that was divided into upper and lower part. Samples were collected in Eppendorf tubes, flash frozen in liquid nitrogen and kept at −70 °C until use.

RNA isolation and relative quantification of mRNA levels by RT-PCR

RNA was isolated by RNAzol. Concentration and purity of RNA was determined spectrophotometrically on a GeneQuant Pro (Amersham Bioscience). Reverse transcription was performed using Ready-To-Go You-Prime First-Strand beads and $pd(N)_6$ primer. PCR for specific receptors was carried out afterward using the primers originally designed or obtained from the references given in Table 1. The initial denaturation was performed at 95 °C for 5 min followed by the process described in detail in Table 1 with the exception of M_2 muscarinic receptors when the denaturation lasting 5 min was followed by 35 s annealing at 60 °C and 45 s polymerization at 72 °C (according to Krejčí and Tuček, 2002 and the process described in Table 1). Polymerization was performed at 72 °C in all cases. The final polymerization lasting 7 min was performed at 72 °C in all cases.

PCR products were analysed on 2% agarose gels and visualised using ethidium bromide. As a control, the housekeeper glyceraldehydes 3-phosphate dehydrogenase (GAPDH) was used. Intensity of the individual bands was evaluated by PCBase software. For semiquantitative evaluation the values were normalised to the signal obtained with GAPDH.

Radioligand binding experiments

Preliminary saturation binding

The tissue was weighted and samples from 2–4 animals was pooled and homogenised for 30–45 s in homogeniser (Ultra-Turrax® T25 basic IKA®-Werke 24,000 r.p.m.) in physiological saline keeping the tissue on ice.

Density of muscarinic binding sites (B_{max}) were computed by non-linear regression of data obtained in saturation experiments with the binding of 31.25–1000 pmol/l [^{3}H]QNB to cardiac tissue homogenates performed in duplicates. The non-specific binding was determined in the presence of 5 μmol/l atropine and was less than 10%.

Density of β-adrenergic binding sites (B_{max}) were computed from saturation experiments with the binding of 62.5–