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Serotonin responsiveness through 5-HT_{2A} and 5-HT_4 receptors is differentially regulated in hypertrophic and failing rat cardiac ventricle

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Abstract

Cardiac ventricular responsiveness to serotonin appears in rat postinfarction congestive heart failure (CHF), mainly mediated by 5-HT_4 receptors in chronic dilated CHF and 5-HT_{2A} receptors in acute CHF. To differentiate between the effects of left ventricular (LV) hypertrophy and failure on 5-HT_{2A}- and 5-HT_4-mediated inotropic serotonin response, male Wistar rats with increasing LV hypertrophy (AB1-3) and failure (ABHF) 6 weeks after banding of the ascending aorta were screened for contractile function *in vivo* (echocardiography) and *ex vivo* in LV papillary muscles, and mRNA expression level determined by RT-PCR. Both AB1-3 and ABHF displayed LV hypertrophy and remodelling. In ABHF, systolic LV and left atrial diameter increased and cardiac output decreased compared to AB3. Serotonin induced a positive inotropic response (PIR) in papillary muscles correlated with the degree of hypertrophy reaching a maximum in ABHF. Both 5-HT_{2A} and 5-HT_4 receptors contributed to the PIR. The 5-HT_{2A} contribution increased with increasing hypertrophy, and the 5-HT_4 contribution increased upon transition to heart failure. No 5-HT_{2B}-mediated PIR was observed, consistent with increased 5-HT_{2B} mRNA only in non-cardiomyocytes. The 5-HT_{2A}, 5-HT_{2B} and 5-HT_4 mRNA levels increased in AB1-3 and increased further in ABHF compared to AB3, but did not correlate with degree of hypertrophy. 5-HT_{2A} mRNA was also increased in LV of terminally failing human hearts. In conclusion, functional 5-HT_{2A} and 5-HT_4 receptors are differentially induced in LV hypertrophy and failure. While the 5-HT_{2A}-mediated PIR is linearly correlated with the degree of hypertrophy, the 5-HT_4-mediated PIR seems to increase with LV dilatation, as also seen in postinfarction CHF.

Keywords: Serotonin receptors; Hypertrophy; Congestive heart failure; Gene expression; Ventricular function

1. Introduction

The neurotransmitter and vasoactive mediator serotonin (5-HT) induces a positive inotropic response (PIR) in the cardiac left ventricle (LV) from rats with chronic postinfarction congestive heart failure (CHF) [1]. This PIR is mediated mainly through the 5-HT_4 receptor, and viable myocardium from the failing hearts has substantially increased levels of 5-HT_4 receptor mRNA compared to controls. A 5-HT_4-mediated PIR is also present in the failing human heart, and 5-HT_4 mRNA is increased 4-fold in explanted failing human hearts compared to donor hearts [2].

The presence of ventricular 5-HT_4 receptors, in many aspects resembling β-adrenergic receptors, mediating a PIR in failing human hearts is of potential clinical interest [3,4], and representative animal models to study the function of ventricular

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5-HT_4 receptors are important [1]. It is also of interest to assess whether induction of serotonin responsiveness in rat ventricles occurs only after myocardial infarction (MI) or whether it also occurs in other types of heart failure and perhaps in cardiac hypertrophy without heart failure. Furthermore, the mechanism of induction of the 5-HT_4 receptor-mediated PIR in heart failure is not clear. In principle, it could be linked either to the cardiac hypertrophy or to LV dilatation and increased wall stress. Even in the absence of heart failure, non-ischemic papillary muscles of infarcted rat hearts displayed a 5-HT_4-mediated PIR to serotonin, increasing with infarction size, demonstrating that CHF is not a prerequisite for the induction of serotonin responsiveness [1]. However, since LV dilatation is present in these hearts in addition to hypertrophy [5], a possible association between hypertrophy and appearance of 5-HT_4 receptors is difficult to assess in a postinfarction model. In this model it is also difficult to assess to which extent the LV dilatation and activation of a stretch-dependent mechanism contribute to the observed 5-HT_4 response. An important aim of this study was therefore to differentiate between hypertrophy and LV dilatation as two possibly independent triggers of 5-HT_4 receptor induction by using another experimental model.

A serotonin-mediated PIR is present not only in chronic, but also in acute CHF after myocardial infarction (MI) [6]. This response was mediated primarily through the 5-HT_{2A} receptor, although a 5-HT_4 receptor-mediated PIR was also observed. The acute post-MI CHF is associated with a compensatory hypertrophic response in the remaining viable myocardium [7]. Thus, our previously used model does not allow differentiation between hypertrophy and failure/dilatation as the primary stimulus for 5-HT_{2A} and 5-HT_4 receptor induction.

To try to distinguish between LV hypertrophy on one hand and dilatation on the other as the main correlate to increased serotonin responsiveness through 5-HT_4 and 5-HT_{2A} receptors, we applied a rat model of aortic banding (AB), characterized by increased afterload and cardiac hypertrophy. Tight aortic banding initially causes a gradually increasing LV hypertrophy, but eventually results in cardiac dilatation and failure. This model is therefore well suited to study independent association of hypertrophy and increased wall stress on the expression pattern of 5-HT receptors and serotonin responsiveness. We found that the PIR induced by 5-HT_{2A} receptor stimulation was highly correlated with the degree of hypertrophy, whereas LV dilatation was an important additional stimulus for increased PIR in response to 5-HT_4 stimulation.

2. Methods

2.1. Animal model

Animals were cared for according to the Norwegian Welfare Act, which conforms with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, Revised 1996). Two animals per cage were housed in a temperature-regulated room at 12:12-h day/night cycle and given access to food and water *ad libitum*.

Male Wistar rats (Møllegård Breeding and Research Center, Skensved, Denmark) weighing 200 g were anesthetized with 68% N_2O, 29% O_2 and 2–3% isoflurane and ventilated on a respirator (Zoovent, Triumph Technical Services, Milton Keynes, UK). The ascending aorta was dissected free through a hemithoracotomy on the right side. A stenosis of the ascending aorta was induced by a ligation (3-0 silk) which included both the aorta and a steel wire (0.9 mm). Immediately after the ligation the steel wire was removed, resulting in a ~0.9 mm aortic diameter. The sham-operated rats (Sham) were subjected to the same surgical procedure without banding the ascending aorta.

After 6 weeks, the rats were again anesthetized and ventilated on the respirator. Echocardiography was performed and the heart excised into saline, weighed and perfused on a modified Langendorff setup. After removal of the LV posterior papillary muscle, the right and LV weights were measured. The aorta-banded (AB) animals were grouped into failing (ABHF) and non-failing (AB). The criteria for inclusion in the ABHF group were increased lung weight (>2.0 g) and left atrial diameter (>5.0 mm). The banded animals that did not fulfill these criteria were divided into 3 groups based on the degree of LV hypertrophy (LV weight to body weight (BW) ratio; AB1 <3.15 g/kg, AB2 3.15–3.50 g/kg and AB3 >3.50 g/kg).

2.2. Human left ventricular tissue

Human left ventricular tissue samples from 25 hearts (5 unused donors, 20 in terminal heart failure) were obtained from patients undergoing heart transplantation at Rikshospitalet University Hospital, Oslo (Ethics approval #S01025) as described in [2].

2.3. Echocardiography

M-mode, two-dimensional and Doppler echocardiography was performed with a VIVID 7 echocardiograph (GE Vingmed Ultrasound, Horten, Norway (GE)) using an M12L 12MHz linear array transducer (GE) and analysed essentially as described in [5].

2.4. Isolated papillary muscles

Posterior left ventricular papillary muscles were prepared and contraction–relaxation cycles (CRCs) recorded and analyzed as previously described [8] with respect to maximal developed force (F_{max}), maximal development of force ($dF/dt)_{max}$, time to peak force (TPF, ms) and relaxation time (RT=time to relaxation to 20% level-TPF, ms) as an index for relaxation. ($dF/dt)_{max}$ was used as an index of contractility and inotropic responses to agonists were expressed by increases in ($dF/dt)_{max}$. The experiments were performed in the presence of blockers (added 90 min prior to agonist) of adrenergic (α_1, prazosin 0.1 μM; β, timolol 1 μM) and muscarinic cholinergic (atropine 1 μM) receptors. Serotonin was added to the organ bath as a single bolus (10 μM to give a complete activation of the serotonin receptors) and the response was measured after 2–