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Review article

# The role of endothelin-1 in the sympathetic nervous system in the heart

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ABSTRACT

Endothelin-1 (ET1) is a peptide that was initially identified as a strong inductor of vascular contraction. In the last 25 years, there have been several biological processes identified in which ET1 seems to play a critical role. In particular, genetic studies have unveiled that ET1 is important for neuronal development, growth and function. Experimental studies identified ET1 as a regulator of the interaction between sympathetic neurons and cardiac myocytes. This might be of clinical importance since patients suffering from heart failure are characterized by disrupted norepinephrine homeostasis in the heart. This review summarizes the important findings on the role of ET1 for sympathetic neurons and norepinephrine homeostasis in the heart.



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## Introduction

ET1 was initially identified by Masashi Yanagisawa in 1988 as a peptide with vasoconstrictive effects and high expression in the intima of the aorta (Yanagisawa et al., 1988). It is generated from a larger precursor protein (big ET1) via proteolysis mediated by endothelin converting enzymes (ECEs) (Yanagisawa et al., 1988). ECEs are a group of heterogenous enzymes that regulate ET1 in different organs (Rubanyi and Polokoff, 1994). This review will focus on the final product ET1, as its bioactivity is higher than that of the precursor protein, which has only been sparsely investigated (Kimura et al., 1989). ET1 binds to endothelin receptor A (ETAR) and endothelin receptor B (ETBR), both of which are G protein-coupled receptors (Rubanyi and Polokoff, 1994). The expression of ET1 is detectable in a variety of organs including the heart, vessels, lung, brain and kidney (Rubanyi and Polokoff, 1994). Genetic studies

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imply that endothelin signaling plays important roles in different organ systems. In patients, it has been shown that ET1 is elevated in various pathological situations including heart failure, myocardial infarction, chronic kidney disease, pulmonary hypertension and hypertension (Kohno et al., 1990; Koyama et al., 1989; Shichiri et al., 1990; Stewart et al., 1991a,b; Clines et al.; Hasue et al., 2005). The development of selective and non-selective ETAR/ETBR antagonists led to multiple clinical trials. However, many of these failed to translate to the clinic (Kohan et al.). Currently, endothelin receptor antagonists are FDA approved for the treatment of pulmonary hypertension and scleroderma digital ulcers (Kohan et al.). Disappointing results in clinical heart failure trials might be explained by a limited understanding of the regulatory role of ET1 in organ cross talk mechanisms. This review will focus on the role of endothelin signaling for the sympathetic nervous system and its interaction with cardiac myocytes in the context of cardiovascular disease.

An activation of the sympathetic nervous system is a hallmark of cardiovascular disease, in particular heart failure. Heart failure is a common and life-threatening disease, which is linked to a poor prognosis despite several therapeutic advancements (Roger et al.). In heart failure, catecholamines are elevated and correlate with the severity of the disease (Benedict et al., 1994; Rockman et al., 2002). The success of the heart failure therapy with β-adrenergic receptor antagonists underscores the pivotal role of the β-adrenergic receptor agonist norepinephrine (NE) in heart failure (Anonymous, 1999). The mechanisms by which NE is released from sympathetic neurons impact on signaling cascades in cardiac myocytes (Nakata et al., 1998; Ogita et al., 2001; Kreusser et al., 2006). In this review we will pay special attention to the regulation of NE release and re-uptake mechanisms through ET1. The consideration of these mechanisms might also lead to a better understanding of the interaction between endothelin receptor antagonists and heart failure drugs. It appears to us that the importance of endothelin signaling for the sympathetic nervous system has been underestimated. Therefore, in this review we will first summarize the important findings on endothelin signaling that have been derived from mouse and rat genetic studies. Then we will describe the effects of pharmacological studies in the context of heart failure. At the end we will focus on the specific roles of the endothelin receptors on the regulatory mechanisms of neurotransmitter release and re-uptake. Finally, we will discuss clinical implications that may have arisen from these findings.

## Lessons from mouse genetic studies

ET1, ETAR and ETBR are expressed in many organs including the heart, vessels, spleen, blood, kidney, lung, gastrointestinal tract, liver, reproductive organs, central nervous system and peripheral nerve system. But what are the cell autonomous functions of ET1 and its receptors? This question can primarily be answered by mouse and rat genetic studies. A variety of global and cell type-specific loss of function studies have been conducted. These studies are summarized in Table 1 and reviewed in more detail as follows.

### *Genetic deletion of ET1*

Genetic studies have shown that the global deletion of ET1 leads to severe developmental defects in the ventricular septum of the heart and in the aortic arch (Kurihara et al., 1995a). Because of the typical developmental phenotypes and additional craniofacial defects, neural crest cells were suspected to be regulated by ET1 during development (Kurihara et al., 1994). Some of the developmental defects in ET1-deficient mice could be explained by a reduced production of neurotrophic factors, such as the nerve growth factor (NGF). In an elegant study, Ieda et al. demonstrated that excessive apoptosis of sympathetic neurons in ET1-deficient mouse hearts could be rescued by transgenic NGF overexpression in the heart (Ieda et al., 2004). The latter findings are discussed in more detail below (see also Fig. 1).

Conditional gene deletion studies revealed several functions of ET1 in different organ systems. Mice lacking ET1 only in cardiac myocytes displayed reduced cardiac hypertrophy in response to hyperthyroidism but also reduced cell survival. Upon pathological pressure overload (induced by transverse aortic constriction, TAC) and aging, these mice develop severe heart failure (Shohet et al., 2004; Zhao et al., 2006), implying that ET1 functions as a cardiac myocyte survival factor. Mice lacking ET1 in the connecting duct of the kidney ET1 were characterized by sodium retention and arterial hypertension (Ahn et al., 2004). The deletion of ET1 in sensory neurons led to an increased sensitivity to acute and persistent pain (Hasue et al., 2005), which is linked to the ETAR in afferent neurons as discussed below.

### *Genetic deletion of the endothelin receptor A (ETAR)*

Similar to the phenotype of mice lacking ET1 globally, the global deletion of the ETAR leads to developmental defects in neural crest-derived tissue resulting in craniofacial, cardiac and aortic arch malformations (Kurihara et al., 1994; Clouthier et al., 1998, 2000). These defects mimic the human conditions collectively termed CATCH 22 or velocardiofacial syndrome.

Conditional deletion of ETAR in nociceptive neurons resulted in a reduction in late nociception (Stosser et al.), highlighting the previous underestimated effect of ET1 on terminally differentiated neuronal tissue. Furthermore, several renal phenotypes were reported by the use of cell-type specific approaches for the nephron and the connecting duct (Stuart et al.; Ge et al., 2005a,b), indicating an involvement of ETAR in the detection of urine osmolality and regulation of fluid retention.

The conditional deletion of ETAR in cardiac myocytes did not lead to an altered a phenotype in response to stress stimuli, in particular isoproterenol or angiotensin II, which typically leads to adverse cardiac remodeling and eventually heart failure (Kedzierski et al., 2003). This was somewhat surprising because the inhibition of the ETAR by pharmacological approaches showed beneficial effects on the heart in vivo, as discussed extensively below (Sakai et al., 1996). Later studies suggested that mice lacking ETAR in cardiac myocytes are protected from age-dependent autophagy and cold-induced cardiac remodeling (Ceylan-Isik et al.; Zhang et al.).

### *Genetic deletion of the endothelin receptor B (ETBR)*

The global deletion of ETBR led to an intestinal phenotype with aganglionosis, megacolon, and spotted coat color, a phenotype that recapitulates the familial Hirschsprung's disease in humans (Hosoda et al., 1994; Gariepy et al., 1996). This disease has been linked to a failure of neural crest cells to migrate. A similar phenotype could be observed in rats with a naturally occurring deletion in the ETBR gene that completely abrogates functional receptor expression. In an elegant study, the lethal phenotype of these rats could be rescued by transgenic expression of the ETBR under the control of the dopamine-β-hydroxylase promoter, directing ETBR expression to sympathetic neurons (Gariepy et al., 2000). The same rescue experiment was successful in mice globally lacking the ETBR. Rescued ETBR mice were then widely in use to study the role of the ETBR in other tissues and organs. Rescued ETBR deficient rodents show for example endothelial dysfunction and salt sensitive hypertension (Gariepy et al., 2000; Quaschning et al., 2005). Global ischemia in isolated perfused hearts (Langendorff preparation) of rescued ETBR deficient rodents resulted in exaggerated norepinephrine (NE) overflow, reduced cardiac function and an increase in cardiac arrhythmias (Yamamoto et al., 2005; Oikonomidis et al.), indicating that the ETBR regulates NE release. However, the cell autonomous ETBR functions are unclear in this context, because it was not investigated whether the exaggerated postischemic NE overflow in rescued ETBR deficient rodents was due to the global lack of the ETBR or the rescue overexpression of ETBR in dopamine-β-hydroxylase positive