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Editorial

New role for endothelin signalling during development

Endothelin-1 (ET-1) is a 21-amino acid peptide produced by the endothelium and other cell types with a variety of physiological functions. Its original description dates back to 1980s, and it is distinguished by a very potent vasoconstrictor activity (Yanagisawa *et al.* 1988, Braunwald *et al.* 2001). Two other isoforms of endothelin have been discovered (ET-2 and ET-3), but endothelium produces only ET-1. Synthesis of ET-1 is complex, starting with a large precursor molecule, pre-proendothelin, which is processed to 'big endothelin' and finally converted by the action of endothelin-converting enzyme (ECE) to the fully active ET-1. In vascular tissues, ET-1 is synthesized in endothelium and secreted on the abluminal side of the vascular wall, acting as an autocrine or paracrine substance. In the heart, ET-1 is also synthesized by cardiac interstitial cells. Although active ET is present in circulation and its levels are increased in patients with heart failure, the half-life of ET is only a few minutes because of rapid clearance by the lung; therefore, ET is an autocrine or paracrine cytokine, not a hormone.

ET-mediated vasoconstriction has been implicated in several human cardiovascular disorders, such as pulmonary hypertension or congestive heart failure (Braunwald *et al.* 2001). At the cellular level, it interacts with other substances influencing vascular tonus, most notably the nitric oxide, also produced by the endothelial cells. Its effects are mediated by two kinds of receptors, ETA and ETB. ETA stimulation leads to proliferation of smooth muscle cells in the vessel wall and their contraction; ETB seems to be involved in contraction of the pulmonary vasculature. Inhibitors of this signalling pathway, such as bosentan or tezosentan, have emerged as a new class of drugs for the treatment of hypertension and heart failure in adult patients.

Endothelin signalling mediated via ETA receptor is essential for normal embryonic patterning in both avians (Kempf *et al.* 1998) and mammals (Kurihara *et al.* 1994, 1995, 1997, Yanagisawa *et al.* 1998a). In both classes of vertebrates, targeting of this pathway leads to perturbation of craniofacial neural crest derivatives and also cardiovascular defects. Ventricular septal defects were noted in 48% of ET-1-null mice (Kurihara *et al.* 1995), and this frequency increased up to 90% with an additional ETA antagonist treatment. Cardiovascular defects in this latter subgroup included reductions in trabeculation and loss of muscle and fibrous tissues at the crest of the interventricular septum. Similar basal/crestal defects to the interventricular septum have been noted in double knockouts in mice of genes encoding ECE-1 and ECE-2, the enzymes responsible for the activation of big-ET (Yanagisawa *et al.* 1998b). While these reports pointed to a primary myocardial defect in response to loss of ET-1 signalling function, no morphological or functional analyses were undertaken specifically on conduction tissues.

Exogenous ET activity can induce conduction cell differentiation in the chick embryonic heart *in vitro* and *in vivo* (Gourdie *et al.* 1998, Takebayashi-Suzuki *et al.* 2000, Gourdie & Watanabe 2004). Mechano-induced upregulation in ET signalling has been correlated with precocious conduction system development *in vivo* (Reckova *et al.* 2003, Hall *et al.* 2004). However, it remains unclear whether ET signalling has an influence on the development or function of the mammalian conduction system, although there are some *in vitro* data supporting the notion that this might be the case (Gassanov *et al.* 2004).

ET signalling appears to be developmentally regulated and is critically dependent on the presence of functional ET receptors (Gourdie *et al.* 1998). This seems to serve to restrict its effects to precisely defined windows for the induction of conduction system differentiation. Similarly, ETA-mediated vasoconstriction is not present during the early postnatal period in mice (Kuwaki *et al.* 2002), while this pathway is of considerable importance in the adult.

Present study by Karppinen *et al.* (2013) explores further the function of ET receptors in control of heart rate during development. They build upon earlier observations linking the ET-1 signalling via inositol triphosphate pathway on calcium oscillations and pacemaking and take on to investigate these effects *in vitro* and *in vivo* during mouse development. Their main finding is that ET-1 serves as a stabilizer of heart rhythm via control of calcium leak through the inositol triphosphate receptors, which occurs through ETB, rather than ETA. This is significant especially during the early stages of development, where I_f alone is not sufficient for maintaining rhythmic membrane voltage oscillations. The authors nicely correlate these findings with quantitative measurements of both ETA

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and ETB expression during development. While ETA mRNA in the heart shows a steady increase from ED 10.5 to birth, ETB levels show a bell-shaped curve with a peak at ED12.5. Application of ET-1 on cardiomyocytes isolated at ED9-11 increased their frequency of beating; however, the higher the initial rate, the smaller was the observed effect. Conversely, blockade of receptors via tezosentan (non-specific inhibitor of both ETA and ETB) leads to decreased frequency and irregular rhythm. Interestingly, ET-1 had no effect on whole isolated ED10.5 embryonic hearts, while the effect of Tezosentan was maintained; the authors suggest that this is due to already maximal activity of the pathway due to the presence of high levels of ET-1 in the intact heart. To dissect whether the inhibitory effect is due to ETA or ETB, the authors went on to probe this issue via specific inhibitors BQ123 and BQ788, respectively, finding that ETB blockade is responsible for oscillation frequency control. Of note, bradycardia after tezosentan infusion was observed also *in utero* in ED12.5 embryos, but not at ED18.5. This further points to narrow critical developmental window and cautions against using these drugs (already contraindicated because of their teratogenicity) during the early stages of pregnancy, where they might cause further detriment via cardiodepressant effect. The final piece of puzzle detailing the downstream signalling cascade comes from mimicking the bradycardia by inhibition of inositol triphosphate receptors, decreasing thus the calcium leak.

The cardiac conduction system is vital for generating and synchronizing the heartbeat. Beginning with Tawara, Einthoven and other pioneering workers, a wealth of information has been collected over the last 100 years on the histological, morphological and physiological characteristics of specialized cardiac tissues. During the last decade, considerable effort has been put into understanding the cellular and molecular mechanisms governing its development and function. Controversies have also arisen as to the nature of the signalling mechanisms involved in induction and patterning of the CCS, particularly with respect to the pathways functioning in mammals. This study furthers our understanding of physiological regulation of spontaneous heart rhythm during development. Understanding the signalling mechanisms that control normal development and function of the conduction system may thus also provide insight into cardiac disease.

Conflict of Interest

No conflict of interest to declare.

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