Expression and function of ET<sub>A</sub> and ET<sub>B</sub> receptors in SSc
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Endothelin (ET) receptors are widely expressed within the human body with one of their major functions being regulation of the vascular tone. Pulmonary arterial hypertension and other complications associated with SSc are related to the function and or dysregulation of these receptors. As ET receptors also play a crucial role in SSc, this review will discuss the expression and physiological functions of ET receptors in the human organism, their signalling pathways, the complications of diseases they are associated with and their importance as a therapeutic target in SSc.

**Key words:** Expression, Function, Endothelin<sub>A</sub> receptors, Endothelin<sub>B</sub> receptors, Endothelin, Systemic sclerosis, Scleroderma, Diseases, Signalling.

**Discovery, expression and physiological functions**

The ET receptors were discovered by pharmacological *in vitro* and *in vivo* studies showing different effects for the three isoforms of ET. To date, there are at least two known human ET receptors: the ETA receptor and the ETB receptor. ETA is usually classified into two subtypes, termed ET<sub>B1</sub> and ET<sub>B2</sub>, which most likely result from alternative splicing, but the evidence is considered controversial, as is the case for the subtypes ETA<sub>1</sub> and ETA<sub>2</sub>. Although ET receptors are expressed almost ubiquitously in humans, distribution and expression of the ET receptors depend on the respective organ, the tissue type and the developmental stage but may also be influenced by a given disease. ETA is primarily expressed in vascular smooth muscle cells and stromal tissues, whereas ETB is mainly expressed in vascular endothelium and epithelial tissues as well as by smooth muscle cells. However, ET receptors are also found in non-vascular structures.

The three isoforms of ET (ET-1, ET-2 and ET-3) bind with different affinities to the two ET receptors. Both ET receptors can bind all endothelin isoforms, but ETA shows a much higher affinity for ET-1, the most abundant ET in human plasma, than for ET-3, which is found at high levels in human brain. ETB, on the other hand, binds all three ET isoforms with equal affinity. The major function of ETA along with its ligand ET-1 consists in maintaining a normal vascular tone by modulating vasoconstriction and retention of sodium. Activation of ETA increases blood pressure. ETB is a more complex molecule because it can exert opposing effects depending on its location: ETB activation of smooth muscle cells results in vasodilation, whereas ETB activation on vascular endothelium causes vasodilatation through the release of nitric oxide by inducing NOS-III. ETB also lowers blood pressure through increased natriuresis and diuresis. ETB can induce the release of prostaglandins. In non-vascular tissues such as kidney, ETB may serve to clear ETs from circulation by receptor-mediated uptake and subsequent lysosomal degradation.

ET receptors can mediate stimulation of proliferation of different cell types. For example, smooth muscle cell proliferation is mainly stimulated via the ETA receptor, while proliferation of astrocytes is stimulated via the ETB receptor. In most cells, ETs exert co-stimulatory effects via their receptors, increasing the effects of other growth factors. The importance of ET receptors in proliferation and development has been underlined by gene disruption and mutational studies in animal models for embryonic development. ET receptors are also involved in modulating inflammation. Macrophages express ETB and upon activation by ET-1 release cytokines, chemokines and free radicals. Other functions include activation of neutrophils and mast cells.

There are many mediators that are able to regulate the expression of ET receptors. Epidermal growth factor, basic fibroblast growth factor, cAMP and hypoxia result in up-regulation of the ETA receptor, while platelet-derived growth factor, angiotensin II, TGF-β and the endothelins themselves down-regulate ETA expression. ETB is regulated in an opposing fashion: its production is up-regulated by angiotensin II and down-regulated by cAMP and catecholamines. The ETB receptor is a regulator itself: ET-1 synthesis is regulated by ETB, thus creating an autoregulatory mechanism.

**Signalling of ET receptors**

ET receptors belong to the group of G-protein-coupled receptors. The dissociated G-protein subunit activates phospholipase C (PLC), which cleaves phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>) to form inositol-1,4,5-trisphosphate (IP<sub>3</sub>) and diacylglycerol (DAG). IP<sub>3</sub> stimulates the release of Ca<sup>2+</sup> from intracellular calcium stores. In a second phase, this opens calcium channels within the plasma membrane, which further increases the intracellular Ca<sup>2+</sup> concentration by influx of calcium ions. In some smooth muscle cells, this also leads to the opening of Ca<sup>2+</sup>-activated potassium channels. DAG along with the elevated Ca<sup>2+</sup> concentrations activates protein kinase C (PKC). The activation of PKC is probably responsible for some of the non-vascular effects of endothelins, such as stimulation of proliferation. Further signalling pathways induced by the ET receptors include phospholipase D (PLD)-, mitogen-activated protein kinase (MAPK)- and p125 focal adhesion kinase (FAK)-dependent pathways. However, much less is known about how the ET receptors actually mediate these pathways. Depending on the distribution and type of ion channels and the signalling network of cells, ETB in particular can mediate different effects in different tissues and organs.

However, it should be mentioned that there may be some 'cross-talk' between the two ET receptors: although ETA and ETB have different affinities for the ET isoforms and can elicit different cellular responses, there is some evidence that ETB may require ETA for fully recognizing and binding ET-1 by forming an ETA–ETB Receptor heterodimer [3].

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Clinical relevance

Endothelin receptors play an important role in various diseases. Receptors and disease are most often associated with over- or underexpression of the receptors. Expression of ET-1 and ETB is increased in certain renal diseases [4]. In scleroderma-associated pulmonary fibrosis, ETA levels were significantly decreased, while ETB levels were slightly increased [5]. Diabetes animal models show overexpression of ETA and ETB in the renal cortex [6]. Most abnormalities concerning the ET receptors are associated with vascular diseases. Ischaemic heart diseases, for example, induce up-regulation of ETA and ETB in human coronary arteries [7]. ET receptors are also involved in inflammatory processes. For example, experimentally induced airway inflammation caused an increase of ETB in bronchial smooth muscle cells [8] and diabetic renal injury could be reduced via an anti-inflammatory mechanism [9].

ET receptors as therapeutic target in SSc

As mentioned previously, ET receptors have several primary sites of expression. Nonetheless, they are widely expressed in many tissues and organs including lung, kidney, liver and skin, which suggests numerous biological effects for the ET receptors in vivo. Vice versa, dysregulation of the ET system can affect many tissues and organs as is the case in SSc. The pathogenesis of SSc is complex but excessive fibrosis and vascular disorders are typical features. These often result in interstitial fibrosis of the pulmonary system and pulmonary vascular disease leading to pulmonary arterial hypertension (PAH) still being a dominant cause of death in SSc. Based on the pathophysiological mechanisms as outlined earlier, ET receptors are an attractive target to improve these sequelae. Several antagonists have been developed for blocking the ET receptors, yet only few are able fulfil the pharmacological and toxicological criteria for successful clinical studies. Amongst these are bosentan, an inhibitor of both ETA and ETB, and sitaxentan and ambrisentan, which are highly selective for ETA.

Many studies are addressing the question whether dual inhibitors or selective ETA inhibitors are the best option for treating SSc and its complications. Selective ETA inhibitors would in theory have the advantage not to reduce the vasodilative effects of ETB and the clearance of ET-1 from circulation by ETB. On the other hand, treatment with dual inhibitors is theoretically favoured of inducing a number of signalling pathways including PLC-, PLD-, PKC-, MAPK- and FAK-dependent pathways.

Besides regulating the vascular tone, the endothelin receptors are also involved in processes of proliferation, inflammation and development.

Endothelin receptors represent a promising therapeutic target for SSc-associated complications such as PAH.

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