Available online at www.sciencedirect.com
ScienceDirect
Vascular Pharmacology 45 (2006) 163 – 170
Vascular Pharmacology
www.elsevier.com/locate/vph

Early-onset endothelin receptor blockade in hypertensive heterozygous Ren-2 rats

Ivana Vaněčková [a,b,*], Herbert J. Kramer [c], Angela Bäcker [c], Stanislava Schejbalová [a,b], Zdena Vernerová [a,d], Václav Eis [a,d], Martin Opočenský [a,b], Pavel Dvořák [a,b], Luděk Červenka [a,b]

[a] *Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic*
[b] *Cardiovascular Research Center, Prague, Czech Republic*
[c] *Section of Nephrology, Medical Policlinic, University of Bonn, Germany*
[d] *Department of Pathology, 3rd Medical Faculty, Charles University, Prague, Czech Republic*

Received 28 February 2006; received in revised form 26 April 2006; accepted 5 May 2006

Abstract

Male heterozygous Ren-2 transgenic rats and Hannover Sprague–Dawley rats fed a normal or high-salt diet were either untreated or treated with the nonselective receptor ET_A/ET_B receptor blocker bosentan or the selective ET_A receptor blocker, ABT-627, known as atrasentan. Survival rate was partly increased by bosentan and fully normalized by atrasentan. Bosentan did not significantly influence the course of hypertension in TGR, whereas atrasentan significantly decreased BP on both diets. Atrasentan substantially reduced proteinuria, cardiac hypertrophy, glomerulosclerosis and left ventricular ET-1 tissue concentration on both diets. Our data indicate that ET_A receptor blockade is superior to nonselective blockade in attenuating hypertension, end-organ damage and improving survival rate.

Keywords: Endothelin receptors; Bosentan; Atrasentan; Ren-2 rats; End-organ damage

1. Introduction

Almost two decades ago, the potent vasoconstrictor peptide endothelin-1 (ET-1) was discovered (Yanagisawa et al., 1988). Its action is mediated by two types of receptors, namely ET_A and ET_B receptors. While ET_A receptors in the vascular system mediate vasoconstriction and are localized on vascular smooth muscle cells, the major function of ET_B receptors, localized mainly on endothelial cells, seems to be vasodilation in addition to its clearance function. Moreover, two distinctive ET_B receptors–ET_{B1} and ET_{B2}–with quite opposing function were identified in the rat (Gellai et al., 1996).

Since its discovery, a growing body of evidence has been accumulated showing that ET-1 plays a pivotal role in several cardiovascular diseases, including chronic heart failure, ischemic heart disease, hypertension, atherosclerosis, pulmonary hypertension and chronic heart failure. In these disease states, the levels of circulating ET-1 are increased, and treatment with ET inhibitors proved to be advantageous (Masaki, 2004). Thus, e.g. the nonspecific ET receptor blocker bosentan has been approved as a therapeutic agent to treat pulmonary hypertension (Kenyon and Nappi, 2003). However, despite the increasing evidence that the ET system plays an important role in the pathogenesis of systemic arterial hypertension, its mechanism of action is still not well understood.

Our present experiments were therefore performed, first, to evaluate the role of ET-1 in the onset and maintenance of hypertension in heterozygous Ren-2 transgenic rats. Heterozygous rats transgenic for the mouse renin gene (TGR) (strain name TGR(mRen2)27), a model of monogenetically defined hypertension (Mullins et al., 1990), exhibit a salt-sensitive component (Callahan et al., 1996). Special emphasis was given to the difference in action between the nonselective ET_A/ET_B and the specific ET_A receptor blockade. The benefit of specific

* Corresponding author. Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Videnska 1958/9, CZ-140 21, Prague 4, Czech Republic. Tel.: +420 261363199; fax: +420 241721666.
E-mail address: ivvn@medicon.cz (I. Vaněčková).

1537-1891/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.vph.2006.05.003

ET_A receptor blockade may relate to the fact, that, contrary to the nonspecific receptor blockade, it does not inhibit the vasodilatory (de Nucci et al., 1988) and natriuretic (Konishi et al., 2002) response to stimulation of ET_B receptors. Besides their vasoconstrictory function, ET_A receptors also mediate cell proliferation of various cell types, especially of vascular smooth muscle cells (Hirata et al., 1989). Therefore, the blockade of ET_A receptors may be beneficial in attenuating vascular alterations leading to end-organ damage. This antiproliferative action of ET_A receptor blockers has even attracted the attention of researchers in the field of cancer research (Salani et al., 2002; Nelson, 2003).

Since dietary sodium plays an important role in the pathogenesis of hypertension not only in humans (Weinberger, 1996) but also in salt-sensitive models of hypertension (Dahl et al., 1968), our second aim was to evaluate the influence of high-salt intake on the course of hypertension, end-organ damage and survival, as well as the potential role of ET-1 in TGR under these conditions. It is generally accepted that young animals are more susceptible to various hypertensinogenic stimuli (Zicha and Kunes, 1999) and also that therapeutic interventions made in these early periods of life are more effective. However, discrepant results were reported in heterozygous TGR, i.e. either no effect (Rothermund et al., 2003a) or hypotensive (Gardiner et al., 2000) effects of nonselective or selective ET receptor blockade were found in adult animals, whereas no effect was found in young animals (Whitworth et al., 1995; Rossi et al., 2000). Therefore, in the present study we evaluated the effects of nonselective as compared to selective ET receptor blockade in TGR on different sodium diets when treatment with receptor blockers was started early in their life.

2. Materials and methods

The protocols in the present study were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" and were approved by Czech Animal Care and Use Committee (Protocols 79/2001 and 923/2003).

2.1. Animals

We used male heterozygous rats transgenic for the mouse renin gene [TGR; strain name TGR(mRen2)27] and male Hannover Sprague–Dawley rats (HanSD) as normotensive controls. Animals were housed under standard conditions and had free access to chow and water. All animals used in this study were bred at the Center for Experimental Medicine of the Institute for Clinical and Experimental Medicine from stock animals supplied from Max Delbrück Center for Molecular Medicine in Berlin, Germany.

2.2. Experimental protocol

Animals were fed either a normal salt (NS, 0.45% NaCl) or high-salt diet (HS, 2% NaCl) starting on day 29 of age. At this time point, either nonselective ET_A/ET_B receptor blockade by bosentan, or selective ET_A receptor blockade by atrasentan was initiated. Bosentan (Actelion, Alschwil, Switzerland) was added to the diet. The concentration in the food was calculated to deliver a dose of 100 mg kg^{-1} day^{-1} (Roux et al., 1999). This dose was previously validated in our laboratory to effectively block ET receptors (Dvorak et al., 2004). The selective ET_A receptor blocker atrasentan (Abbott, Chicago, USA) was added to the drinking fluid; the dose was adjusted weekly to provide a concentration of 5 mg kg^{-1} day^{-1} (Mulder et al., 2000), which is generally accepted to effectively block ET_A receptors (Opgenorth et al., 1996; D'Angelo et al., 2005). At the start of the experiments, the animals were allotted to eight groups receiving either normal salt (NS) or high-salt diet (HS). As controls age-matched HanSD rats on the same regimens were investigated.

The following experimental groups were studied:

HanSD + NS (n = 24)
TGR + NS (n = 18)
TGR + NS + bosentan (n = 18)
TGR + NS + atrasentan (n = 18)
HanSD + HS (n = 24)
TGR + HS (n = 24)
TGR + HS + bosentan (n = 18)
TGR + HS + atrasentan (n = 23).

2.3. Blood pressure, proteinuria and tissue weight

From day 29 onwards, regular measurements of body weight and systolic BP (SBP) were made at weekly intervals using the tail plethysmography method (Hatteras Instruments, Cary, North Carolina, USA). At the age of 50 and 80 days, animals were housed in metabolic cages so that fluid intake could be monitored and urine collected. Urinary protein concentration in 24 h urine was measured by a biuret method (Lachema, Czech Republic).

By day 90, animals were weighed, anesthetized with thiopental sodium (50 mg kg^{-1}) and mean arterial pressure (MAP) was monitored directly in the carotid artery using the data acquisition system PowerLab (ADInstruments, Mountain View, California, USA). Kidneys and hearts were weighed. Ratios of kidney weight/body weight (KW/BW) and heart weight/body weight (HW/BW) were used as indices of organ hypertrophy.

2.4. Tissue ET-1 concentrations

Left heart ventricles were rapidly removed and cortex from the right kidney was quickly dissected. Both tissues were immediately frozen in liquid nitrogen for ET-1 determination using an enzyme-linked immunosorbent assay test (ELISA) (Amersham, Braunschweig, Germany).

2.5. Histological examination

The left kidney was quickly removed, fixed in 4% buffered formaldehyde, dehydrated and embedded. Paraffin sections