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Brief communication

A major role for AT_{1b} receptor in mouse mesenteric resistance vessels and its distribution in heart and neuroendocrine tissues

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Abstract

In mice, angiotensin (Ang) II type-1 (AT_1) receptors exist as AT1a, and AT_{1b} subtypes. In an effort to understand the role of AT_{1b} in regulating vascular function, AT_1 subtype mRNA and its functional relevance in the mesenteric resistance vessels were determined using wildtype (WT) and AT_{1a} knockout ($AT_{1a}^{-/-}$ mice) mice. With RT-PCR followed by restriction-enzyme digestion, we found that AT_{1b} accounted for almost all (98%) of AT_1 receptors in the mesenteric resistance vessels of WT mice. Also, the Ang II response in the vessels of $AT_{1a}^{-/-}$ mice was comparable to that of WT mice, suggesting an important role for AT_{1b} in regulating vasoreactivity. To further characterize AT_{1b} receptor distribution, several other tissues were examined. Among them, AT_{1b} is only predominantly expressed in hypothalamus, whereas AT_{1a} exists exclusively or as a major subtype in heart, pituitary, adrenal glands and brainstem. These results further underscore a tissue-specific role for AT_{1b} receptor in mice.
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1. Introduction

Angiotensin (Ang) II is a multi-functional peptide produced by the rennin–angiotensin system that plays an important role in regulating blood pressure [1]. To date, two types of Ang II receptors designated as AT_1 and AT_2 have been identified. While AT_2 receptors play an important role in the early stages of life, AT_1 receptors are thought to account for most of the biological effects of Ang II in adult [1]. In mice, AT_1 receptors are subdivided into two pharmacologically identical subtypes, AT_{1a} and AT_{1b}, which have been suggested to exhibit different patterns of expression [2–4].

Recently, gene knockout models for AT_1 receptor subtypes have been used to differentiate Ang II-mediated signaling in health and disease [5–10]. Previous in vivo studies suggest that AT_{1a} is primarily responsible for the regulation of blood pressure and cardiovascular function. This has been largely because AT_{1a} knockout ($AT_{1a}^{-/-}$), but not AT_{1b}-deficient ($AT_{1b}^{-/-}$), mice were found to exhibit low blood pressure and attenuated Ang II pressor response [6,11,12]. However, using isolated mouse abdominal aorta and femoral artery, we have recently found that AT_{1b} predominantly mediates Ang II induced contraction [8,13]. In this study, we further report experiments demonstrating that AT_{1b} plays a major role in mouse mesenteric resistance vessels, suggesting an important role for AT_{1b} in the regulation of vascular function. In addition, experiments were also performed to determine the distribution of AT_{1b} receptors in other mouse tissues, in which AT_1 receptor subtype composition has not been characterized.

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2. Material and methods

2.1. Buffers and chemicals

The ionic composition of physiologic salt solution (PSS) was as follows (in mM): NaCl 123, KCl 4.7, $NaHCO_3$ 15.5, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 1.25 and D-glucose 11.5. The 60 mM K^+-PSS (K^+) was prepared by replacing an equal molar of NaCl with KCl. Ang II was purchased from Sigma (St. Louis, MO). AT1 receptor antagonist losartan was bought from Merck & Co. (Whitehouse Station, NJ).

2.2. $AT_{1a}^{-/-}$ mice and tissue preparation

Male wildtype (WT) or $AT_{1a}^{-/-}$ mice (16–20 weeks age) were generated by mating AT_{1a} heterozygous breeding pairs purchased from The Jackson Laboratory (Bar Harbor, ME). Genotyping was performed with PCR reaction on DNA isolated from tail biopsies. Mice were euthanized with 95% CO_2 inhalation in accordance with the Animal Research Ethics Committee of The Ohio State University.

Branches of lower mesenteric artery with a diameter less than 150 μm were isolated, and the fat, connective tissue or the adventitia was mechanically removed under a binocular microscope. For mRNA analyses, vessels was frozen and stored at –80 °C. For functional studies, vessels with a diameter of 90–130 μm were cut transversely into approximately 1.5 mm segments. In addition, several other tissues, including heart, adrenal gland, pituitary, hypothalamus, and brainstem, were isolated for mRNA analyses.

2.3. Analyses of AT_1 subtype mRNA

Total RNA preparation and RT-PCR analyses were performed using an Absolutely RNA RT-PCR Miniprep Kit (Stratagen, La Jolla, CA), according to the manufacturer's instructions. Primers for AT_1 receptor were designed from common sequences of AT_1 isoforms [8,14,15]: 5′-CCAAAGTCACCTGCATCATC-3′ (PCR sense) and 5′-CACAATCGCCTAATTATCCTA-3′ (RT and PCR antisense). Protocols for RT-PCR reaction and enzymatic digestions were described elsewhere [8,14]. The expected size of AT1 PCR products was 305 bp. Eco RI cuts AT_{1a} PCR products at nucleotide 128, while Hpa II digests both isoforms, with AT_{1a} at nucleotide 125, and AT_{1b} at nucleotide 51, respectively. PCR products or digested fragments were separated with 2% agarose gel and visualized with ethidium bromide staining. Band densities were quantified using a Biochemi System (UVP Inc., Upland, CA). In certain experiments, β-actin mRNAs were amplified as previously described to serve as internal controls for the PCR analyses [14].

2.4. Isometric force measurement

The method for isometric force measurement was described elsewhere [8,14]. Briefly, the segment of mesenteric resistance vessels was mounted onto two tungsten wires in a 37 °C water-circulating tissue bath, of which one was fixed and the other was connected to a force transducer (AE 801, Horten, Norway). To remove the endothelial cells, vessel segments were moved around two tungsten wires for three times, with passive tension kept at approximately 100 mg prior to the experiment. Thereafter, vessels were stimulated with 60 mM K^+ every 15 min (five times), and the resting tension was increased in a stepwise manner. After the equilibration, the resting tension was adjusted to approximately 250–300 mg, at which 60 mM K^+ causes a maximum contraction. Ang II was used only once in each specimen and its response was expressed as a percentage of 60 mM K^+ induced contraction, assuming the value in the PSS (5.9 mM K^+) and 60 mM K^+ to be 0% and 100%, respectively.

3. Results and discussion

3.1. AT1 subtype mRNA expression in WT mesenteric resistance vessels and the heart

A major goal of this study was to define the function of AT_{1b} in mouse vasculature. Therefore, AT_1 mRNAs in mouse mesenteric resistance vessels were amplified using RT-PCR with primers common to both AT_{1a} and AT_{1b} isoforms. Then, the contribution of each subtype was determined using restriction-enzyme analyses. In parallel, we studied the heart muscle, in which the expression of AT_1 receptor subtypes has not been characterized.

As shown in Fig. 1, PCR products of expected size (305 bp) were clearly seen in both the vessels and the heart. Eco RI, which specifically cuts the products of AT_{1a}, only minimally (2%) digested the PCR products in the vessels, while it digested most of products in the heart. On the other hand, Hpa II, which cuts the PCR products of both isoforms, produced an almost complete digestion both in the heart and in the vessels. Interestingly, in the vessels 98.2% of the Hpa II-digested fragments corresponded to AT_{1b} (51 and 254 bp), whereas in the heart all fragments were associated with AT_{1a} subtype (125 and 180 bp). These results suggest that AT_{1b} is the predominant subtype in mouse mesenteric resistance vessels. AT_{1a}, on the other hand, is abundantly expressed in the heart muscle.

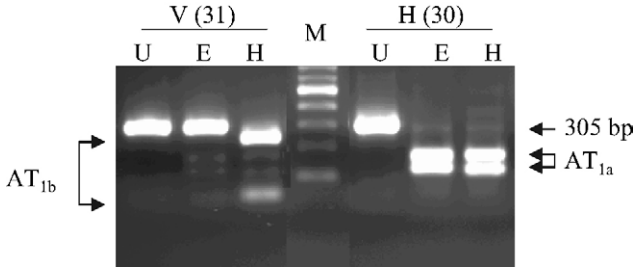

Fig. 1. Analyses of AT1 subtype mRNA in the mesenteric resistance vessels (V, 31 cycles) and the heart (H, 30 cycles) of WT mice. U, E, and H indicate undigested PCR products (305 bp), those cut with Eco RI, and Hpa II, respectively. M represents 100 bp-ladder size markers (NEB, Beverly, MA).

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