Neuron-targeted (pro)renin receptor deletion attenuates the development of deoxycorticosterone acetate (DOCA)-salt-induced hypertension.

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Abstract

Although (pro)renin receptor (PRR) is highly expressed in the brain, the physiological importance of brain PRR remains to be determined. We previously showed that PRR mRNA levels were up-regulated in the brain regions involved in blood pressure (BP) regulation in the DOCA-salt-induced hypertensive mice. To elucidate the physiological importance of brain PRR, we developed a neuron-specific PRR knockout mouse model (Neth-PRR). Using real-time PCR and immunofluorescence, we observed PRR deletion throughout the brain. Neth-PRR mice and Neth-cre mice with wild type PRR gene (WT, n/group) were implanted with telemetric probes. BP and heart rate (HR, bpm) were recorded for baseline and following DOCA-salt treatment (50mg DOCA, 0.9%NaCl drinking solution, 21 days). Neth-PRR mice exhibited normal baseline BP and HR. Following DOCA-salt treatment, BP was significantly lower in Neth-PRR mice (±3% ±6.2% at WT mice (±3 ±6.2% ±9.7% in Neth-PRR mice) compared to WT mice (±4 ±6% ±10% at WT mice). The cardiac sympathetic tone (HR response to propranolol, AVP, 0.9%NaCl) and the vasomotor sympathetic tone (BP response to chlorisondamine, APB, 0.9%NaCl) were lower in Neth-PRR mice than WT mice after DOCA-salt treatment. Moreover, HR response to methylxanthine (AHR 96±8 to 43±9) was greater in Neth-PRR mice compared to WT mice. In addition, DOCA-salt treatment increased plasma vasopressin (AVP) levels (3.6 ±3.3% ±3.0% ±3.0% in WT mice. PRR deletion significantly attenuated the increase in plasma AVP levels (19.0 ±2.0% ±2.0% ±2.0% induced by DOCA-salt. The data suggest PRR deletion prevents the development of DOCA-salt hypertension and is associated with improved autonomic function and reduction of plasma AVP levels.

Introduction

The renin-angiotension system (RAS) plays an important role in blood pressure regulation and body fluid homeostasis. Many studies have shown the importance of the brain RAS in the maintenance of normal blood pressure (BP) and the development of hypertension. Recently, a component of the brain Ras, which is a receptor for both prorenin and renin, was discovered and named the (pro)renin receptor (PRR). The binding of renin or prorenin to PRR promotes angiotension II (Ang II) generation and activates Ang II independent signaling pathways leading to organ injury. Recent studies have revealed that PRR plays essential pathophysiologic roles in renal and cardiovascular diseases. However, there is limited information available on the function of the PRR in the central nervous system. The presence of PRR mRNA in brain nuclei involved in BP regulation and body fluid homeostasis suggests that PRR may play a regulatory role in the central regulation of blood pressure and cardiovascular function.

Assess the effect of neuron-targeted PRR deletion on hypertension.

Materials and Methods

Transgenic mice generation: PRR-floxed mice were generated at Dr. Atsuhiko Ichihara’s laboratory. The PRR floxed 2 gene was deleted by breeding PRR floxed mice with mice that express Cre recombinase under the control of neuron-specific neurofilament-H (Neft) promoter (Neft-Cre mice from Jackson laboratory). The resulting PRR-neft/cre(-/-) mice represent neuron-specific PRR knockout (Neth-PRR) mice.

Characterization of Neth-PRR mice: Tail tissues were used for genotyping to identify the expression of Cre recombinase and the loss of the Loop site. Different areas of the brain were used for real time PCR and immunofluorescence to measure the RNA and protein levels of the PRR.

DOCA-salt hypertension modeling: Male Neth-PRR mice and control mice, 6-10 weeks old. Following anesthesia (0.75-1.5% isoflurane in oxygen at 15/min), mice were surgically instrumented with a radio telemetry probe (PA-C15, DSi) for blood pressure (BP) recording. Upon recovery (2 weeks), baseline blood pressure was recorded for 4 days. Mice were then treated with 50mg DOCA pellets with access to 0.9% NaCl solution for 21 days. Automatic function was assessed by using methylxanthine (1mg/kg), propranolol (5mg/kg) and chlorisondamine (5mg/kg). Changes in HR or BP was calculated after administration of the antagonists.

Vasopressin measurement: Pepsin was then extracted and concentrated from the plasma using a Sep-pak extraction kit (Waters). Plasma vasopressin concentration was then measured using an AVP Fluorescent Immunoassay kit (Phoenix Pharmaceuticals). The relative fluorescence unit was measured by a fluorescence reader with wavelength 340nm for excitation and 450nm for emission.

Results

Characterization of Neth-PRR mice

Figure 1. Generation of neuron-targeted PRR conditional knockout mice. The mouse PRR exon 2 gene was deleted by breeding PRR floxed mice with mice that express Cre recombinase under the control of neuron-specific neurofilament-H (Neft) promoter. The resulting PRR-neft/cre(-/-) mice represent neuron-specific PRR knockout (Neth-PRR) mice.

Figure 2. PRR expression in Neth-PRR mice. PRR expression decreases in brain nuclei involved in BP regulation including the subfornical organ (SFO), the paraventricular nucleus (PVN), and the area postrema (AP), as well as in non-cardiovascular regulatory brain regions in Neth-PRR mice compared to WT mice. At higher magnification, representative pictures showed that PRR is deleted in Neth-PRR mice where the cre recombinase is expressed.

Figure 3. Baseline parameters. The Neth-PRR and control mice were implanted with telemetry transmitters. Following 2 weeks recovery, baseline blood pressure and heart rate was recorded. The Neth-PRR mice exhibited normal BP (A), HR (B), and locomotor activity (C).

Figure 4. PRR deletion decreases BP and improves autonomic function in DOCA-salt hypertension. Mice were implanted with telemetry transmitters for BP recording and with 50 mg DOCA-pellet (21 days release). 0.9%NaCl was provided in drinking solution by A and HR (B) were continuously recorded in conscious mice. The cardiac parasympathetic tone (HR response to 1mg/kg Atropine, sp), sympathetic tone (HR response to 1mg/kg Propranolol, sp), and vasomotor sympathetic tone (BP response to 1mg/kg Chlorisondamine, sp) were assessed before and after DOCA-salt treatment. n=4/group, *P<0.05 vs. Neth-PRR in figure A, *P<0.05 vs. WT and #P<0.05 vs. WT-DOCA in figure C and D.

Figure 5. PRR deletion in the neurons decreases plasma AVP levels during DOCA-salt stimulation. Baseline AVP levels were similar between Neth-PRR and WT mice. DOCA-salt increased AVP levels in the WT mice. PRR deletion in the neurons attenuated the increase of AVP induced by DOCA-salt. n=4/group, *P<0.05 vs. WT and #P<0.05 vs. WT-DOCA.

Summary

> Neth-PRR mice exhibit normal basal blood pressure, heart rate and locomotor activity.
> Neuron-targeted PRR deletion attenuates the development of DOCA-salt hypertension.
> PRR deletion improves parasympathetic tone, decreases cardiac and vasomotor sympathetic tone during DOCA-salt hypertension.
> PRR deletion in the neurons attenuates the increase of AVP induced by DOCA-salt.

Conclusions

> PRR plays an important regulatory role in the central regulation of blood pressure.
> PRR may represent a therapeutic target for the treatment of hypertension.

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