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Research Article

Age-dependent regulation of renal vasopressin V_{1A} and V_2 receptors in rats with genetic hypertension: implications for the treatment of hypertension

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Abstract

The role of arginine vasopressin (AVP) as a hypertensive hormone remains controversial. We have previously reported that intervention with a V_{1A} receptor antagonist in 6-week-old prehypertensive spontaneously hypertensive rats (SHR) for 4 weeks attenuated the subsequent development of hypertension in adult SHR. This study assessed the age-dependent regulation of plasma AVP levels and kidney V_{1A} and V_2 receptor expression during the development of hypertension in SHR and in normotensive Sprague Dawley rats. Systolic blood pressure (SBP), plasma AVP, and plasma renin activity (PRA) and kidney V_{1A} and V_2 receptor expression were assessed. SHR were studied at three ages: prehypertensive (6 weeks), developed hypertension (10 weeks), and established hypertension (16 weeks). SBP increased with age in SHR ($P < .01$) and both plasma AVP ($P < .01$) and PRA ($P < .05$) were increased in 10-week-old SHR. Renal medulla V_{1A} receptor gene expression decreased in 10-week and 16-week-old SHR ($P < .01$), with a reduction in V_{1A} receptor protein in the inner medulla of 16-week-old SHR ($P < .05$) compared with young SHR. There was no change in V_2 receptor expression during the development of hypertension. In normotensive rats, plasma AVP, PRA, and kidney V_{1A} and V_2 receptor expression were unchanged over time. These data suggest that in SHR, activation of plasma AVP and the renal V_{1A} receptor occurs during developing hypertension, with downregulation when hypertension is established. The use of V_{1A} receptor antagonists in prehypertension may provide a unique opportunity for the prevention of hypertension in high-risk individuals. J Am Soc Hypertens 2013;7(1):3–13.

Keywords: Arginine vasopressin; hypertension; prehypertension; kidney; SHR; vasopressin receptor antagonists.

Introduction

Arginine vasopressin (AVP) is a hormone that plays an important role in blood pressure control and salt and water homeostasis through its potent vasoconstrictor effects at the vascular V_{1A} receptor and its antidiuretic actions at the renal V_2 receptor.[1–4]

Although the role of AVP in the pathophysiology of mineralocorticoid hypertension is well recognized,[5] its contribution to the pathogenesis and maintenance of genetic hypertension remains controversial. The finding that hypertension develops in the stroke-prone spontaneously hypertensive rat (SHR) crossbred with Brattleboro rats[6] has long been a powerful argument against the importance of AVP, but the finding of AVP-like immunoreactivity indistinguishable from authentic AVP in peripheral tissues of the Brattleboro rat[7] has cast doubt on the validity of that argument.

Several other lines of evidence support a role for AVP in the pathogenesis of genetic hypertension. Our own studies have shown that intervention with a V_{1A} receptor antagonist in the prehypertensive 6-week-old SHR reduced blood pressure during the 4 weeks of treatment and led to a persistent reduction in blood pressure in adult SHR after treatment withdrawal.[8,9] Both intravenous[10] and renal intramedullary[11]

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infusion of a $V1_{1A}$ receptor agonist resulted in sustained hypertension, and V_{1A} receptor knockout mice have reduced blood pressure with a suppressed renin angiotensin system[12] and decreased susceptibility to salt-induced hypertension.[13]

The V_2 receptor may play a role in the development of hypertension. Long-term AVP infusion into the renal medulla did not cause sustained hypertension[14] because of stimulation of renal vasodilatory V_2 receptors,[14,15] and V_2 receptor–mediated release of paracrine hormones from renal medullary interstitial cells[16] that offset the vasoconstrictor and hypertensive actions of AVP. We have reported that administration of an oral V_2 receptor antagonist, OPC-31260, to prehypertensive SHR increased blood pressure in adult SHR,[9] adding to the evidence that renal V_2 receptor stimulation may be involved in preventing the full expression of hypertension.

In adult SHR with established hypertension, the role of AVP is unclear; there are reports that circulating vasopressin concentrations are increased and AVP receptor blockade lowers blood pressure as well as reports to the contrary.[1,2,5,9] We reported that the oral V_{1A} receptor antagonist OPC-21268 had no effect on blood pressure in adult 18-week-old SHR, indicating that vasopressin is not involved in the maintenance of blood pressure once high blood pressure has developed.[5]

Despite the potential importance of AVP via actions at both the V_{1A} and the V_2 receptor in the kidney, there have been few studies to assess the regulation of both receptor subtypes during the development of hypertension in SHR. Most kidney studies in SHR have focused on the expression of V_{1A} receptors in the resistance vessels in the renal cortex,[17–19] whereas localization studies suggest that maximal V_{1A} receptor binding occurs in the medulla in the vasa recta and nephron segments.[16,20–23] Many of the studies that examined AVP receptors in the renal medulla used the agonist radioligand [^{3}H] AVP, which binds to both V_{1A} and V_2 receptors.[17,24,25] To date no study has assessed concomitantly assessed both V_{1A} and V_2 receptor binding in the kidney during the development of hypertension.

The aim of this study was to use selective V_{1A} and V_2 receptor antagonist radioligands[20,26,27] to discriminate changes in renal medullary V_{1A} and V_2 receptor levels that may occur during the development of genetic hypertension using the SHR. Three separate groups of SHR were studied; in the prehypertensive stage (age 6 weeks), at 10 weeks when hypertension had developed, and when hypertension was established in adult SHR (age 16 weeks). Systolic blood pressure (SBP), water intake, urinary output, plasma and urinary osmolality and sodium, plasma AVP, plasma renin, and kidney V_{1A} and V_2 receptor gene and protein expression were assessed.

There has been much debate around the appropriate normotensive control for the SHR strain.[28,29] Many investigators have used the normotensive Wistar-Kyoto (WKY) rat but these rats were not strictly inbred and the genetic variation between the two strains makes them unsuitable as a "control." In this article, we have studied the parameters of interest longitudinally across time, making the prehypertensive SHR a form of control for the adult SHR. We have also assessed the normotensive Sprague Dawley (SD) rat to take into account the effect of growth and increased body weight per se on AVP and blood pressure.

Methods

Animals

Experimental procedures were performed in accordance with the National Health and Medical Research Council of Australia guidelines for animal experimentation, and approved by the Animal Ethics Committee, Austin Health. Rats were obtained from the Austin Biological Research Laboratories, Austin Health, Melbourne, Australia. To confirm their inbred status, SHR colonies were regularly tested for polymorphic markers. Animals were housed at 20 to 22°C in a 12-hour light/dark cycle with access to a standard rat chow (0.6% sodium, 2% chloride, 2% calcium, Norco, Melbourne, Australia) and tap water ad libitum, unless otherwise indicated.

Experimental Protocol

Study 1: SBP, vasopressin, and the SHR

SBP was measured by tail-cuff plethysmography (38L flatbed recorder, model 229 Amplifier, IITC Life Science, Woodland Hills, CA) in male SHR age 6, 10, and 16 weeks (n = 26 rats/age group) over 3 consecutive days and the three readings were averaged. A subset of rats from each age group was placed into metabolic cages to assess 24-hour food and water intake, urine output, and urinary sodium and osmolality (n = 10 rats/age group). Rats were killed by decapitation and trunk blood collected into prechilled lithium heparin tubes for measurement of plasma osmolality, sodium, renin, and AVP (n = 10 rats/age group). Kidneys were weighed and rapidly frozen in isopentane for real-time polymerase chain reaction (RT-PCR) (n = 6 rats/age group) and in vitro receptor autoradiography of V_{1A} and V_2 receptors (n = 4 rats/age group). The heart was dissected into left and right ventricle and weighed.

Study 2: SBP, vasopressin, and the SD rat

The same protocol was followed in a normotensive cohort of male SD rats (age 6, 10, and 16 weeks; n = 8 rats/age group).

Analytical Methods

Plasma AVP was measured by radioimmunoassay as previously described.[30] The interassay and intra-assay coefficients of variation were less than 8% and the limit of