Renal Dopamine-1 Receptors in Hypertensive Inbred Rat Strains With and Without Hyperactivity

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Renal dopamine-1 (DA-1) receptors are involved in the regulation of sodium transport in several nephron segments, including the proximal convoluted tubule (PCT). DA-1 receptors in the PCT and cortical collecting duct of normotensive rats are linked to the stimulation of adenylyl cyclase (AC). We have reported a defect in the DA-1 receptor/AC coupling in the PCT of the spontaneously hypertensive rat (SHR) of the Okamoto-Aoki strain. Hyperactivity and hypertension are both expressed in the SHR. To determine if the DA-1 receptor coupling defect is associated with hyperactivity or hypertension, we studied the DA-1 receptor in the PCT of two new inbred rat strains derived from the SHR: the hyperactive WKHA and the hypertensive WKHT rat. Tail-cuff blood pressures taken at 4 weeks indicated that WKHT rats were not hypertensive (86±3 mm Hg, n=6), whereas at 12 weeks systolic pressures in both SHR and WKHT rats exceeded 150 mm Hg. Hyperactivity, however, was noted in WKHA rats even at this early age. Basal AC activity was similar in WKHA and WKHT PCT in either age group. In the older rats, the DA-1 agonist fenoldopam (10⁻⁷ mol/L) stimulated AC activity in WKHA (70.6±16.1 fmol per 3 mm PCT per 20 minutes, n=3) but not in WKHT PCT (43.3±3.6 fmol per 3 mm PCT per 20 minutes, n=4). Gpp(NH)p (10⁻⁵ mol/L), a nonhydrolyzable GTP analogue, stimulated AC activity to a similar extent in WKHA and WKHT PCT. Forskolin (10⁻³ mol/L) also stimulated AC activity to a similar extent in PCT from both strains. Similar results were obtained in the younger, prehypertensive rats. The specific binding of the DA-1 antagonist [¹²⁵I]-Sch 23982 was concentration dependent and saturable in both strains. The dissociation constant (Kd) and maximum receptor density (Bmax) were similar in WKHA PCT (23.9±1.7 nmol/L and 0.52±0.04 pmol/mg protein, n=5) and WKHT PCT (23.8±3.8 nmol/L and 0.58±0.14 pmol/mg protein, n=5). These data demonstrate that the coupling defect between the DA-1 receptor and AC in the PCT from these new inbred rat strains is associated with the hypertensive phenotype. The DA-1 receptor in PCT may be important in the pathogenesis of genetic hypertension. (Hypertension 1993;21:485–490)

Key Words • receptors, dopamine • rats, inbred SHR • hyperkinesis • kidney tubules, proximal • adenosine cyclic monophosphate • adenylyl cyclase

Dopamine receptors have been characterized in specific segments of the nephron3-4 and presumably act as a target for dopamine synthesized in the kidney.5-7 Dopamine receptors of the dopamine-1 (DA-1) subtype stimulate adenylyl cyclase (AC) activity and reduce tubular reabsorption of sodium by inhibiting both Na⁺,K⁺-ATPase activity8,4 and Na⁺-H⁺ exchange activity.8,9 Other dopamine receptor subtypes (DA-2, DA₃) may also affect sodium transport.10,11 Under conditions of “moderate” sodium excretion, dopamine produced by the renal tubules acts as a paracrine or autocrine agent that regulates sodium excretion.12,13 Several animal models of essential hypertension are initiated or aggravated by increased sodium load.14-16 The decreased natriuretic response of the spontaneously hypertensive rat (SHR) of the Okamoto-Aoki strain to a sodium load has been attributed in part to an attenuated DA-1-mediated action.17 The natriuretic effect of DA-1 agonists is also reduced in the SHR.17 We and others have reported a decreased ability of DA-1 agonists to stimulate AC activity in the proximal tubule of the SHR and Dahl salt-sensitive rat.18-20 The defective DA-1 receptor/AC coupling seems to be specific to the proximal tubule of the kidney, because the defect was not found in the striatum of the brain or the cortical collecting duct of the kidney.21 This defective receptor in the proximal tubule may explain the decreased ability of dopamine and DA-1 agonists to inhibit Na⁺-H⁺ exchange activity in the renal tubular brush border membranes22 and in proximal tubules.20 Other reports also suggest that the ability of dopamine to inhibit Na⁺,K⁺-ATPase activity in the proximal tu-
FIGURE 1. Bar graph shows blood pressure (BP) and activity testing at 12 weeks of age in spontaneously hypertensive rats (SHR), Wistar-Kyoto (WKY) rats, and inbred hyperactive (HA) and hypertensive (HT) rat strains as previously described. Systolic pressure was determined by tail plethysmography in restrained rats warmed in a 37°C chamber. Spontaneous activity was determined in an activity cage equipped with light beams and photodetectors; scores represent the number of light beams crossed in 15 minutes. Values are mean±SEM for two WKY rats, two SHR, eight HA rats, and six HT rats. *p<0.05 compared with WKY; dp<0.05 compared with SHR; and §p<0.05 compared with HA (analysis of variance, Bonferroni correction).

Adenylyl Cyclase Studies

In adult rats, basal AC activity in WKHT PCT (43.5±4.9 fmol per 3 mm PCT per 20 minutes, n=4) was significantly higher than that in WKY PCT (22.3±3.3, n=6) but similar to that noted in WKHA PCT (35.6±5.6, n=3). Fenoldopam (10⁻⁷ mol/L) stimulated AC activity in PCT from WKHA rats (70.6±16.1, n=3) and WKY rats (46.8±10.1, n=6) but not from WKHT rats. To determine whether this inability of fenoldopam to stimulate AC activity in WKHT PCT was related to the G, protein, we studied the ability of Gpp(NH)p to stimulate AC activity. Gpp(NH)p (10⁻³ mol/L) stimulated AC activity to a similar extent in WKHA PCT (84.9±15.9, n=3) and WKHT PCT (84.4±8.5 fmol per 3 mm PCT per 20 minutes, n=4) (Figure 3). To determine whether the function of the AC enzyme per se was altered, we studied forskolin, which directly stimulates the catalytic subunit of AC. Forskolin stimulated AC activity to a similar extent in WKHA PCT (8.9±2.7-fold over basal, n=3) and WKHT PCT (10.0±1.2-fold over basal, n=4) (Figure 4).

When examined in the prehypertensive age group, basal AC activity was similar in WKHA and WKHT PCT (Table 1). However, the ability of fenoldopam to stimulate AC activity in PCT was already markedly attenuated in prehypertensive WKHT PCT, in contrast with WKHA PCT, where fenoldopam (10⁻⁷ mol/L)
Radioligand Binding Studies

Specific binding of 125I-Sch 23982 to PCT was concentration dependent and saturable in both young WKHA and WKHT rats (Figure 5, insets). The \( K_d \) and \( B_{max} \) values were similar in both strains. \( K_d \) was 23.9±1.7 and 23.8±3.8 pmol/L and \( B_{max} \) was 0.52±0.04 and 0.58±0.14 pmol/mg protein in WKHA \((n=5)\) and WKHT \((n=5)\) rats, respectively (Figure 5). These \( K_d \) and \( B_{max} \) values are similar to those previously reported from our laboratory using WKY rats and SHR obtained from commercial sources.18

Discussion

We have previously reported that the ability of DA-1 agonists to stimulate AC activity in the microdissected PCT of SHR is attenuated compared with WKY PCT.18 This report has since been confirmed by Gesek and Schoolwerth using proximal tubular segments. The preliminary studies we have also detected expression of the DA-A receptor gene in rat proximal tubules.40 These two subtypes of dopamine receptors are linked to AC activation. The genes coding for these receptors have been assigned to human chromosomes 5 and 4, respectively.41-42 The linkage studies need not eliminate an important contribution of a dopamine receptor gene in blood pressure elevation. The \( Bpl \) locus in rat chromosome 10 (human chromosome 17), the \( Bp2 \) locus in rat chromosome 18, and the rat X chromosome could account for only about 40% of the blood pressure elevation.38,39 Thus, other important genes are likely to contribute to the hypertension in the SHR.30 The \( Bpl \) locus assigned to rat chromosome 10 had the greatest influence on blood pressure. Because a rat microsatellite marker of angiotensin converting enzyme was mapped to this chromosome with the region containing the \( Bpl \) locus, this enzyme was suggested as a candidate gene in primary hypertension. Dopamine receptors can also influence the renin-angiotensin system, and their interaction with the renin-angiotensin system can also influence sodium excretion and blood pressure regulation.1,13

In summary, we report a cosegregation of a defective DA-1/AC coupling in the PCT with hypertension, using a new strain of SHR in which the hyperactive and hyperreactive traits were genetically separated from the hypertensive trait.

References


Figure 5. Scatchard plot of specific 125I-Sch 23982 binding in proximal convoluted tubule (PCT) of adult inbred hyperactive (WK-HA) and hypertensive (WK-HT) rats. Dissociation constants \((K_d)\) and maximum binding \((B_{max})\) are listed in Table 1. 125I-Sch 23982 demonstrated high-affinity binding in PCT of both rat strains. There was no difference in \( B_{max} \) between WK-HA and WK-HT PCT. Data points shown are representative data. Nonspecific bindings were determined using the selective dopamine-1 antagonist Sch 23390 \((10^{-5} \text{ mol/L})\). Inset graphs show concentration dependence of specific bindings averaged for all five experiments of each rat. Values are mean±SEM (error bars). B, specifically bound 125I-Sch 23982; F, free 125I-Sch 23982.
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