Impaired renal D1-like and D2-like dopamine receptor interaction in the spontaneously hypertensive rat

CECILIA A. LADINES,1 CHUNYU ZENG,1 LAUREANO D. ASICO,1 XIAOGUANG SUN,1 FELICE POCCHIARI,2 CLAUDIO SEMERARO,2 JOSEPH PISEGNA,3 STEPHEN WANK,3 IKUYO YAMAGUCHI,1 GILBERT M. EISNER,4 AND PEDRO A. JOSE1,5

Departments of 1Pediatrics, 5Physiology and Biophysics, and 4Medicine, Georgetown University Medical Center, Washington, District of Columbia 20007; 2Zambon Group, 20091 Bresso (MI), Italy; and 3Developmental Endocrinology Branch, National Institute of Child Health and Human Development, Bethesda, Maryland 20892

Received 8 March 2000; accepted in final form 22 May 2001

Ladines, Cecilia A., Chunyu Zeng, Laureano D. Asico, Xiaoguang Sun, Felice Pocchiarri, Claudio Semeraro, Joseph Pisegna, Stephen Wank, Ikuyo Yamaguchi, Gilbert M. Eisner, and Pedro A. Jose. Impaired renal D1-like and D2-like dopamine receptor interaction in the spontaneously hypertensive rat. Am J Physiol Regulatory Integrative Comp Physiol 281: R1071–R1078, 2001.—D1-like (D1, D5) and D2-like (D2, D3, D4) dopamine receptors interact in the kidney to produce a natriuresis and a diuresis. Disruption of D1 or D2 receptors in mice results in hypertension that is caused, in part, by a decreased ability to excrete an acute saline load. We studied D1-like and D2-like receptor interaction in anesthetized spontaneously hypertensive rats (SHR) by the intrarenal infusion of Z-1046 (a novel dopamine receptor agonist with rank order potency of D3>D2>D1>D5). Z-1046 increased glomerular filtration rate (GFR), urine flow, and sodium excretion in normotensive Wistar-Kyoto rats but not in SHRs. The lack of responsiveness to Z-1046 in SHRs was not an epiphenomenon, because intrarenal cholecystokinin infusion increased GFR, urine flow, and sodium excretion to a similar extent in the two rat strains. We conclude that renal D1-like and D2-like receptor interaction is impaired in SHRs. The impaired D1-like and D2-like receptor interaction in SHRs is not caused by alterations in the coding sequence of the D2 receptor, the D2-like receptor expressed in rat renal tubules that has been shown to be involved in sodium transport. Because the diuretic and natriuretic effects of D1-like receptors are, in part, caused by an interaction with D2-like receptors, it is possible that the decreased Z-1046 action in SHRs is secondary to the renal D1-like receptor dysfunction in this rat strain.

D1-like receptors; D2-like receptors; natriuresis; diuresis; cholecystokinin; hypertension

SEVERAL CANDIDATE GENES have been shown to be important in the pathogenesis of hypertension in human and rodent models of genetic hypertension, but the etiology of essential hypertension remains to be determined (22). In several models of hypertension, an impairment in the ability of the kidney to regulate fluid and electrolyte balance is important in the pathogenesis of the high blood pressure (12). There is ample evidence that under conditions of moderate sodium loading, endogenous renal dopamine, acting via D1-like dopamine receptors with contributions by D2-like dopamine receptors, is important in the regulation of sodium excretion (13, 17, 18, 29, 37). The five dopamine receptors that have been cloned are expressed in the kidney: two belong to the D1-like family (D1 and D5) and three belong to the D2-like family (D2, D3, and D4) (17, 18). Although dopamine or the selective D1-like receptor agonist fenoldopam elicits a decrease in proximal tubular sodium reabsorption in normal humans or in Wistar-Kyoto (WKY) rats, this effect is not seen in the spontaneously hypertensive rat (SHR) and in humans with essential hypertension (1, 7, 10, 26). In mice, disruption of the D1-receptor gene led to the development of hypertension (1).

However, abnormalities in the D1-like receptor alone may not explain salt-sensitive hypertension. Although D2-like receptors exert an antinatriuretic effect when stimulated independently of D1-like receptors, D1-like and D2-like receptors may participate in complex interactions at different levels and through different mechanisms, resulting in a potentially greater inhibition of the sodium pump (4, 5, 14, 17, 18, 33, 34, 36, 39). For instance, in striatal neurons and proximal tubules (but not in medullary thick ascending limbs or cortical collecting ducts), both D1-like and D2-like receptors were required to inhibit Na+–K+-ATPase activity (4, 33). More recently, we reported that D1-like receptors exerted a renal vasodilatory effect while D2-like receptors exerted a renal vasoconstrictor effect (16), in agreement with the studies of Siragy et al. (37). We also found that costimulation of D1-like and D2-like receptors resulted in a diuresis and a natriuresis greater than stimulation of D1-like receptors alone, as predicted by combined ability of these receptors to inhibit Na+–K+-ATPase activity in renal proximal tubules (4, 16, 33).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
We previously showed that disruption of the D_3-receptor gene in mice produces renin-dependent hypertension and impairment in the excretion of an acute saline load (3). In the SHR, there is also evidence of a defective interaction between D_1- and D_2-like receptors (42). We, therefore, sought to determine whether the ability of D_1-like and D_2-like receptors to inhibit sodium transport in vivo is impaired in the SHR. We compared the effect of Z-1046 (a novel dopamine receptor agonist with rank order potency of D_2 > D_3 > D_5 > D_1) (16) on glomerular filtration rate (GFR), urine flow (V), and absolute (U_NaV) and fractional (FE_{N_a}) sodium excretion between the WKY rat and its hypertensive counterpart, the SHR. We also studied the coding sequence and expression of the D_3 receptor, the D_2-like receptor expressed in renal tubules involved in sodium transport (17, 23, 27).

**METHODS**

Male WKY and SHRs (12–16 wk old) were maintained on a regular Purina rat chow diet and unlimited water intake. Food but not water was then withheld 24 h before the study. The rats were anesthetized with pentobarbital sodium (50 mg/kg body wt), placed on a heated table to maintain core body temperature at ~37°C, and tracheotomized (PE-240) (16). Catheters (PE-50) were placed into the external jugular and femoral veins and left carotid artery for fluid administration and blood pressure monitoring. Systemic blood pressure was monitored electronically using Cardiomax II (Columbus Instruments, Columbus, OH). Laparotomy was performed to expose the left and right ureters, which were then catheterized for urine collection. The right suprarenal artery (which originates from the right renal artery) was located and catheterized (PE-10, heat stretched to 180 μm). In some animals, a Transonic Systems flow probe was secured around the right renal artery (Transonic Systems, Ithaca, NY); the abdomen was closed with surgical clips. Fluid losses throughout the experiment were replaced with 5% albumin at 1% body wt over 30 min. The animals then received an intravenous infusion of saline containing [14C]inulin (0.01 mCi/10 ml infusate; New England Nuclear, Boston, MA) at a rate of 5 ml/h for three periods where the vehicle, normal saline, was infused. The vehicle alone was again infused during the recovery period, during which the vehicle alone was again infused. The infusate was changed 10 min before each period to account for the dead space in the renal arterial catheter. All infusions were given at a rate of 40 μl/h.

**Cholecystokinin group.** To determine whether there is receptor selectivity in any differential effect of Z-1046 between WKY and SHRs, we also tested the effect of cholecystokinin. Cholecystokinin receptors, similar to D_1-like receptors, are linked to G_sα and G_q (2, 17). After two baseline periods with vehicle infusion, cholecystokinin was given to achieve a final renal arterial blood concentration of 10^{-7} mol/l for three periods. Thereafter, the infusion was changed to the vehicle during the recovery period as in the Z-1046 group.

Blood samples were obtained before the first collection period, before the fifth collection period, and at the end of the experiment. Radioactivity, sodium, and potassium concentrations were assayed in the blood and urine samples. The position and patency of the intrarenal arterial catheter were verified with lissamine green infusion at the conclusion of the experiment.

**Immunoblotting Studies**

The antipeptide polyclonal affinity-purified rabbit anti-rat D_3 antibody was raised against the specific 19 amino acids in the third cytoplasmic loop of the D_3-dopamine receptor at a concentration of 1 μg/μl (D3RI2-A, Alpha Diagnostic International, San Antonio, TX). Membranes derived from kidney homogenates or brush-border membranes (BBM) were mixed with Laemmli sample buffer, boiled for 5 min, subjected to electrophoresis on 7.5% SDS-PAGE, and transferred electrophoretically to nitrocellulose membranes. Nonspecific binding was blocked with 10% nonfat dry milk in Tris-HCl-saline-Tween 20 buffer. The membrane was then probed with the D_3 receptor, the D_2-like receptor expressed in renal tubules involved in sodium transport (17, 23, 27).

![Table 1. Effect of vehicle on renal function in the infused right kidney of the SHR](http://ajpregu.physiology.org/)
sequently, PCR was performed to amplify the coding region within groups was made using ANOVA for repeated measures.

Table 3. Effect of Z-1046 on renal function in the infused right kidney of the WKY rat

<table>
<thead>
<tr>
<th>Periods</th>
<th>MAP, mmHg</th>
<th>GFR, ml·g kidney⁻¹·min⁻¹</th>
<th>V₁, μl/min</th>
<th>U₅₀V, nEq/min</th>
<th>FE₅₀Na, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z₁</td>
<td>101.0 ± 2.7</td>
<td>1.9 ± 0.1†‡</td>
<td>9.03 ± 0.51†‡</td>
<td>1,120 ± 199*</td>
<td>0.39 ± 0.07*</td>
</tr>
<tr>
<td>Z₂</td>
<td>102.3 ± 2.0</td>
<td>1.7 ± 0.1*</td>
<td>9.07 ± 0.48†‡</td>
<td>1,120 ± 199*</td>
<td>0.39 ± 0.07*</td>
</tr>
<tr>
<td>R₁</td>
<td>101.3 ± 2.6</td>
<td>1.3 ± 0.1</td>
<td>7.13 ± 0.65</td>
<td>818 ± 186</td>
<td>0.38 ± 0.08</td>
</tr>
<tr>
<td>R₂</td>
<td>101.8 ± 3.3</td>
<td>1.2 ± 0.1</td>
<td>6.07 ± 0.63</td>
<td>725 ± 178</td>
<td>0.34 ± 0.07</td>
</tr>
<tr>
<td>R₃</td>
<td>102.6 ± 3.0</td>
<td>1.4 ± 0.1</td>
<td>5.69 ± 0.45</td>
<td>700 ± 147</td>
<td>0.30 ± 0.06</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. WKY, Wistar-Kyoto (body wt 388 ± 13 g, n = 10); periods Z₁–Z₃, Z-1046 infusion periods; periods R₁–R₃, recovery periods (vehicle infusion). *P < 0.05 vs. Z₁ or Z₂, repeated-measures ANOVA (ANVR). Newman Keuls test. †P < 0.05 vs. R₁ or R₂, t-test with Bonferroni correction.

Effect of Z-1046 Infusion on Renal Function

Table 2. Effect of Z-1046 on renal function in the infused right kidney of the SHR

<table>
<thead>
<tr>
<th>Periods</th>
<th>MAP, mmHg</th>
<th>GFR, ml·g kidney⁻¹·min⁻¹</th>
<th>V₁, μl/min</th>
<th>U₅₀V, nEq/min</th>
<th>FE₅₀Na, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z₁</td>
<td>101.6 ± 2.7</td>
<td>1.9 ± 0.1†‡</td>
<td>9.03 ± 0.51†‡</td>
<td>1,120 ± 199*</td>
<td>0.39 ± 0.07*</td>
</tr>
<tr>
<td>Z₂</td>
<td>102.3 ± 2.0</td>
<td>1.7 ± 0.1*</td>
<td>9.07 ± 0.48†‡</td>
<td>1,120 ± 199*</td>
<td>0.39 ± 0.07*</td>
</tr>
<tr>
<td>Z₃</td>
<td>102.6 ± 3.0</td>
<td>1.4 ± 0.1</td>
<td>5.69 ± 0.45</td>
<td>700 ± 147</td>
<td>0.30 ± 0.06</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. SHR body wt 296 ± 16 g, n = 7.
cholerectokinin were evident in SHRs whether basal sodium excretion was the same as or higher than in the WKY rats. Because the baseline $V_\text{Na}$, $V_\text{NaV}$, $F_\text{ENa}$, and GFR were slightly different between WKY and SHRs, we also expressed the effects of cholerectokinin as percent maximum response compared with baseline period 2. The maximum increases in $V_\text{Na}$, $V_\text{NaV}$, $F_\text{ENa}$, and GFR after cholerectokinin were not different among the groups, regardless of whether basal sodium excretions were the same or higher in SHRs compared with WKY rats. (Fig. 1).

**DISCUSSION**

Our studies show that the ability of Z-1046, a dopamine receptor agonist with the rank order potency $D_3 \geq D_1 > D_2 > D_5 > D_1$, to increase $V_\text{Na}$, $V_\text{NaV}$, $F_\text{ENa}$, and GFR in the normotensive WKY rat is not present in its hypertensive counterpart the SHR. Previous studies in our laboratory have shown that the natriuretic and diuretic effects of Z-1046 are $D_1$- and $D_2$-like receptor mediated (16). Thus the infusion of the $D_1$-like antagonist SCH-23390 or the $D_2$-like antagonist domperidone prevented the natriuresis and diuresis caused by Z-1046 alone in the WKY rat. In the same study, it was shown that the increase in GFR with Z-1046 was caused by a $D_1$-like receptor action (16). The inability of Z-1046 to affect renal function in the SHR is consistent with an impaired interaction between $D_1$- and $D_2$-like receptors.

Several studies have shown an impaired function of the $D_1$-like receptor in the kidney in rodent genetic and human essential hypertension (6–10, 17, 18, 24, 26). The defective $D_1$-like function in the kidneys of SHRs is caused by an uncoupling of the $D_1$-dopamine receptor from its G protein/effecter complex (see review, Refs. 17, 18). The uncoupling has been suggested to be caused by a “hyper” serine phosphorylation of the $D_1$ receptor (31, 43). An important role of the $D_1$-receptor gene in regulating blood pressure was supported by the development of hypertension in mice in which the $D_1$-receptor gene was disrupted (1). It is possible that the failure of the preferential $D_2$-like agonist Z-1046 to alter GFR or produce a
diuresis and natriuresis in the SHR was caused by a defective D_{1}-receptor that prevented a normal interaction with the D_{2}-like receptor (4, 16, 19, 23, 33, 34, 42). In the current studies, Z-1046 also had no effect on renal blood flow in the SHR (data not shown).

D_{2}-like receptors have been shown in cells and in tissues in vitro to increase the inhibition of the sodium pump via synergistic interactions with D_{1}-like receptors (4, 5, 30, 33). In vivo, in the WKY rat, the D_{2}-like antagonist domperidone prevented the expected Z-1046-stimulated natriuresis and diuresis seen, indicating participation of D_{2}-like receptors (16). The major D_{2}-like receptor expressed in rat renal proximal tubules and renal vessels is the D_{3} receptor (17, 23, 27). D_{3} receptors in the kidney appear to be located prejunctionally in dopaminergic nerves (11, 17), and the D_{4} receptor is mainly expressed in collecting ducts (17, 40). D_{3} but not D_{4} long receptors have been identified in rat juxtaglomerular cells, and there is evidence that the D_{3} receptor may be the dopamine receptor subtype responsible in regulating renin release (32). Mice whose D_{3}-receptor gene has been disrupted exhibit renin-dependent hypertension (3). Increased activity of the renin-angiotensin system resulting in increased reabsorptive action in the kidney has been reported in the SHR (41). Thus a defective action of D_{3} receptors in the kidney of the SHR may play a role in the pathogenesis of the hypertension in this rat strain. The exact defect in the D_{3} receptor is not clear from these studies. However, it is not due to differences in D_{3}-receptor sequence between WKY and SHRs. Two molecular sizes, ≈45 and ≈90 kDa, were observed in the renal BBM of both WKY and SHRs. There were no differences in the ≈90-kDa protein between WKY and SHR. However, less ≈45-kDa D_{3}-receptor protein was found in the BBM of SHRs. The cause of the differences in one size but not in another was not determined in these studies.

The apparent lack of responsiveness of the SHR to Z-1046 infusion is not an epiphenomenon, because the diuretic and natriuretic effects of cholecystokinin seen in the WKY rats were also observed in the SHRs. The effects of parathyroid hormone on cAMP and phosphate excretions were also similar in WKY and SHRs (28). Cholecystokinin-A receptors and parathyroid hormone receptors, similar to D_{1}-like receptors, are linked to G_{S} and G_{q/11} (2, 17). Polymorphisms in G_{S}- and G_{β}-subunits have been reported in some populations with essential hypertension (15, 35). However, of these receptors, only D_{1}-like receptor function is impaired in the kidney in genetic hypertension, suggesting that G protein dysfunction is probably not a critical component in the rodent models of genetic hypertension and human essential hypertension (17, 32).

In conclusion, we showed that the natriuresis and diuresis caused by Z-1046, a dopamine receptor agonist with preferential affinity at D_{2}-like more than D_{1}-like receptors, are abrogated in the SHR. This deficiency was receptor specific, because renal functional effects of cholecystokinin acting at another G protein-coupled receptor, were intact in the SHR.

**Perspectives**

Abnormal regulation of blood pressure by dopamine receptor subtypes has been reported in genetic hyper-

### Table 6. Effect of cholecystokinin on renal function in the infused right kidney of the SHR

<table>
<thead>
<tr>
<th>Periods</th>
<th>MAP, mmHg</th>
<th>GFR, ml/g kidney·min⁻¹</th>
<th>U_{Na}V, nEq/min</th>
<th>FE_{Na}, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>162.6 ± 3.8</td>
<td>1.0 ± 0.1</td>
<td>4.40 ± 0.45</td>
<td>412 ± 72</td>
</tr>
<tr>
<td>B2</td>
<td>158.4 ± 6.1</td>
<td>1.2 ± 0.1</td>
<td>4.61 ± 0.48</td>
<td>398 ± 86</td>
</tr>
<tr>
<td>CCK1</td>
<td>159.4 ± 4.9</td>
<td>1.6 ± 0.1*</td>
<td>7.49 ± 1.01*</td>
<td>887 ± 212</td>
</tr>
<tr>
<td>CCK2</td>
<td>160.4 ± 5.3</td>
<td>1.5 ± 0.1</td>
<td>7.95 ± 1.07*</td>
<td>1,077 ± 253*</td>
</tr>
<tr>
<td>CCK3</td>
<td>156.6 ± 4.9</td>
<td>1.6 ± 0.1*</td>
<td>6.86 ± 0.69*</td>
<td>845 ± 179</td>
</tr>
<tr>
<td>R1</td>
<td>155.6 ± 4.8</td>
<td>1.5 ± 0.2</td>
<td>6.08 ± 0.64</td>
<td>804 ± 249</td>
</tr>
<tr>
<td>R2</td>
<td>156.0 ± 5.5</td>
<td>1.5 ± 0.3</td>
<td>5.72 ± 0.80</td>
<td>817 ± 230</td>
</tr>
<tr>
<td>R3</td>
<td>155.1 ± 4.7</td>
<td>1.3 ± 0.2</td>
<td>4.95 ± 0.56</td>
<td>696 ± 209</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. SHR body wt 329 ± 12 g, n = 8. *P < 0.05 vs. B1 or B2, ANVR, Newman Keuls test. †P < 0.05 vs. R3, ANVR, Newman Keuls test.

### Table 7. Effect of cholecystokinin on renal function in the infused right kidney of the SHR with a lower basal sodium excretion than those observed in Table 6

<table>
<thead>
<tr>
<th>Periods</th>
<th>MAP, mmHg</th>
<th>GFR, ml/g kidney·min⁻¹</th>
<th>U_{Na}V, nEq/min</th>
<th>FE_{Na}, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>191.3 ± 3.1</td>
<td>1.1 ± 0.1</td>
<td>2.60 ± 0.13</td>
<td>165 ± 22</td>
</tr>
<tr>
<td>B2</td>
<td>185.3 ± 4.0</td>
<td>1.2 ± 0.1</td>
<td>2.74 ± 0.20</td>
<td>145 ± 7</td>
</tr>
<tr>
<td>CCK1</td>
<td>187.2 ± 5.1</td>
<td>1.6 ± 0.2*</td>
<td>5.10 ± 0.52*</td>
<td>391 ± 102</td>
</tr>
<tr>
<td>CCK2</td>
<td>189.5 ± 4.4</td>
<td>1.7 ± 0.2*</td>
<td>5.36 ± 0.39*</td>
<td>521 ± 163*</td>
</tr>
<tr>
<td>CCK3</td>
<td>184.8 ± 5.8</td>
<td>1.7 ± 0.2*</td>
<td>5.49 ± 0.38*</td>
<td>689 ± 196*</td>
</tr>
<tr>
<td>R1</td>
<td>184.8 ± 5.2</td>
<td>1.3 ± 0.1</td>
<td>4.95 ± 0.62*</td>
<td>705 ± 198*</td>
</tr>
<tr>
<td>R2</td>
<td>185.5 ± 4.5</td>
<td>1.4 ± 0.1</td>
<td>4.82 ± 0.44*</td>
<td>740 ± 204*</td>
</tr>
<tr>
<td>R3</td>
<td>180.6 ± 4.6</td>
<td>1.5 ± 0.1</td>
<td>4.50 ± 0.21*</td>
<td>575 ± 150</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. SHR body wt 344 ± 7 g, n = 5. *P < 0.05 vs. B1 or B2, ANVR, Newman Keuls test.
tension (17, 18). In the SHR, we have reported that the uncoupling of the D1-like receptors, specifically the D1 subtype, in renal proximal tubules from its G protein/effect complex is important in the pathogenesis of hypertension (17, 18, 31). However, abnormalities of D2-like receptors have also been described in genetic hypertension (21, 38). D1- and D2-like receptors may interact to regulate cardiovascular and renal function (4, 5, 16, 33). We now report that there is an impaired interaction in the kidney of D1- and D2-like receptors in the SHR, resulting in impaired renal vasodilatory, diuretic, and natriuretic effects of a dopamine receptor agonist with selectivity to all the dopamine receptor subtypes but not to other G protein-coupled receptors (16). The major renal tubular D2-like receptor is the D3 receptor (reviewed in Refs. 17, 18, 23, 27). The absence of any differences in D3 sequence between WKY and SHRs suggests a primary abnormality of the D1-like receptor. There is also a possibility that the D3 receptor shares a similar posttranslational modification with the D1 receptor that results in impaired renal dopamine receptor function. D3 receptors can form homooligomers depending on where the receptors are expressed: the brain striatum expresses oligomers, whereas only monomers are expressed in the ventral midbrain (19, 25). Jung et al. (19) suggested that the D3 autoreceptors may be expressed only as monomers. The significance of the decreased expression of monomeric D3 receptors in the SHR remains to be determined. However, a decreased D3-autoreceptor function.

Fig. 2. A: immunoblot of lysates from renal cortical or brush-border membranes (BBM) from WKY and SHRs. Protein (100 μg) was subjected to immunoblot with anti-D3 receptor antiserum (1:250). Two species of D3 receptors (~45 and 90 kDa) were noted in renal BBM; only the ~45 kDa was noted in renal cortical membranes. The 45- and 90-kDa bands were completely or almost completely blocked when the antibodies were preadsorbed with immunizing peptide (1:5 vol/vol). Lane 1 = renal BBM, WKY; lane 2 = renal BBM, SHR; lane 3 = renal cortical membrane, WKY; lane 4, renal cortical membrane, SHR; 50 and 98 kDa = molecular sizes. B: immunoblot of lysates from renal cortical or BBMs from WKY and SHRs. Because the ~45-kDa bands were much denser in WKY than in SHR, the amount of protein loaded was increased in the SHRs relative to the WKY rats. Lane 1 = renal BBM (50 μg), WKY; lane 2 = renal BBM (75 μg), SHR; lane 3 = renal cortical membrane (100 μg), WKY; lane 4 = renal cortical membrane (200 μg), SHR; 50 kDa, molecular sizes. With less protein, the 90 kDa was no longer apparent in BBMs; the 45-kDa bands were completely or almost completely blocked when the antibodies were preadsorbed with immunizing peptide (1:5 vol/vol). C: quantitation of D3 receptor immunoblots from WKY (open bars) and SHRs (solid bars). The %area occupied by the 45-kDa band was decreased in both renal cortical and BBMs in SHR compared with WKY. No differences were noted in the 90-kDa band. *P < 0.05 vs. SHR, t-test.
may be a mechanism for an increased sympathetic activity in the SHR. D1 receptors have been reported to upregulate D3 receptors but not vice versa (20). Whether the defective D1-receptor function in the SHR has a role in the differential expression of the 45-kDa D3-receptor protein remains to be determined. It is also not known whether the renal D3 receptor shares with the renal D1 receptor the same abnormalities in post-translational modification (e.g., serine phosphorylation) seen in genetic hypertension (31, 43).

These studies were supported in part by Grant HL-58536 from the National Heart, Lung, and Blood Institute and by a National Kidney Foundation Grant, National Capital Affiliate.

REFERENCES


