Dopamine D₃ Receptors and Salt-Dependent Hypertension

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Abstract. Alterations in the dopaminergic system may contribute to the pathogenesis of hypertension. Dopamine D₃ receptors have been shown to be involved in the regulation of sodium balance and hemodynamics in rodents. For determining the role of D₃ receptors in salt-dependent hypertension, clearance experiments were performed in anesthetized salt-sensitive (DS) and salt-resistant (DR) Dahl rats that were fed a standard diet with either normal (0.2%) or high (4%) sodium content for 21 to 26 d, which induced hypertension in DS but not in DR rats. The D₃ receptor agonist R(+)-7-hydroxydipropyl-aminotetralin (7-OH-DPAT) increased GFR by up to 35% and urinary sodium excretion by up to 4.4-fold in DR rats that were on both normal and high-sodium diet. 7-OH-DPAT-induced natriuresis also was observed in DS rats that were on normal diet but not in hypertensive DS rats that were on high-salt diet. No GFR response to 7-OH-DPAT was found in DS rats, irrespective of sodium diet. The diminished functional response to D₃ receptor stimulation in DS rats was associated with a significantly lower [³H]-7-OH-DPAT binding to renal membrane protein when comparing DS with DR rats. Consequently, DR rats were treated with BSF 135170, a novel, highly selective D₃ receptor antagonist, for 29 d. Whereas no change in systolic BP was observed during normal diet, high sodium intake significantly increased BP by almost 40 mmHg. In summary, both expression and function of the renal dopamine D₃ receptor are impaired in salt-sensitive Dahl rats. Together with the induction of salt-dependent hypertension in genetically salt-resistant Dahl rats by D₃ receptor blockade, the data strongly suggest that the deficiency in dopamine D₃ receptors represents an important pathophysiological factor in the development of salt-dependent hypertension.

An abnormal renal handling of sodium plays a major role in some forms of essential hypertension, however, the exact nature of the renal defect remains unclear (1). Because dopamine is involved in the regulation of renal function and systemic hemodynamics, an impaired renal dopaminergic system may contribute to the pathogenesis of hypertension (2). In the kidney, filtered L-DOPA is reabsorbed and converted to dopamine in the proximal tubular cells by L-amino acid decarboxylase (3). Although the functional role of dopamine secreted into the tubular lumen seems to be of minor significance (4,5), a release of dopamine into the interstitial space might be more important but awaits further investigation. So far, five distinct dopamine receptors have been identified and are separated into two families: the D₁-like and the D₂-like dopamine receptors (6). The D₁-like receptors, including the D₁A and D₁B, are coupled to stimulation of adenylyl cyclase, whereas the D₂-like receptors (D₂, D₃, and D₄) may inhibit cyclase activity; however, the data are not uniform (7). On the mRNA level, all of these dopamine receptors have been shown to be present in the kidney (8–11); on the protein level, the data are not that clear. Recently, a defective D₁A receptor was suggested to contribute to genetic hypertension (12), most likely by affecting renal sodium excretion. However, D₁ receptors seem to regulate tubular sodium excretion only in part and under certain conditions of extracellular volume (13).

More recently, the dopamine D₃ receptor was suggested to be present in tubular, glomerular, and vascular structures of the rat kidney (14). In normal rats, pharmacologic D₃ receptor stimulation decreased tubular sodium resorption, suggesting an involvement of this receptor subtype in the excretory function of the kidney (15). In transgenic mice with disrupted D₃ receptor gene, an increase in systemic BP was observed (16). The present investigation was carried out to test the hypothesis that an impaired D₃ receptor function is involved in the development of salt-dependent hypertension. For this purpose, the D₃ receptor-dependent changes in renal function were characterized in anesthetized salt-sensitive (DS) and salt-resistant (DR) Dahl rats that were kept on normal or high-sodium diet. Furthermore, the renal D₃ receptor density was studied by radioligand binding using the specific D₃ ligand [³H]-7-OH-DPAT in these animals. In addition, DR rats were chronically treated with a selective dopamine D₃ receptor antagonist to confer salt sensitivity to this genetically salt-resistant rat strain.

Materials and Methods
All animal experimentation was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the German Law on the Protection of Animals. Male DS and DR Dahl rats that were aged 7 to 8 wk and weighed 125 to 175 g were purchased.
from M&B (Ry, Denmark). The animals were kept under controlled environmental conditions (21°C; 12-h dark/light cycle). According to the experimental protocol, the animals received ad libitum a standard rat diet (1324; Altromin, Lage, Germany) with either normal (0.2%) or a high (4%) sodium content for 21 to 26 d.

**Clearance Experiments**

The animals were anesthetized with thiopental sodium (80 mg/kg; Byk Gulden, Constance, Germany) and prepared for clearance study as described previously (15). In brief, catheters were inserted into the right jugular vein for infusion, into the carotid artery for blood sampling and monitoring of cardiovascular function, and into the bladder for urine collection. A tracheostomy was performed to keep the airways free. Via the first intravenous catheter, Ringer’s saline solution (in mmol/L: 111 NaCl, 30 NaHCO₃, 4.7 KCl) was infused at a rate of 0.8 ml/100 g body wt per h. Via the second catheter, [³H]-inulin (1.2 μCi/ml) dissolved in Ringer was infused at a rate of 0.6 ml/h throughout the entire experiment for assessment of GFR. The rats were allowed to recover from surgical procedures for 80 to 100 min before the measurements were started. Two consecutive clearance periods were carried out to obtain baseline values. Thereafter, Ringer infusion was switched to the specific D₃ receptor agonist R(+)-7-hydroxy-dipropyl-aminotetralin (7-OH-DPAT; Biotrend, Cologne, Germany), dissolved in Ringer, in two consecutive doses of 0.01 and 0.1 μg/kg × min) while the infusion rate was maintained. Ten minutes after onset of each 7-OH-DPAT infusion, two clearance periods were performed. After the second period with 7-OH-DPAT administration, the infusion was switched back to the vehicle; after 10 min, another clearance period, designated as recovery, was carried out. Each clearance period comprised a 20-min urine collection, with a midpoint blood sample of 180 μl. An additional blood sample of 80 μl for the determination of plasma renin activity (PRA) was drawn during the second baseline period.

**Radioligand Binding Studies**

After normal or high-sodium diet of 21 d, rats were decapitated and the kidneys were removed rapidly through a midline abdominal incision and immediately placed on ice. Kidneys were freed of connecting tissue and homogenized in a motor-driven glass-Teflon Potter (Braun, Melsungen, Germany) with 1200 rpm for 4 strokes in buffer containing (mmol/L) Tris/HEPES 40 (pH 7.4), sucrose 320, ethylenediaminetetraacetate 0.5, and phenylmethylsulfonyl fluoride 0.01. The homogenate was centrifuged at 1000 × 15 min at 4°C (Biofuge; Heraeus, Hanau, Germany). The supernatant was discarded while the pellet was resuspended in the homogenization buffer and recentrifuged again (1000 × 15 min, 4°C). The second supernatant was centrifuged at 100,000 × g for 30 min at 4°C. Preliminary studies showed that this preparation was the most efficient for a high membrane/protein ratio (data not shown). The final pellet was suspended in the homogenization buffer in the absence of sucrose and ethylenediaminetetraacetate and stored at −80°C until binding experiments.

For binding studies, 100 μg of kidney membrane protein was used. The experiments were performed in buffer solution (pH 7.4) containing 25 mmol/L Tris and 40 mmol/L HEPES. Saturation assays were performed with 0.5 to 50 nmol/L [³H]-7-OH-DPAT (Amersham, Buckinghamshire, UK) and 10 μmol/L unlabeled 7-OH-DPAT for assessment of nonspecific binding. After incubation for 1 h at 20°C, the reaction was terminated by rapid filtration through glass-fiber filters (GF/B; Whatman, Maidstone, UK), presoaked in 0.3% polyethylenimine. Radioactivity adsorbed on the filters was determined by liquid scintillation counting. Specific binding was calculated as the difference between total binding and nonspecific binding. The conditions described derived from preliminary experiments: association studies were carried out at 0°C, 20°C, and 30°C. In these experiments, binding equilibrium was achieved at 20°C within 1 h. Thereafter, dissociation was started by adding 10 μmol/L unlabeled 7-OH-DPAT, showing a rapidly reversible 7-OH-DPAT binding. In addition, the dissociation constants (Kᵦ values) estimated in these kinetic studies were similar to those calculated from the saturation experiments (data not shown).

**Chronic Dopamine D₃ Receptor Inhibition**

DR rats (n = 6) were treated for 29 d with BSF 135170 (Knoll AG, Ludwigshafen, Germany), a novel selective dopamine D₃ receptor antagonist (patent registration number WO 9602520, priority date July 15, 1994). The high D₃ selectivity of this compound was demonstrated in competition studies using human dopamine D₂, D₄, and D₅ receptors expressed in human embryonic kidney cells, and native bovine caudate dopamine D₃ receptors as well. In comparison to a Kᵦ value of 13 nmol/L for the dopamine D₃ receptor, 50-fold, >800-fold, and >150-fold lower affinities were determined for the D₂, D₄, and D₅ receptors, respectively.

BSF 135170 was dissolved in 40 μl of acetic acid (100%) and added to 500 ml of drinking water. The concentration was adjusted daily to achieve a dose of 40 mg/kg per d. The animals were kept on normal rat diet for the first week, and tail-cuff BP measurements were performed on days 0 and 7 of BSF 135170 treatment. After the BP measurement on day 7, the high-sodium diet was initiated and BP was assessed on days 8, 15, 22, and 29 of the study. DS and DR rats that were on high-sodium diet without drug treatment (only vehicle in the drinking water) served as controls for salt sensitivity and salt resistance, respectively.

**Analytical Procedures**

Sodium concentrations were determined by flame photometry (ELEX 6361; Eppendorf, Hamburg, Germany). The [³H]-inulin and the [³H]-7-OH-DPAT radioactivity were measured by liquid phase scintillation counting (2550 TR; Canberra-Packard, Frankfurt, Germany). PRA was determined by its capacity to generate angiotensin I with addition of renin substrate, as described previously (17). The protein concentration was determined using Coomassie blue and bovine serum albumin as a standard according to the method of Bradford (18).

**Statistical Analyses**

Dissociation constant and maximum receptor density in the binding experiments were calculated using LIGAND (19) and nonlinear regression analysis. In clearance experiments, renal excretory and hemodynamic values were calculated per gram of kidney wet weight. Results were expressed as mean ± SEM. Differences within and between groups were compared using the ANOVA with Bonferroni correction. P < 0.05 was considered statistically significant.

**Results**

**Clearance Experiments**

As shown in Table 1, mean arterial BP (MAP) of DS and DR rats that were on normal sodium did not differ under anesthesia. When animals were fed the high-sodium diet, DS rats developed hypertension, whereas MAP did not change in DR animals. Heart rate was similar when comparing DS and DR rats. Infusion of the dopamine D₃ receptor agonist 7-OH-
DPAT slightly (by 5%) lowered BP and heart rate with no significant differences when comparing DS and DR rats as well as the diets. Body weight was significantly higher in DS than in DR rats, on both normal and high-sodium diet. Baseline sodium plasma concentration, however, did not significantly differ among all groups (Table 1). On high-sodium diet, kidney weight corrected for body weight was significantly greater in DS than in DR rats, whereas no difference was observed on normal sodium diet. As expected, PRA, which was measured at baseline, was significantly lower during high-sodium intake as compared with normal diet. However, there were no significant differences in PRA when comparing DS and DR rats (Table 1).

At baseline, GFR and urinary flow rate (UV) were slightly but not significantly lower in DS than in DR rats. The high-
sodium condition significantly decreased GFR and UV in both DR and DS rats (Table 1). No significant difference in absolute or fractional sodium excretion (UNaV and FENa, respectively) occurred in DS as compared with DR rats (Table 1). Both values were moderately but not significantly higher in rats that were on the high-sodium diet. Infusion of 7-OH-DPAT in DR rats that were on normal sodium significantly increased GFR by up to 10% and UV by up to 1.8-fold. These effects were even more pronounced during the high-salt diet (Figure 1). Both GFR and UV tended to return to baseline after discontinuation of the 7-OH-DPAT infusion. In contrast to DR rats, there was no 7-OH-DPAT-induced increase in GFR in animals of the DS strain, irrespective of sodium diet (Figure 1). Similarly, the UV response to 7-OH-DPAT in DS animals was attenuated during the normal and absent during the high-
sodium condition (Figure 1). In DR rats that were fed the normal sodium diet, 7-OH-DPAT dose-dependently increased UNaV and FENa by up to 4.4-fold and 3.4-fold, respectively, whereas these effects were less pronounced during the high-sodium intake (Figure 2). 7-OH-DPAT elevated UNaV and FENa also in DS rats that were on normal diet. However, this effect was absent on the high-sodium diet (Figure 2).

Table 1. Baseline measurements of clearance experimentsa

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal Sodium Diet</th>
<th>High-Sodium Diet</th>
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<tbody>
<tr>
<td></td>
<td>Dahl Salt-Resistant</td>
<td>Dahl Salt-Sensitive</td>
</tr>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>272 ± 5</td>
<td>291 ± 8c</td>
</tr>
<tr>
<td>Kidney wet weight (g/100 g body wt)</td>
<td>0.80 ± 0.02</td>
<td>0.85 ± 0.03</td>
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<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>122 ± 3</td>
<td>123 ± 6</td>
</tr>
<tr>
<td>Heart rate (1/min)</td>
<td>415 ± 7</td>
<td>410 ± 4</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.49 ± 0.1</td>
<td>0.52 ± 0.1</td>
</tr>
<tr>
<td>Sodium plasma concentration (mmol/L)</td>
<td>146 ± 2</td>
<td>144 ± 2</td>
</tr>
<tr>
<td>GFR (ml/min per g KW)</td>
<td>1.09 ± 0.04</td>
<td>0.98 ± 0.06</td>
</tr>
<tr>
<td>Urinary flow rate (µl/min per g KW)</td>
<td>18.7 ± 2.3</td>
<td>17.9 ± 2.1</td>
</tr>
<tr>
<td>Urinary sodium excretion (µmol/min per g KW)</td>
<td>0.67 ± 0.21</td>
<td>0.54 ± 0.16</td>
</tr>
<tr>
<td>Fractional urinary sodium excretion (%)</td>
<td>0.47 ± 0.17</td>
<td>0.44 ± 0.16</td>
</tr>
<tr>
<td>Plasma renin activity (ng A I/ml per h)</td>
<td>2.74 ± 0.50</td>
<td>2.97 ± 0.52</td>
</tr>
</tbody>
</table>

a Values represent means ± SEM. KW, kidney wet weight.

b P < 0.05 versus respective Dahl strain fed a normal sodium diet.

c P < 0.05 versus respective Dahl salt-resistant rats.
pharmacologic blockade of dopamine D₃ receptors conferred salt sensitivity to DR rats. In addition, an attenuated renal response to D₃ receptor activation was observed in DS rats. Thus, this study provides strong functional evidence that deficient dopamine D₃ receptors are involved in the development of salt-dependent hypertension.

Transplantation of kidneys from DS to DR rats can lead to salt-induced hypertension, and, conversely, DR kidneys that are transplanted into DS rats can normalize BP, indicating that renal dysfunction contributes to hypertension in this model (20). Several lines of evidence indicate that hypertension in DS rats results from the inability to excrete sodium adequately in response to a salt challenge (21). It was proposed that the retention of sodium and the consecutive expansion of extracellular volume triggers mechanisms that affect BP, e.g., cardiac output, total peripheral resistance, activity of the sympathetic nervous system (22,23). However, the underlying mechanisms are unsatisfactorily understood. Several neuroendocrine systems, because of their role in the regulation of sodium excretion, might be involved in the cause of Dahl hypertension. In this context, the renin-angiotensin system was proposed because plasma and renal renin activity were lower in DS than in DR rats (24). In contrast, PRA in the present study did not differ between DS and DR rats but responded appropriately to

**Figure 1.** Effect of 7-OH-DPAT on GFR (A) and urinary flow rate (UV; B) in Dahl salt-resistant (DR) and Dahl salt-sensitive (DS) rats that were fed either normal (NS) or high-sodium (HS) diet. Depicted is the absolute change related to baseline values (Table 1) in GFR and UV in response to infusion of two different doses of 7-OH-DPAT or Ringer solution (REC). Values represent means ± SEM; n = 8 to 10 per group. *P < 0.05 versus baseline (see Table 1); †P < 0.05 versus respective DR rats.

**Figure 2.** Effect of 7-OH-DPAT on absolute urinary sodium excretion (UₙaV; A) and fractional excretion of sodium (FEₙa; B) in anesthetized Dahl rats. See Figure 1 for further abbreviations.
changes in sodium intake. This observation does not support the idea that renin is involved in the pathogenesis of Dahl hypertension, however, with the limitation that renal renin was not assessed in the present study.

Abnormalities in the renal dopaminergic system, in view of its potential natriuretic actions, were suggested to contribute to the development of salt-dependent hypertension. Transgenic mice that lack one or both D1 alleles developed high BP (12). In addition, a defective coupling of the D1-like receptor was suggested in DS rats: D1-like receptor agonists were unable to stimulate adenylate cyclase in proximal tubules of kidneys from DS rats, whereas forskolin, as a direct cyclase activator, stimulated the enzyme in both DS and DR rats (25).

In vivo evidence for a defective dopamine D1 receptor in prehypertensive DS rats was reported by Hansell (26). More recently, also derived from observations in a transgenic mouse model, deficiency of the D3 receptor was suggested to cause hypertension (16). However, the pathophysiology induced by disruption of D1 or D3 receptors seems to be clearly different because the renal renin activity was much greater in mice that lack D3 receptors in comparison to wild-type controls (16), whereas dopamine D1 receptor activation has been reported to increase renin release (27).

In the present study, the renal response of DR and DS rats to 7-OH-DPAT was significantly different. Regarding the relatively high structural homology between the D2 and the D3 receptor protein, one might question the selectivity of 7-OH-DPAT as a D3 agonist. However, binding experiments showed a 100-fold D3-over-D2 selectivity of 7-OH-DPAT (28). In a recent study, 7-OH-DPAT-induced changes in renal function in normotensive Sprague-Dawley rats were attenuated by U-99194A, a selective D2 antagonist, but not by the D2 receptor blocker S(-)-sulpiride (15). The 7-OH-DPAT-induced changes in GFR observed in that study also were seen in the
The marked reduction of dopamine D₃ receptors induced by high-salt diet, as indicated by [³H]-7-OH-DPAT binding, was diminished in animals of the DS strain that were on a high-sodium diet, whereas in prehypertensive DS rats that were on normal sodium intake, the tubular excretory response to D₃ receptor activation was not affected. Even if these functional variations were paralleled by changes in [³H]-7-OH-DPAT binding, the obliging hypothesis that in the Dahl model of salt-dependent hypertension a deficient dopamine D₃ receptor expression, via an impaired tubular electrolyte handling and consequently sodium retention, leads to extracellular volume expansion and thus to systemic arterial hypertension seems to be somewhat simplifying.

The D₃ receptor density in kidneys of DS rats, as indicated by [³H]-7-OH-DPAT binding, was reduced in comparison with DR rats, whereas receptor affinity was not altered. In contrast to the present data, Barili et al. (30) reported 10- to 20-fold lower K_D values for binding of [³H]-7-OH-DPAT to sections of rat renal cortex. This might be due to differences in the tissue preparation or to experimental details, e.g., unspecific binding. Because the binding kinetics and the saturation experiments of the present study delivered almost identical K_D values and reasonable characteristics of the unspecific binding, the B_max values seem to be plausible. Because, so far, no other data have been reported on D₃ receptor affinity in renal tissue, additional studies in this context are desirable. In accordance with the present data on [³H]-7-OH-DPAT binding, we found recently that renal dopamine D₃ receptor mRNA expression was diminished to the same extent in animals of the DS strain when compared with DR rats (31).

To summarize, the data suggest that both expression and function of the renal dopamine D₃ receptor deficiency derives from the induction of salt-dependent hypertension in genetically salt-resistant Dahl rats by chronic treatment with a selective D₃ antagonist. Even if these rats did not reach the same level of hypertension as did rats of the DS strain, their net BP increase was identical. In this context, Shigetomi et al. (38) reported that the unsel ective D₂-like antagonist metoclopramide induces hypertension in salt-loaded Wistar rats possibly via inhibition of endogenous Na⁺-K⁺-ATPase. In good accordance with our experiments, this study indicates that dopaminergic receptors of the D₂-like family are involved in controlling BP and sodium handling of the kidney. The high selectivity of the pharmacologic tools used in the present study, however, clearly points toward the D₃ type of this receptor family as the major pathologic principle.

Nonetheless, striking evidence for the involvement of an impaired sodium handling in the pathogenesis of hypertension as a result of D₃ receptor deficiency derives from the induction of salt-dependent hypertension in genetically salt-resistant Dahl rats by chronic treatment with a selective D₃ antagonist. Even if these rats did not reach the same level of hypertension as did rats of the DS strain, their net BP increase was identical. In this context, Shigetomi et al. (38) reported that the unsel ective D₂-like antagonist metoclopramide induces hypertension in salt-loaded Wistar rats possibly via inhibition of endogenous Na⁺-K⁺-ATPase. In good accordance with our experiments, this study indicates that dopaminergic receptors of the D₂-like family are involved in controlling BP and sodium handling of the kidney. The high selectivity of the pharmacologic tools used in the present study, however, clearly points toward the D₃ type of this receptor family as the major pathologic principle.

To summarize, the data suggest that both expression and function of the renal dopamine D₃ receptor are decreased in salt-sensitive Dahl rats. The idea that this deficiency contributes to the pathogenesis of hypertension is supported by the phenotypical conversion of salt-resistant into salt-sensitive Dahl rats by pharmacologic receptor inhibition. Because both renal vascular regulation and tubular excretory function are affected by the dopamine D₃ receptor deficiency, additional investigational effort is needed on the exact pathomechanisms.
and on the possibility that additional factors independent of the obvious receptor defect are involved.

Acknowledgments
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