Original Article

Arterial Blood Pressure and Renal Sodium Excretion in Dopamine D_3 Receptor Knockout Mice

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Alterations in the dopaminergic system may contribute to the development of hypertension. Recently, it has been reported that pentobarbital-anesthetized mice with deficient dopamine D_3 receptors showed renin-dependent elevation in blood pressure. In a series of experiments, we evaluated the contribution of the dopamine D_3 receptor to the renal sodium excretion and arterial blood pressure behavior in conscious as well as anesthetized dopamine D_3 receptor knockout (–/–) mice. The blood pressure measuring study was designed as a cross-over trial to investigate the influence of different sodium loads. The animals were fed a normal salt diet (0.6% NaCl, NS) for 1 week and afterwards a low (0.2% NaCl, LS) or a high salt diet (4.6% NaCl, HS) for 2 weeks. After the third week, the animals were switched to the corresponding protocol. Systolic blood pressure in conscious (–/–) mice measured by tail-cuff plethysmography was not different from that of wild-type (+/+) animals, irrespective of the time course or the salt diet. In another experiment, challenge of an acute sodium loading per gavage in conscious D_3 receptor (–/–) and (+/+) animals on HS or NS diet did not show significant differences in renal sodium excretion between the two genotypes. Additionally, animals were fed an NS diet for 1 week and an HS diet for another week. As expected, sodium excretion significantly increased after the change from the NS to the HS diet. A slightly lower urinary sodium excretion was observed when comparing D_3 receptor (–/–) mice to their corresponding (+/+) mice, both on an HS diet. Clearance experiments with anesthetized D_3 receptor (–/–) and (+/+) mice were performed to investigate the renal sodium excretion capacity, when exposed to a moderate volume expansion (VE). Urinary sodium excretion increased in response to the VE; however, no difference were observed between the two genotypes. Taking these results together, we conclude that in the present animal model renal dopamine D_3 receptors are not significantly involved in the regulation of blood pressure associated with a deficiency in renal sodium elimination. (*Hypertens Res* 2007; 30: 93–101)

***Key Words*: hypertension, dopamine D_3 receptor, renal sodium excretion, glomerular filtration rate, knockout mice**

Introduction

Essential hypertension is a widespread disease in Western countries and the origin of several serious complications, *e.g.*, myocardial infarction, stroke and end-stage renal disease. It has been suggested that alterations in the dopaminergic system may be involved in the development of hypertension (*1*).

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The effects of dopamine are mediated by G-protein–coupled receptors, which are classified into two major subfamilies, *i.e.*, D_1-like and D_2-like. D_1-like receptors include the products of the D_1 and D_5 receptor genes (D_{1A} and D_{1B} in rodents), whereas the D_2-like subfamily consists of the D_2, D_3 and D_4 receptors. Agonist binding to the D_1-like subfamily stimulates the G_s protein and consecutively adenylate cyclase (AC). On the other hand, activation of the D_2-like subfamily produces multiple effects, including activation of G_i proteins and inhibition of AC.

Dopamine has been suggested to be involved in the regulation of renal hemodynamics and urinary sodium excretion by activating peripheral receptors. In the kidney, D_1-like receptor agonists increase renal blood flow and glomerular filtration rate (GFR), as well as urinary sodium and water excretion (*2*, *3*). The D_2 and D_5 receptors regulate systemic blood pressure by interacting with the sympathetic nervous system, whereas the D_1, D_3 and D_4 receptors also influence the renin-angiotensin system (*4*). The dopamine D_3 receptor has been suggested to be present in tubular, glomerular and vascular structures of the rat kidney (*5*) and to influence renal hemodynamics as well as sodium reabsorption (*6*). Two alternatively spliced isoforms of the dopamine D_3 receptor have been identified in the mouse (*7*), but not in other species (*8*).

In anesthetized mice, D_3 receptor deletion causes renin-dependent hypertension due to the lack of renal sodium excretion (*9*). Since anesthetic conditions markedly influence blood pressure regulation (*10*), conclusions regarding hypertensive behavior should be based on studies in conscious animals. Therefore, in the present study the effects of a dopamine D_3 receptor deletion on arterial blood pressure and on renal sodium handling were studied in conscious mice using acute and chronic sodium loading. The data were supplemented by volume expansion (VE) experiments in pentobarbital-anesthetized D_3 receptor knockout (−/−) and the corresponding wild-type (+/+) mice.

Methods

Animals

The experiments were performed in male dopamine D_3 receptor knockout (−/−) mice and the corresponding wild-type (+/+) animals. Generation of dopamine D_3 receptor (−/−) mice was performed as described recently (*11*). In brief, the genomic DNA was modified by the introduction of the tetracyclin-responsive transcriptional activator protein (tTA) and a neomycin resistance gene cassette downstream of the D_3 gene promoter, both replacing most of the first exon, including the translational initiation site of the dopamine D_3 receptor and the splice donor sequence. The resulting DNA fragment was transfected into mouse embryonic stem (ES) cells (strain 129SVJ). Chimeric mice were generated by the injection of successfully targeted ES cells into C57BL/6-derived blastocysts followed by the transfer into C57BL/6 foster mothers. Chimeric males were backcrossed to C57BL/6 females and heterozygous animals (+/−) were obtained. Repeated backcrossing (to the sixth generation) of (+/−) males to C57BL/6 females was performed to establish transgenic mice with a genetic background similar to that of C57BL/6 mice. The homozygous D_3 receptor (−/−) mice and (+/+) littermates used in the present experiments were generated by breeding heterozygous animals.

The animals were housed individually in cages (Tecniplast, Hohenpeissenberg, Germany) in a room with a 12-h-light, 12-h-dark artificial lighting cycle and free access to rodent chow (Altromin, Lage, Germany) and tap water. The mice were 3 months old, and their body weights (wts.) ranged between 24 and 30 g. All animal experimentation was performed according to the German Law on the Protection of Animals.

Design of the Different Experimental Series

This study is composed of four different experiments: 1) a long-term cross-over experiment investigating blood pressure regulation, 2) an acute loading of sodium on mice fed a normal salt diet (NS, 0.6% NaCl; Altromin) or high salt diet (HS, 4.6% NaCl; Altromin), 3) a chronic sodium loading experiment and 4) clearance experiments with VE in anesthetized mice.

Long-Term Cross-Over Experiment in Conscious Mice

This study was designed as a cross-over trial to observe the effects of sodium chloride (NaCl) load on blood pressure. During the first week the animals (D_3 receptor (−/−) and (+/+) mice, $n=12$ per group) received an NS diet. After this week, the animals were randomized into two study arms consisting of 6 D_3 receptor (−/−) and 6 (+/+) mice per arm. The low salt diet (LS) group received two initial injections of furosemide 0.6 mg/kg body wt. (Lasix, Aventis, Frankfurt, Germany), on day 1 and 4 to increase sodium elimination (*12*) and a low salt diet (LS, 0.2% NaCl; Altromin). The high salt group was fed an HS diet and received a daily injection of 1.0 mg/kg body wt. deoxycorticosteroneacetate (DOCA; Sigma, Taufkirchen, Germany) to accumulate NaCl (*13*). At the beginning of the fourth week the animals were switched to the other protocol for another 2 weeks. Systolic blood pressure (SBP) was measured in conscious mice by tail-cuff plethysmography (Model 179 blood pressure analyzer; Hugo Sachs Elektronik, Hugstetten, Germany). For this purpose the mice were trained for a period of 2 weeks to get used to the measuring procedure. The SBP was measured 3 times a week. During the experiments, the animals were kept in restrainers, which were placed in a box of acryl glass, heated to 32°C. After 10 min of equilibration the blood pressure was measured consecutively 5 times per mouse. The arithmetic mean of the values in the respective mouse represented the blood pressure of one examination per mouse and day.

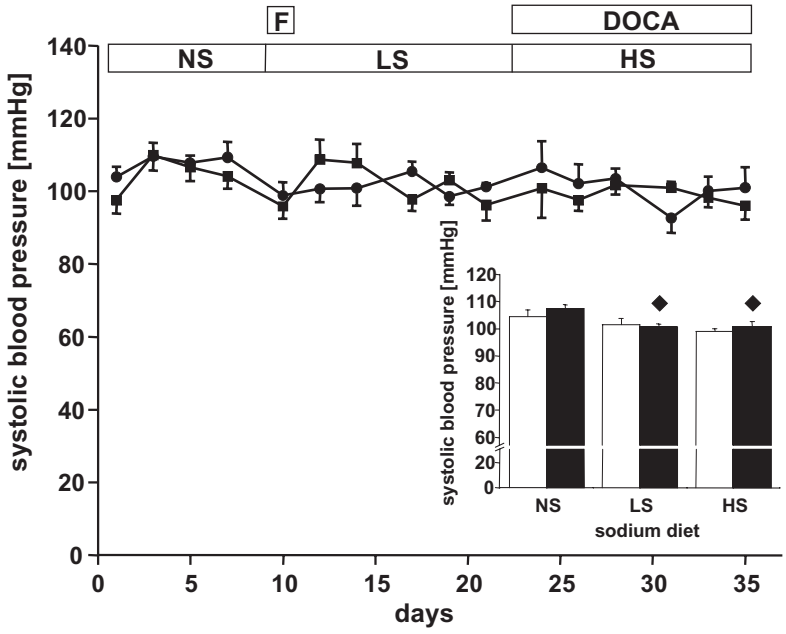

Fig. 1. *Systolic blood pressure values of dopamine D_3 receptor (−/−) (circles, filled bars) and (+/+) (squares, open bars) mice on a normal salt (0.6% NaCl, NS), low salt (0.2% NaCl, LS) or high salt diet (4.6% NaCl, HS) (F, furosemide treatment; DOCA, deoxycorticosteroneacetate treatment). The inset shows the means of the periods on the respective sodium diet. Values are the means ± SEM;* n = *6 per each group.* ♦$p < 0.05$ vs. *the respective NS group.*

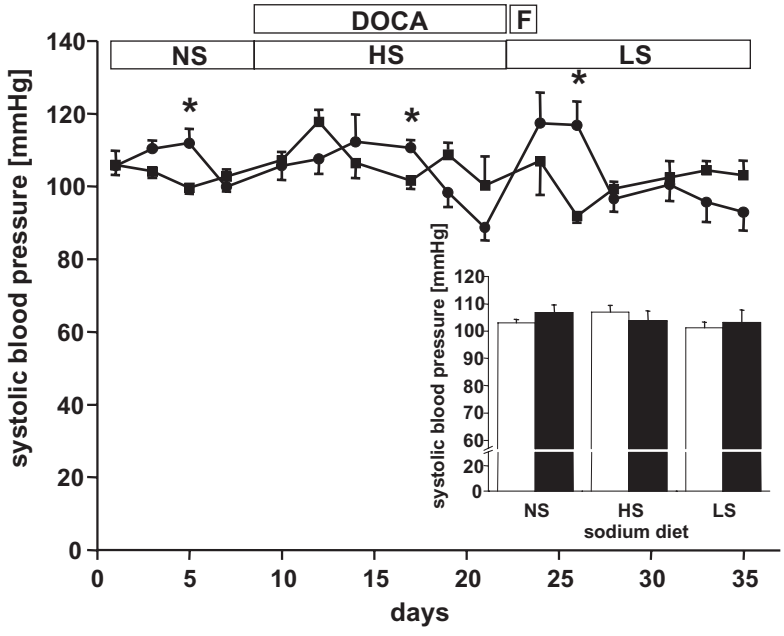

Fig. 2. *Systolic blood pressure values of dopamine D_3 receptor (−/−) (circles, filled bars) and (+/+) (squares, open bars) mice on a normal salt (0.6% NaCl, NS), high salt (4.6% NaCl, HS) or low salt diet (0.2% NaCl, LS) (F, furosemide treatment; DOCA, deoxycorticosteroneacetate treatment). The inset shows the means of the periods on the respective sodium diet. Values are the means ± SEM;* n = *6 per each group.* *$p < 0.05$ vs. *D_3 receptor (+/+).*

Acute Sodium Loading of Conscious Mice on Different Salt Diets

To investigate the effects of an acute sodium load in conscious D_3 receptor (−/−) mice and (+/+) animals (n=9 per group) on an NS diet (0.6% NaCl), an NaCl-containing solution (0.6% NaCl [NSL] or 4.6% NaCl [HSL] in drinking water) according to a volume of 5% of body wt. was applied per gavage. Another group of D_3 receptor (−/−) and (+/+) mice (n=9 per group), which had been on an HS diet (4.6% NaCl) for 1 week received an acute high sodium load (4.6% NaCl [HSL] in drinking water) of 5% of body wt. per gavage. The animals were placed in metabolic cages for 4 h with free access to water, while chow was restricted. The urine was collected in the first hour after the volume load and the following 3 h with determination of the renal sodium excretion. The metabolic cages allowed separate collection of urine and feces. The room in which the metabolic cage experiments was performed had a constant temperature of 21°C and a humidity of 57%.

Chronic Sodium Loading of Conscious Mice

Dopamine D_3 receptor (+/+) and (−/−) mice (n=6 per group) were examined for 2 weeks in metabolic cages. During the first week the animals received an NS diet (0.6% NaCl; Altromin) followed by an HS diet in the second week (4.6% NaCl; Altromin). The animals had free access to water and the corresponding diet. Daily measurement of urine excretion, food and water intake as well as determination of body wt. were performed.

Clearance Experiments with VE

At the beginning of the experiments, two consecutive clearance periods were performed in dopamine D_3 receptor (+/+) and (−/−) mice (n=5–10 per group) and are referred to as the baseline periods. At the beginning of the three experimental periods the infusion rate was increased in the VE groups to a volume of 4.5% of body wt. per hour while the control animals (CON) remained on the infusion rate of the baseline periods for the entire experiment. Each clearance period consisted of a 30-min urine collection, with a blood sample (40 µl) taken at midpoint. Arterial blood pressure was monitored continuously (Linear Corder Mark V; Hugo Sachs Elektronik, Hugstetten, Germany). For the surgical preparation the mice were anesthetized by i.p. injection of sodium pentobarbital (100 mg/kg body wt.; Sigma, St. Louis, USA) and placed on a servo-controlled heated table (Effenberger, München, Germany) to maintain the rectal temperature at 37.2°C. Additional sodium pentobarbital up to 20 mg per kg body wt. was applied if required. A tracheostomy (all catheters: Portex tubing, Portex, Hythe Kent, Great Britain; outer diameter 0.9 mm) was performed to facilitate spontaneous breathing. Another two catheters (original outer diameter 0.6 mm and additionally reduced by heat-stretching) were inserted into the right jugular vein for infusion of Ringer solution (NaCl 111 mmol/l, $NaHCO_3$ 30 mmol/l, KCl 4.7 mmol/l) with 2.25% bovine serum albumin (BSA; Sigma). *Via* the first catheter, [^{3}H]-inulin (10 µCi/ml) in Ringer/BSA was infused at a rate of 180 µl/h, and *via* the second catheter, Ringer/BSA was infused at a rate of 150 µl/h during the baseline periods (for a combined infusion rate of ~1.0% of body wt. per hour). In the left carotid artery another catheter was used to collect blood samples and to continuously monitor systemic blood pressure (Linear Corder Mark V; Hugo Sachs Elektronik). A catheter inserted into the bladder (outer diameter 0.9 mm) served for urine collection. The mice were allowed to recover from the surgical procedures for about 40 min before starting the measurements.

Analytical Methods

Urine volume, body and kidney wt., and water and food intake were quantified gravimetrically. Blood samples were centrifuged, and the hematocrit was assessed. Urinary and plasma concentrations of sodium were determined by flame photometry (ELEX 6361; Eppendorf, Hamburg, Germany), and the [^{3}H]-inulin radioactivity was measured by liquid scintillation counting (2550 TR; Canberra-Packard, Frankfurt, Germany).

Calculations and Statistics

The metabolic cage and blood pressure measuring experiments in D_3 receptor (+/+) and (−/−) mice were tested by the unpaired Student's t-test or by analysis of variance (ANOVA). In the clearance experiments baseline periods were determined individually and summarized as the means±SEM. Differences between D_3 receptor (+/+) and (−/−) mice, as well as differences between mice receiving sodium diets or sodium loads were tested by the unpaired Student's t-test. The statistical significance of the differences between the baseline and experimental periods was calculated by analysis of variance (ANOVA) with post-test correction according to Bonferroni. $p<0.05$ was considered statistically significant.

Results

Long-Term Cross-Over Experiment in Conscious Mice

SBP was measured in conscious 3-month-old D_3 receptor (+/+) and (−/−) mice using a cross-over protocol with diets containing different amounts of sodium chloride. During the first week, while the animals received an NS diet, SBP of the D_3 receptor (+/+) and (−/−) mice was similar. The mean values ranged from 89 to 118 mmHg (Figs. 1 and 2). Over the following 2 weeks, neither the feeding of an LS diet (Fig. 1)

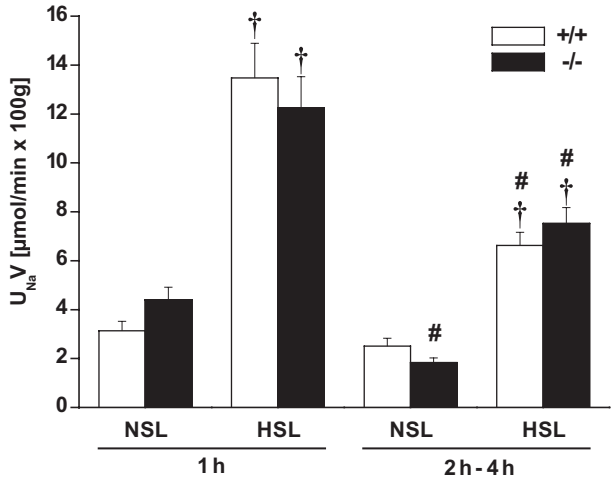

Fig. 3. *Urinary sodium excretion ($U_{Na}V$) of conscious dopamine D_3 receptor (−/−) and (+/+) mice in the first hour and the following 3 h after either an acute normal sodium load (0.6% NaCl, NSL, 5% of body wt.) or a high sodium load (4.6% NaCl, HSL, 5% of body wt.). The animals were fed a normal salt (0.6% NaCl) diet. Values are the means ± SEM;* n = *9 per group.* $^{\dagger}p < 0.05$ vs. *respective NSL;* $^{\#}p < 0.05$ vs. *1 h after sodium load.*

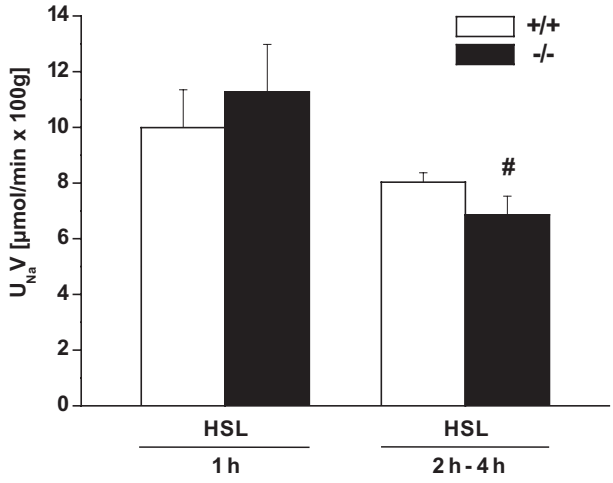

Fig. 4. *Urinary sodium excretion ($U_{Na}V$) of conscious dopamine D_3 receptor (−/−) and (+/+) mice in the first hour and the following 3 h after a high sodium load (4.6% NaCl, HSL, 5% of body wt.). The animals were fed a high salt (4.6% NaCl) diet. Values are the means ± SEM;* n = *9 per group.* $^{\#}p < 0.05$ vs. *1 h after sodium load.*

nor the feeding of an HS diet (Fig. 2) resulted in any remarkable changes when compared to the use of an NS diet, either in D_3 receptor (−/−) or D_3 receptor (+/+) mice. After the cross-over at the beginning of the fourth week, the change from an LS to an HS diet also did not cause a significant alteration in SBP in D_3 receptor (+/+) animals. However, a slight but significant decrease of SBP was observed in D_3 receptor (−/−) mice during the feeding of the LS and HS diets compared to the feeding of the NS diet (Fig. 1). The change from an HS to an LS diet, in contrast, had no effect on SBP in either mouse strain (Fig. 2). The summary of the respective diet periods (Figs. 1 and 2) clearly shows that SBP was similar in D_3 receptor (−/−) mice when compared to the corresponding D_3 receptor (+/+) animals and that SBP was only slightly influenced by the applied sodium diet.

Acute Sodium Loading of Conscious Mice on Different Salt Diets

The excretory response to an acute sodium load was investigated in the D_3 receptor (−/−) and corresponding (+/+) mice, both on an NS diet. The 0.6% sodium load (NSL) per gavage did not induce significant differences in the renal sodium excretion comparing between the two genotypes either during the first hour after the load nor during the following 3 h (Fig. 3). As expected, the 4.6% sodium load (HSL) induced a significantly higher sodium excretion than the NSL. The urinary sodium excretion after the HSL was significantly higher in the first hour than during the following 3 h. However, no significant differences in sodium excretion were observed when comparing D_3 receptor (−/−) with (+/+) mice, neither during the first hour nor during the following 3 h after the sodium load (Fig. 3). The fraction of the applied sodium load that was eliminated during the experiment was calculated as the sodium balance and expressed as a percentage. The sodium balance of the D_3 receptor (+/+) mice, which received an NSL, was 72.9 ± 7.9%, and thus was not significantly different from that of the D_3 receptor (−/−) mice (66.2 ± 6.1%). The sodium balance of the D_3 receptor (+/+) mice loaded with an HSL was 58.0 ± 1.6% and similar to that of the D_3 receptor (−/−) mice (60.6 ± 4.2%).

A different group of D_3 receptor (−/−) and corresponding (+/+) mice were fed an HS diet. The animals received an acute sodium load of 4.6% NaCl (HSL) in a volume corresponding to 5% of body wt. per gavage. The urinary sodium excretion during the first hour was significantly higher than that during the following 3 h in D_3 receptor (−/−) but not in (+/+) mice. However, no significant differences in sodium excretion were observed between D_3 receptor (−/−) and (+/+) mice (Fig. 4). During the entire experiment the sodium balance of the D_3 receptor (+/+) mice, which received an HSL, was 60.3 ± 3.2% and was not significantly different from that of the D_3 receptor (−/−) mice (53.0 ± 5.0%).

Chronic Sodium Loading of Conscious Mice

In metabolic cage experiments, the effect of different sodium diets on daily urinary sodium excretion was observed. Animals were fed an NS diet for 1 week and an HS diet for another week. Urinary sodium excretion was similar in both

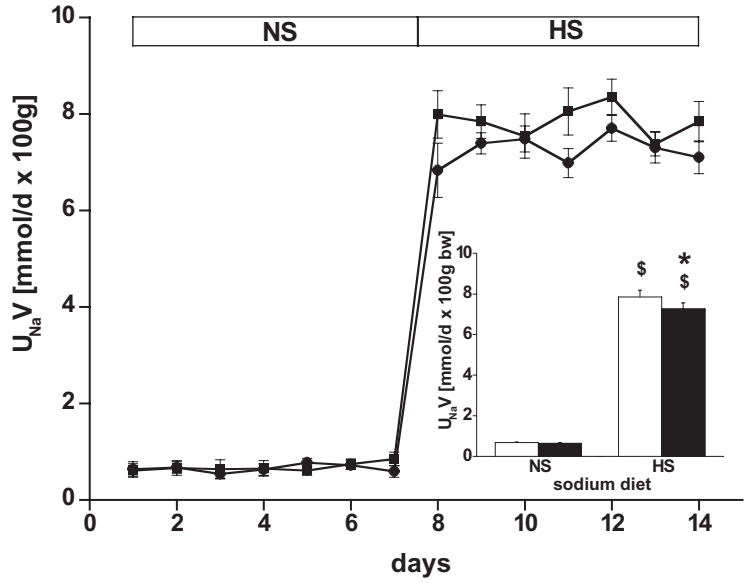

Fig. 5. *Urinary sodium excretion ($U_{Na}V$) of dopamine D_3 receptor (−/−) (circles, filled bars) and (+/+) (squares, open bars) mice on a normal salt (0.6% NaCl, NS) and high salt diet (4.6% NaCl, HS), each for 1 week. The inset shows the means of the periods on the respective sodium diet. Values are the means±SEM;* n = *6 per group.* *p < *0.05* vs. *D_3 receptor (+/+);* $p < *0.05* vs. *NS.*

genotype groups, approximately 0.7 mmol/day per 100 g body wt. (Fig. 5). As expected, sodium excretion significantly increased after the change to the HS diet, ranging from 7.4 to 8.4 and 6.8 to 7.7 mmol/day per 100 g body wt. in D_3 receptor (−/−) and (+/+) animals, respectively. Interestingly, a lower sodium excretion was observed in the D_3 receptor (−/−) mice on an HS diet, when compared to their corresponding (+/+) mice (Fig. 5). On both diets, the sodium balance was lower in the D_3 receptor (−/−) mice than in the (+/+) animals (23.7±1.5% *vs*. 27.6±1.4% on the NS diet), and this difference reached the level of statistical significance in mice fed an HS diet (42.6±1.5% *vs*. 49.1±1.2%).

Clearance Experiments with VE

Clearance experiments with dopamine D_3 receptor (−/−) and wild-type (+/+) animals on either an NS diet (0.6% NaCl) or an HS diet (4.6% NaCl) were performed to assess the influence of an increased sodium and water load on renal hemodynamics and the excretory function of the kidney. After a basal period (infusion rate of 1.0% of body wt. per hour), VE was performed by increasing the infusion rate to 4.5% of body wt. per hour. CON of the D_3 receptor (−/−) genotype, which received no VE, showed a slightly higher basal mean arterial blood pressure (MAP) when compared to the (+/+) animals. Heart rate (HR), GFR, urinary flow rate (UV), total ($U_{Na}V$) and fractional renal sodium excretion (FE_{Na}) at baseline did not differ between the D_3 receptor (−/−) and (+/+) animals (Table 1, Fig. 6). During the entire experiment these variables did not change in either CON group, suggesting that the experimental conditions were stable in this setting (Table 1, Fig. 6).

VE in mice on an NS diet partially induced a significant increase in UV, $U_{Na}V$ and FE_{Na} when compared to the basal periods. MAP, HR and GFR were not affected by VE in these mice (Table 1, Fig. 6). Compared to the (+/+) animals, MAP and HR were slightly but not significantly higher in D_3 receptor (−/−) mice. In mice on an HS diet, VE induced a significant increase in UV, $U_{Na}V$ and FE_{Na} when compared to the basal periods in both genotypes. MAP, HR and GFR were not affected by the VE in the mice on an HS diet (Table 1, Fig. 6). The diuretic and natriuretic effect of VE was more distinct in mice on an HS diet than in animals that were fed an NS diet.

Discussion

Alterations in the dopaminergic system may contribute to the development of hypertension. Previously, it has been shown in anesthetized mice that the renal dopamine D_3 receptor is involved in the regulation of blood pressure and urinary sodium excretion (*9*). However, in the present experiments no relevant blood pressure difference was observed in conscious dopamine D_3 receptor knockout (−/−) mice when compared to wild-type (+/+) animals, irrespective of whether an NS, HS or LS diet was fed. In additional experiments designed to investigate renal sodium excretion as a marker of sodium handling, the response to an acute sodium load per gavage was similar in both genotypes. Dopamine D_3 receptor (−/−) mice that were fed an HS diet for 1 week showed a reduced urinary sodium elimination when compared to (+/+) animals, indicat-

Table 1. Clearance Experiments of Anesthetized Dopamine D_3 Receptor (–/–) and the Corresponding (+/+) Animals

Group	Period	MAP (mmHg)	Heart rate (bpm)	GFR (ml/min/g kw)	UV (µl/min/g kw)	$U_{Na}V$ (µmol/min/g kw)
CON-NS-WT (n=10)	Basal	90.2±3.6	493.9±28.1	0.50±0.05	11.0±3.3	0.97±0.35
	EP I	93.6±4.5	523.9±22.0	0.45±0.05	16.9±3.3	1.38±0.47
	EP II	93.8±4.5	513.9±25.2	0.52±0.06	17.8±3.8	2.17±0.52
	EP III	91.9±3.8	548.6±25.7	0.45±0.04	15.8±3.7	2.27±0.51
CON-NS-KO (n=10)	Basal	104.8±4.2*	511.6±10.4	0.52±0.04	7.3±2.2	0.89±0.23
	EP I	105.3±3.6	515.0±14.7	0.52±0.04	13.6±3.5	1.84±0.40
	EP II	105.8±4.2	520.9±16.6	0.48±0.06	18.4±3.2	2.52±0.40$^{\#}$
	EP III	105.6±4.4*	542.5±16.4	0.47±0.12	16.3±5.4	2.13±0.70
VE-NS-WT (n=7)	Basal	94.6±4.0	500.7±29.8	0.56±0.04	9.8±2.8	0.62±0.17
	EP I	100.4±4.2	522.7±29.8	0.56±0.04	27.1±8.6	2.51±0.78
	EP II	97.9±6.1	530.7±22.8	0.57±0.05	48.4±14.5$^{\#,\dagger}$	5.83±1.90$^{\#,\dagger}$
	EP III	93.0±5.1	562.9±24.0	0.57±0.04	39.9±9.4†	5.46±1.32$^{\#,\dagger}$
VE-NS-KO (n=8)	Basal	99.3±4.5	514.7±13.0	0.48±0.06	9.3±2.9	1.79±0.94
	EP I	98.5±3.9	521.1±08.8	0.61±0.05	28.1±6.2†	3.56±1.61
	EP II	106.3±4.2	536.7±10.1	0.63±0.04	47.6±9.7$^{\#,\dagger}$	4.30±1.95
	EP III	104.8±4.7	540.0±16.9	0.54±0.04	50.2±8.9$^{\#,\dagger}$	6.26±2.62
VE-HS-WT (n=5)	Basal	96.8±4.4	491.3±23.6	0.49±0.07	11.2±1.5	1.24±0.47
	EP I	93.6±4.4	479.5±24.1	0.47±0.05	20.7±3.1	2.24±0.25
	EP II	99.0±4.2	558.8±31.9	0.64±0.09	50.8±6.0$^{\#,\$}$	7.65±0.92$^{\#,\$}$
	EP III	89.8±4.4	586.3±12.2$^{\#}$	0.49±0.05	50.5±5.9$^{\#,\$}$	7.81±0.96$^{\#,\$}$
VE-HS-KO (n=7)	Basal	100.6±5.1	492.1±21.9	0.51±0.04	12.4±4.2	2.04±0.67
	EP I	108.0±4.8	520.7±20.4	0.58±0.04	16.4±2.9	4.14±0.48*$^{,\$}$
	EP II	107.4±4.4	544.3±17.7	0.48±0.05	53.2±5.6$^{\#,\$}$	8.15±0.96$^{\#,\$}$
	EP III	102.0±2.4*	541.7±08.6*	0.53±0.04	58.4±5.0$^{\#,\$}$	8.85±0.88$^{\#,\$}$

Values represent means±SEM. WT, wild-type mice (+/+); KO, dopamine D_3 receptor knockout mice (–/–); MAP, mean arterial blood pressure; GFR, glomerular filtration rate; UV, urinary flow rate; $U_{Na}V$, urinary sodium excretion; CON, control animals; VE, volume expansion; NS, normal salt diet; HS, high salt diet; EP, experimental period; bw, body weight; kw, kidney weight. $^{\#}p<0.05$ *vs*. basal period (ANOVA); *$p<0.05$ *vs*. respective WT (*t*-test); $^{\dagger}p<0.05$ *vs*. respective CON groups (*t*-test); $^{\$}p<0.05$ *vs*. respective CON groups (*t*-test).

ing that, under certain conditions, dopamine D_3 receptors might influence renal sodium excretion. Studies using a clearance design with a moderate VE in anesthetized mice provided a similar total as well as fractional sodium excretion rate in both genotypes.

It has been reported that dopamine receptors are involved in the development of hypertension (*1*). Several experiments have shown that a deletion of the D_1-like as well as the D_2-like receptors is associated with high blood pressure levels (*3*). Shigetomi *et al*. (*14*) observed that dopamine D_2-like receptor blockade with metoclopramide induces the development of hypertension in saline-loaded Wistar rats. The selective blockade of dopamine D_3 receptors in salt-resistant Dahl rats with a peripheral D_3 receptor antagonist in combination with an HS diet induced a salt-dependent hypertension (*15*), whereas an NS diet did not increase blood pressure in this Dahl strain. These results indicate that blood pressure regulation is associated with D_3 receptor–modulated sodium handling, but this association appears to depend on the animal model. In this respect, pharmacological activation of D_3 receptors in spontaneously hypertensive rats (SHR) did not show significant differences in sodium excretion when compared to normotensive Wistar-Kyoto rats (*16*). In a dopamine D_3 receptor knockout mouse model, a reduced sodium excretion was reported in response to an acute NaCl load, which was accompanied by elevated blood pressure (*9*). Some of the genetically determined phenotypes associated with essential hypertension have been ascribed to an impaired ability of the kidney to excrete sodium after a chronic loading (*17*). It has been discussed that the inability of D_1-like receptors to affect normal sodium excretion may be due to a need for a complementary D_2-like action, since co-stimulation of D_1-like and D_2-like receptors is needed to inhibit Na^+/K^+-ATPase activity in renal proximal tubules (*18*, *19*) and thus induces a higher diuresis and natriuresis than the stimulation of D_1-like receptors alone (*20*).

Because anesthetic conditions might influence blood pressure regulation (*10*), conclusions regarding hypertensive factors should be based on studies in conscious animals. Therefore, in the present study the effects of a dopamine D_3

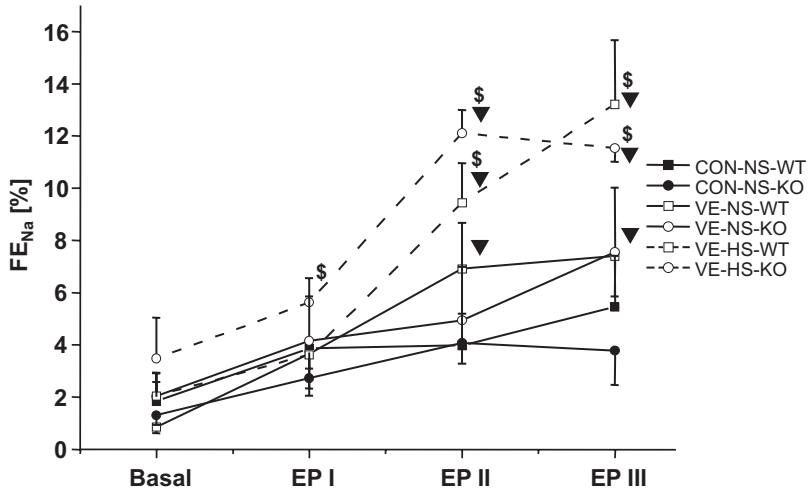

Fig. 6. *Clearance experiments showing the fractional urinary sodium excretion (FE_{Na}) of dopamine D_3 receptor knockout (KO) and wild-type (WT) mice at baseline and during volume expansion (VE, EP I–III). Control mice (CON) did not receive VE. The animals were fed either a normal salt diet (0.6% NaCl, NS) or a high salt diet (4.6% NaCl, HS). Values are the means±SEM;* n = *5–10 per group.* ▼$p < 0.05$ vs. *the baseline period (ANOVA);* $^{\$}p < 0.05$ vs. *the respective CON group.*

receptor deletion on arterial blood pressure were investigated in non-anesthetized mice. These conscious dopamine D_3 receptor (−/−) mice showed no relevant difference in SBP when compared to the corresponding (+/+) animals. This effect did not change during different salt diets. A possible reason for the discrepancy between the present study and the previously reported study (*9*), which showed elevated blood pressure in mice with defective D_3 receptors, might be differences in the knockout model or experimental conditions, *i.e.*, anesthesia *vs*. a conscious state. Taken together, these findings support the assumption that anesthesia is an important factor which has to be considered when assessing blood pressure studies. It is possible that stress during the preparation of the mice for blood pressure detection might have increased the secretion of catecholamines and thus blood pressure. In this respect, it is worth noting that presynaptically located dopamine D_3 receptors have been reported to inhibit neural noradrenaline release (*21*). Therefore, the knockout of D_3 receptors might be responsible for the higher blood pressure values in these anesthetized mice when compared to the wild-type animals.

In the present experiments only marginal differences in renal sodium excretion were observed between D_3 receptor (−/−) and (+/+) mice after an acute NSL as well as an HSL, irrespective of whether the animals were fed an NS or an HS diet. When comparing the fraction expressed as a percentage of the applied sodium load that was eliminated during the experiment, which is a suitable index of renal sodium handling, no significant differences were observed between the two genotypes. However, the experiments in which a chronic HSL followed a 1-week NS diet showed a significantly reduced urinary sodium excretion in D_3 receptor (−/−) when compared to (+/+) mice. This finding might result from the challenge of the D_3 receptor (−/−) mice with an HS diet and the inability of these mice to sufficiently handle a chronic sodium loading, whereas an acute load was not associated with a difference in sodium excretion between the two genotypes. The reasons for the discrepant results regarding the acute and the chronic sodium loading are not clear but compensating factors might be unmasked during a longer sodium challenge. It is possible that differences in intestinal excretion of sodium might play a role; however, this issue has to be evaluated in further studies. Irrespective of the underlying mechanism, the significantly retarded renal sodium excretion in D_3 receptor (−/−) mice was not sufficient to induce elevated blood pressure levels as shown in the cross-over study. Since blood pressure regulation depends on various mechanisms, *e.g.*, baroreceptor reflex, the renin-angiotensin-system and aldosterone release, compensation of blood pressure effects could not be excluded. In this respect, it is worth noting that the hypertension observed in a previous study on anesthetized mice with D_3 receptor deletion was associated with an increase in renal renin activity (*9*). However, renin activity was not measured in the present experiments. According to the acute sodium load experiments, the volume expansion of anesthetized mice did not show significant differences in total or fractional urinary sodium excretion between the D_3 receptor (−/−) and (+/+) genotypes. This effect was independent of whether the mice were fed an NS or an HS diet, supporting the assumption that a chronic sodium load is necessary to induce changes in renal sodium excretion.

To summarize, conscious dopamine D_3 receptor (−/−) mice did not show alterations in blood pressure levels when compared to wild-type animals. Renal sodium excretion was sim-

ilar in both genotypes and differed during a chronic high sodium loading. We conclude that in the present animal model renal dopamine D_3 receptors are not significantly involved in the regulation of blood pressure associated with a deficiency in renal sodium elimination. Whether a compensating mechanism might play a role remains to be investigated.

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