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Inhibitors of 20-hydroxyeicosatetraenoic acid (20-HETE) formation attenuate the natriuretic effect of dopamine

Maria M Federik Fernandez[a,1], **Daniel Gonzalez**[a], **Jan M Williams**[b], **Richard J Roman**[b], and **Susana Nowicki**[a]

[a]Centro de Investigaciones Endocrinólogicas (CEDIE-CONICET), Gallo 1360, C1425EFD Buenos Aires, Argentina

[b]Department of Pharmacology and Toxicology, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, US

Abstract

Endogenous renal dopamine is a major physiological regulator of renal ion transport; however its intracellular signaling pathways are not thoroughly understood. The present study examined the role of 20-hydroxyeicosatetraenoic acid (20-HETE), the major cytochrome P450 (CYP4A) metabolite of arachidonic acid formed in the renal cortex, on the natriuretic response to dopamine in Sprague Dawley rats. Infusion of dopamine (1.5 ug/kg/min, i.v.) increased urine flow (1.9 fold over basal), sodium excretion (UNaV, 2.7 fold), fractional sodium excretion (FENa, 3.3 fold) and proximal and distal delivery of sodium by 1.5 and 2-fold respectively. Administration of two inhibitors of the synthesis of 20-HETE, 1-aminobenzotriazole (ABT) and N-hydroxy-N'-(-4-butyl-2-methylphenyl)formamidine (HET0016) reduced the response to dopamine by 65%. Induction of the renal expression of CYP4A enzymes with clofibrate did not alter the response to dopamine. The natriuretic response to dopamine was lower in Dahl salt-sensitive rats in comparison to an SS.BN5 consomic strain in which transfer of chromosome 5 from Brown Norway to Dahl salt-sensitive rats upregulates the renal expression of CYP4A protein and the production of 20-HETE. Treatment with HET0016 blocked the renal effects of dopamine in SS.BN5 rats. We also examined the influence of 20-HETE in the natriuretic response to acute volume expansion that is in part mediated via the release of endogenous dopamine. The increase in urine flow, UNaV, FENa and distal FENa following volume expansion was markedly reduced in rats treated with ABT. These results suggest that 20-HETE plays at least a permissive role in the natriuretic response to dopamine.

Keywords

dopamine; 20-HETE; CYP4A; sodium excretion

Corresponding Author: Dra. Susana Nowicki, Centro de Investigaciones Endocrinólogicas, CEDIE-CONICET, Gallo 1360, C1425EFD Buenos Aires, Argentina, TE: +54 11 4963 5931 ext 110, FAX: +54 11 4963 5930, snowicki@cedie.org.arnowickisusana@hotmail.com.
[1]Present address: Servicio de Clínica Médica, Hospital Alemán, C1118AAT Buenos Aires, Argentina

1. Introduction

Previous studies have indicated an important role of renal dopamine in the regulation of sodium excretion and blood pressure (Ashgar et al., 2011; Harris RC, 2012; Zeng and Jose, 2011; Zhang et al., 2011). Dopamine is produced in proximal tubule cells via the decarboxylation of filtered L-dopa and enters the cell via a sodium transporter in the apical membrane (Carranza et al., 2000). Dopamine can exit the cell through either the apical or basolateral membrane; however, newly formed dopamine preferentially crosses the apical membrane such that the urinary concentration of dopamine far exceeds the levels of dopamine in the interstitial space (Wang et al., 1997). The biological effects of dopamine are mediated by receptors linked to the stimulation (dopamine D_1-like) or inhibition (dopamine D_2-like) of adenylyl cyclase in the proximal tubule, medullary thick ascending limb and to a lesser extent in the cortical collecting duct. Dopamine reduces sodium and water reabsorption by inhibiting the Na^+/H^+ exchanger, Na^+-phosphate co-transporter and Na^+,K^+-adenosine triphosphatase (Na^+,K^+-ATPase) in the proximal tubule and in the medullary thick ascending limb and to a lesser extent in the cortical collecting duct (Banday and Lokhandwala, 2008; Zeng et al., 2009; Zeng and Jose, 2011).

The role of cytochrome P450 (CYP450) metabolites of arachidonic acid in the renal handling of sodium has been thoroughly studied over the past few years and its importance as a target for the treatment of hypertension has been recently reviewed (Williams et al., 2010). 20-hydroxyeicosatetraenoic acid (20-HETE), the main CYP450 metabolite of arachidonic acid produced in the proximal tubule and the thick ascending limb, inhibits sodium transport in both of these nephron segments (Williams et al., 2010). Moreover, deficiencies in the renal formation of 20-HETE have been reported to contribute to sodium retention and the development of salt-sensitive hypertension in both man and experimental animal models (Elijovich, 2006; Williams et al., 2010).

The inhibitory effect of 20-HETE on sodium reabsorption in both the proximal tubule (Nowicki et al., 1997) and the medullary thick ascending limb (Yu et al., 2007) is in part secondary to inhibition of Na^+,K^+-ATPase. Like dopamine (Nowicki et al., 2000), the inhibitory action of 20-HETE on Na^+-K^+-ATPase activity is dependent on protein kinase C (Nowicki et al., 1997). Several studies have suggested that dopamine activates phospholipase A_2 and stimulates the release of arachidonic acid (Bhattacharjee et al., 2005; Hussain and Lokhandwala, 2003; Vial and Piomelli, 1995). Based on these results, we hypothesized that 20-HETE may be involved in the inhibitory actions of dopamine on Na^+,K^+-ATPase. In a previous paper, we demonstrated that 20-HETE plays at least a permissive role in the activation of protein kinase C to inhibit Na^+-K^+-ATPase activity in proximal tubule cells incubated with dopamine (Kirchheimer et al., 1997). The aim of the present study was to investigate the role of 20-HETE in mediating the natriuretic effect of an i.v. infusion of dopamine and the natriuretic response to isotonic saline volume expansion, which is in part dependent on the release of endogenous dopamine (Hegde et al., 1989).

2. Materials and methods

Experiments were performed on 8–10 week old, male Sprague Dawley rats that were purchased from the Animal Breeding Facility from the School of Biochemistry, University of Buenos Aires. The rats were housed in an Animal Care Facility at the Ricardo Gutierrez Children's Hospital. Other experiments were performed on Dahl salt sensitive rats and a SS.BN5 consomic strain in which the CYP4A genes on chromosome 5 of Brown Norway rats were transferred into the Dahl salt sensitive genetic background (SS.BN5 rats) (Williams et al., in press). These animals were obtained from breeding colonies maintained at the University of Mississippi Medical Center. We have previously reported that the renal

expression of CYP4A protein and the formation of 20-HETE is reduced in Dahl salt sensitive rats (Williams et al., 2008). More recently, we have reported that transfer of the CYP4A genes found on chromosome 5 from the Brown Norway rat into the Dahl salt sensitive genetic background in an SS.5BN consomic strain increases the renal expression of CYP4A protein and the production of 20-HETE while epoxygenase activity is not significantly different in Dahl salt sensitive and SS.5BN rats (Williams et al., in press). The protocols were reviewed by Institutional Animal Welfare Committes in Buenos Aires and at the University of Mississippi Medical Center and were performed according to the guidelines recommended by the National Institutes of Health.

2.1 Effects of CYP4A induction or inhibition on the natriuretic response to infusion of dopamine

These experiments were performed on 4 groups of Sprague Dawley rats that were treated with vehicle, clofibrate (400 mg/kg per day, p.o.) for 2 weeks which has been reported to induce the renal expression of the CYP4A enzymes and the production of 20-HETE (Ishizuka et al., 2003; Roman et al., 1993), a nonselective inhibitor of the renal formation of 20-HETE, 1-aminobenzotriazole (ABT, 50 mg/kg, i.p.), 18 h before the acute experiment to block the renal formation of EETs and 20 HETE (Dos Santos et al., 2004; Su et al., 1998; Williams et al, 2007), and a highly selective inhibitor of the synthesis of 20-HETE, N-hydroxy-N'-(-4-butyl-2methylphenyl) formamidine (Hoagland et al., 2003; Miyata et al., 2001) (HET0016, 10 mg/kg/day, i.p.) for 3 consecutive days prior to the experiment. Similar experiments were performed in SS.BN5 rats that were pretreated with vehicle or HET0016. The effects of these treatments on renal cortical 20-HETE synthesis has been previously documented: Clofibrate has been reported to increase the renal production of 20-HETE in the renal cortex by 142 % (Ishizuka et al., 2003) and by 170 % (Roman et al., 1993) compared to control, whereas ABT reduced cortical 20-HETE formation by 80–90% of control (Dos Santos et al., 2004; Su et al., 1998; Williams et al, 2007), and HET0016 to approximately 10% of control animals (Hoagland et al., 2003).

The rats were anesthetized with thiopental (70 mg/kg, i.p.) and the trachea was cannulated to facilitate breathing. The carotid artery and femoral vein were catheterized to measure mean arterial pressure and for i.v. infusions, respectively. The rats received an i.v. infusion of 0.9% NaCl solution containing FITC-labeled inulin (4 mg/ml) and LiCl (5 mM) at a rate of 3 ml/hr. After surgery and a 45 min stabilization period, urine and a blood sample (0.4 ml) were collected during a 30 min control period. Then, dopamine was infused at a dose of 1.5 ug/kg/min and urine and plasma samples were collected during 2–30 min clearance periods. Glomerular filtration rate was calculated from the clearance (C) of FITC-Inulin (IN). Sodium delivery out of the proximal tubule was estimated by CLi^+, and fractional delivery of sodium from the proximal tubule was calculated as: FE Na^+ prox= CLi^+/CIN. Fractional delivery from the distal nephron was calculated as: FE Na^+ dist= CNa^+/CLi^+ (Christensen et al., 1986).

2.2 Effects of ABT on the natriuretic response to acute volume expansion

These experiments were performed in Sprague Dawley rats pretreated with vehicle or ABT (50 mg/kg, i.p.), 18 h before surgery to block the renal formation of EETs and 20-HETE. We have previously reported that this treatment reduces the renal formation of both 20-HETE and EETs in Sprague Dawley rats by 80 and 60 %, respectively (Dos Santos et al., 2004). The rats were surgical prepared for clearance experiments as described above. Urine and plasma samples were collected during a 30 min control period and in 2–30 min clearance periods during administration of an i.v. infusion of 5% of the rat's body weight in 0.9% NaCl solution.

2.3 Analysis of samples

The concentration of FITC-inulin in urine and plasma samples was measured using a Synergy HT multiplate reader (BioTek Instruments, Inc. Winooski, VT, US) using an excitation and emission wavelengths of 480 and 530, respectively. Dopamine was extracted from urine using alumina, separated by reverse-phase high-pressure liquid chromatography using a 4.6x150 mm, 5um Zorbax C18 column (Agilent Life Sciences and Chemical Analysis, CA, US) and quantified amperometrically using a triple-electrode system (ESA, Bedford, MA, US) (Eisenhofer et al., 1986). Sodium concentration in plasma and urine was measured by flame photometry, and lithium levels were determined by Atomic Emision Spectrophotometry using a Varian 240 FS atomic absorption spectrometer (Agilent Technologies Inc., CA, US).

2.4 Expression of CYP4A proteins

Samples of liver and renal cortex from control and clofibrate- treated rats were homogenized in a 10 mM potassium buffer (pH 7.7) containing (in mM) 250 sucrose, 1 EDTA, and 0.1 phenylmethylsulfonylfluoride (PMSF). The homogenate was centrifuged at 3,000 *G* for 5 min and the supernatant was centrifuged at 11,000 *G* for 15 min. Protein concentration was measured using the Bradford method. Equal amounts of protein (15 g) were separated by 8.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride (PVDF) membranes and immunodetected using a rabbit polyclonal antibody to cytochrome P450 4A (1:1500, Abcam, Cambridge, UK). The membranes were washed and incubated with an anti-rabbit IgG, HRP-linked antibody (1:4000, Cell Signalling Technology Inc., MA, US). The bands were detected by enhanced chemiluminescence using the ECL Advance Western Blotting Detection Kit (GE Healthcare, Buckinghamshire, UK). The membranes were stripped in a buffer containing 0.2M glycine, 0.1% sodium dodecyl sulfate, 1% Tween 20, pH 2.2 (room temperature, 2–10 min incubations), washed, and membranes were reblotted with anti- Tubulin primary antibody (1:3,000; SigmaAldrich Co, US). Membranes were incubated with an anti-mouse IgG, HRP-linked antibody (1:12,000; Amersham Biosciences, Sweden) and examined as previously described. The X-ray films were scanned using a HP Scan Jet 5100C and HP Precision Scan software (Hewlett-Packard, Palo Alto, CA).

2.5 Statistics

Data are presented as mean values S.E.M. Comparisons were done by 2-way repeated measures ANOVA followed by a Student-Newman-Keuls post hoc test. *P* value 0.05 was used as the level of significance.

3. Results

3.1 Effect of CYP4A inhibitors on the renal response to dopamine in Sprague Dawley rats

A comparison of the effects of ABT and HET0016 on the renal response to dopamine in Sprague Dawley rats is presented in Fig. 1. In control rats, dopamine elicited a 1.9 fold increase in urine flow and a 2.7 fold increase in sodium excretion in the absence of changes in glomerular filtration rate 30 min after the initiation of the infusion. These values remained elevated for the duration of the experiment. Urine flow, sodium excretion, glomerular filtration rate and fractional sodium excretion were not significantly altered in time control experiments in rats given vehicle alone, or by ABT or HET0016 administration.

The diuretic and natriuretic response to dopamine was markedly reduced in rats pretreated with both, ABT or HET0016 (Fig. 1, A, B and C). The small increase in glomerular filtration rate elicited by dopamine after 60 min infusion (1.3 fold) was also inhibited by ABT and HET0016. Mean blood pressure values during the basal period were similar

among groups (in mmHg: Control 126±6; ABT 136±6; HET0016 139±14) and were not significantly altered by infusion of dopamine. Fractional sodium excretion increased from 0.6 to 2.0% of the filtered load in the animals infused with dopamine and this was associated with a large increase in the fractional excretion of lithium (a marker of proximal tubular sodium delivery) from 52.9 to 78.9% of the filtered load. The ratio of the CNa^+/CLi^+ which is an estimate of the renal handling of sodium in the distal nephron, increased from 1.5 to 3.0% of the filtered load. The inhibition of tubular sodium reabsorption produced by dopamine was reduced by more than 50% in rats pretreated with ABT and HET0016 (Fig. 1, D, E and F).

3.2 Effect of the upregulation of CYP4A on the renal response to dopamine in Sprague Dawley rats

Clofibrate pretreatment markedly increased the expression of CYP4A protein in liver and the renal cortex (Fig. 2). The response to dopamine was not statistically different in control and clofibrate-treated rats if compared to vehicle-treated rats in all the analyzed parameters with the exception of glomerular filtration rate and fractional proximal sodium excretion (Fig. 3). A modest increase in glomerular filtration rate value was evident after 30 min of the beginning of dopamine infusion in clofibrate- treated animals. It reached statistical significance after 60 min (1.6 fold increase over control, $P<0.05$)) (Fig. 3C). The fractional excretion of lithium was also higher in clofibrate- treated than control animals after 60 min of dopamine infusion (88.9% vs. 71.7%, $P<0.05$) (Fig. 3E). Clofibrate treatment did not modify mean blood pressure either in the control period or during infusion of dopamine (in mmHg: Basal 131±4; dopamine 30 min 130±5; dopamine 60 min 134±10).

3.3 Natriuretic response to infusion of dopamine in Dahl salt sensitive and SS.BN5 consomic rats

The effect of dopamine on renal sodium handling was studied also in Dahl salt sensitive rats and a SS.BN5 consomic strain. Dopamine produced a much smaller increase in urine flow and sodium excretion in Dahl salt sensitive rats that have been reported to be deficient in the renal formation of 20-HETE (Williams et al., 2008), than the response seen in Sprague Dawley rats. The diuretic and natriuretic response to dopamine was much greater in the SS.BN5 rats than in Dahl salt sensitive rats (fold increase in SS.BN5 over Dahl salt sensitive rats: diuresis, 1.7; natriuresis, 1.4; $P<0.05$ for both). Pretreatment of the SS.BN5 rats with HET0016 completely blocked the natriuretic response to infusion of dopamine (Fig. 4, A and B). Dopamine and treatment with HET0016 had no significant effect on glomerular filtration rate in any of the groups (Fig. 4C).

3.4 Effect of ABT on the natriuretic response to acute volume expansion in Sprague Dawley rats

Previous studies have indicated that release of dopamine plays an important role in the natriuretic response to an acute volume expansion (Hegde et al., 1989). We therefore studied whether blockade of the synthesis of 20-HETE with ABT would blunt the natriuretic response to the endogenous release of dopamine following volume expansion. Acute volume expansion with 0.9% sodium chloride (5% body weight) increased the urinary excretion of dopamine equally in both control and ABT- treated animals (Fig. 5). However, the increase in urine flow and sodium excretion was much greater in the control rats than that seen in ABT- treated rats (Fig. 6, A and B). The natriuretic response to volume expansion was associated with a marked increase in glomerular filtration rate and fractional sodium excretion in control rats and both responses were decreased significantly in the ABT- treated animals (Fig. 6, C and D). Both proximal and distal fractional sodium excretion increased following volume expansion (1.5 and 2.9 fold over basal respectively, $P<0.01$ for both) in the rats treated with vehicle. ABT reduced the increase in sodium delivery from the distal

nephron ($P<0.01$ vs. control) but not the increase seen following volume expansion in the proximal tubule level (Fig. 6, E and F). Mean blood pressure was not significantly altered following volume expansion in either the control or ABT- treated animals.

4. Discussion

The present study examined the role of 20-HETE in the natriuretic response to dopamine. We found that infusion of a low dose of dopamine (1.5 g/kg/min) that does not alter arterial pressure increased urine flow and sodium excretion in the absence of major changes in glomerular filtration rate indicating that the natriuretic response was mostly due to inhibition of tubular sodium reabsorption. Dopamine also increased the fractional excretion of lithium indicating that it inhibited sodium transport in the proximal tubule. These results are consistent with the previous in vitro findings indicating that dopamine inhibits Na^+,K^+-ATPase activity, Na^+/H^+ exchange and the Na^+/phosphate co-transporter in proximal tubular cells (Bacic et al., 2005; Bobulescu et al., 2010; Pedemonte et al., 2005). Several previous studies have also indicated a role of phospholipase A_2 in mediating the effects of dopamine on Na^+,K^+-ATPase and the sodium transporters *in vitro* and suggested a role for the endogenous metabolites of arachidonic acid in these responses (Bhattacharjee et al., 2005; Hussain and Lokhandwala, 2003; Vial and Piomelli, 1995). However, very few studies have attempted to verify that dopamine increases the synthesis or action of 20-HETE (Kirchheimer et al., 2007). Moreover, the role of 20-HETE in mediating the actions of dopamine *in vivo* has not been studied previously. In the current study, administration of two chemically dissimilar inhibitors of the formation of 20-HETE, ABT and HET0016 markedly inhibited the natriuretic response to infusion of dopamine in Sprague Dawley rats. This finding is consistent with the view that dopamine likely activates phospholipase A_2 to release arachidonic acid and increase the production of 20-HETE which reduces sodium transport in the proximal tubule. Although ABT has been reported to block the synthesis of both EETs and 20-HETE, our finding that the very selective inhibitor of the synthesis of 20-HETE, HET0016, had the same effect to blunt the natriuretic response to dopamine suggests that 20-HETE rather than EETs is the likely mediator of this response. These results are also consistent with the view that 20-HETE has the same effect as dopamine to increase protein kinase C activity to phosphorylate the serine 23 residue on Na^+, K^+-ATPase to inhibit its action in proximal tubule cells (Nowicki et al., 1997) and in the thick ascending limb in vitro (Grider et al., 2003).

We next explored whether induction of the renal expression of the CYP4A enzymes that produce 20-HETE with clofibrate would enhance the response to infusion of dopamine. We found that administration of clofibrate had the expected effect to markedly increase the expression of CYP4A protein both in microsomes prepared from the renal cortex and liver. Despite the higher expression of CYP4A in clofibrate- treated rats, the natriuretic response to infusion of dopamine was similar in control and clofibrate- treated rats. This suggest that the expression of CYP4A protein is not limiting for the production of 20-HETE. This conclusion is consistent with previous reports that the rate limiting step in the formation of 20-HETE, as with other eicosanoids, is the activation of phospholipase A_2 and the availability of the substrate, arachidonic acid. However, we did observe a significant increase in glomerular filtration rate and fractional excretion of sodium after 60 min of dopamine infusion in clofibrate- treated rats. The effects at the proximal tubule might be explained by a locally circumscribed higher availability of 20-HETE. The effect on glomerular filtration rate might be due to an increase in the formation of nitric oxide or epoxyeicosatrienoic acids in the vasculature since peroxisome proliferator-activated receptor (PPAR) agonists like clofibrate have also been reported to increase the expression of epoxygenase enzymes of the 2C family (Sankaralingam et al., 2006) and nitric oxide synthase (eNOS) (Newaz et al., 2004) in endothelial cells. Upregulation of the formation of

nitric oxide or epoxyeicosatrienoic acids might enhance the renal vasodilatory response to dopamine D_1 receptor stimulation.

Dahl salt sensitive rats have been reported to have a deficiency in the renal expression of CYP4A protein and produce less 20-HETE relative to other strains of rats (Williams et al., 2008). They also exhibit a poor natriuretic and diuretic response to acute volume expansion as well as with the exogenous administration of dopamine (Nishi et al., 1993; Roos et al, 1984). The impaired response has been linked to defective dopamine D_1 like receptor function and to an impaired Na^+,K^+-ATPase inhibition by dopamine (Hussain and Lokhandwala, 2003; Nishi et al., 1993). This latter effect might be related to the deficiency in the renal formation of 20-HETE in this strain (Ma et al., 1994), since 20-HETE serves as a second messenger of dopamine receptor action in the proximal tubule (Kirchheimer et al., 2007). To test this hypothesis, we compared the renal response to infusion of dopamine in Dahl salt sensitive rats and the SS.BN5 consomic strain in which chromosome 5 containing the CYP4A alleles from Brown Norway rats were introgressed into the Dahl salt sensitive genetic background. We have recently shown that transfer of chromosome 5 increases the renal expression of CYP4A protein and the production of 20-HETE in the SS.BN5 consomic strain (Williams et al., in press). As expected the diuretic and natriuretic responses to dopamine were higher in the SS.BN5 strain than in Dahl salt sensitive rats. These results are consistent with previous studies indicating that the induction of CYP4A with fenofibrate improved the pressure-natriuresis relationship in this strain (Alonso-Garcia et al., 1998). In that study the higher sodium excretion secondary to the increase in renal perfusion pressure was primarily due to an elevation in glomerular filtration rate with not significant effects on tubular sodium transport. The role of 20-HETE in mediating the enhanced natriuretic response to dopamine in the SS.BN5 consomic strain was confirmed by the demonstration that administration of the selective inhibitor of 20-HETE formation HET0016, completely eliminated the natriuretic response to dopamine in this strain.

We also tested the role of 20-HETE in the natriuretic response to acute volume expansion with isotonic saline since this response is known to stimulate local release of endogenously produced dopamine in the kidney that contributes to the inhibition of proximal sodium reabsorption following volume expansion (Hegde et al., 1989). We confirmed that the urinary excretion of dopamine increased similarly following acute volume expansion in the control and ABT- treated rats. However, the natriuretic and diuretic responses and inhibition of tubular sodium reabsorption in response to acute volume expansion were markedly blunted in ABT-treated rats. Taken together, these results indicate that 20-HETE also plays a critical role in the natriuretic response to locally synthetized dopamine.

In a previous paper we postulated that 20-HETE is part of an integrated network of intracellular signals triggered by the stimulation of both dopamine D_1 and D_2 receptors and that it synergizes the actions of diacylglycerol to activate protein kinase C. Stimulation of adenylyl cyclase, which is coupled to the dopamine D_1 receptor, leads to the activation of protein kinase A. Finally, the combined activation of protein kinase A and protein kinase C results in the inhibition of Na^+-K^+-ATPase activity (Kirchheimer et al., 2007).

Overall, results from the present study support the findings of previous *in vitro* studies and together indicate that 20-HETE plays an important role in mediating the natriuretic response to dopamine.

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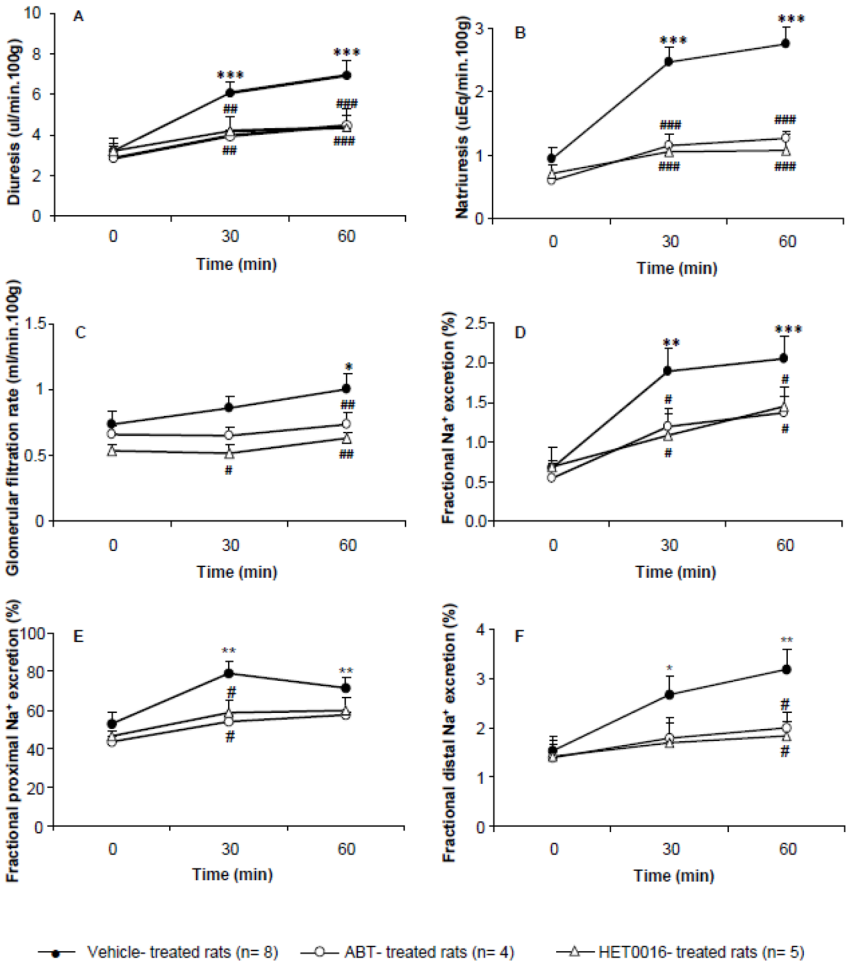

Figure 1.

Comparison of the effects of ABT (50 mg/kg, i.p.) and HET0016 (10 mg/kg/day, i.p.) on the natriuretic response to an i.v. infusion of dopamine (1.5 ug/kg/min) in Sprague Dawley rats. Numbers in parentheses indicate the number of animals studied per group. Values are means ± S.E.M. *, **, *** indicate $P<0.05$, 0.01 and 0.001 vs. the control value within the same group (0 min). #, ##, ### indicate $P<0.05$, 0.01, 0.001 vs. the corresponding value in the vehicle- treated rats.

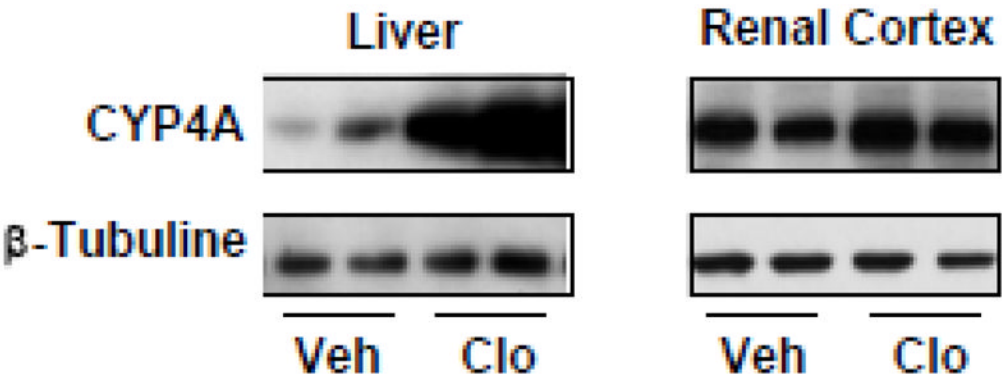

Figure 2.
Representative immunoblot for the expression of CYP4A and -Tubulin in homogenates of liver and renal cortex prepared from vehicle- (Veh) or Clofibrate- (Clo) treated Sprague Dawley rats.

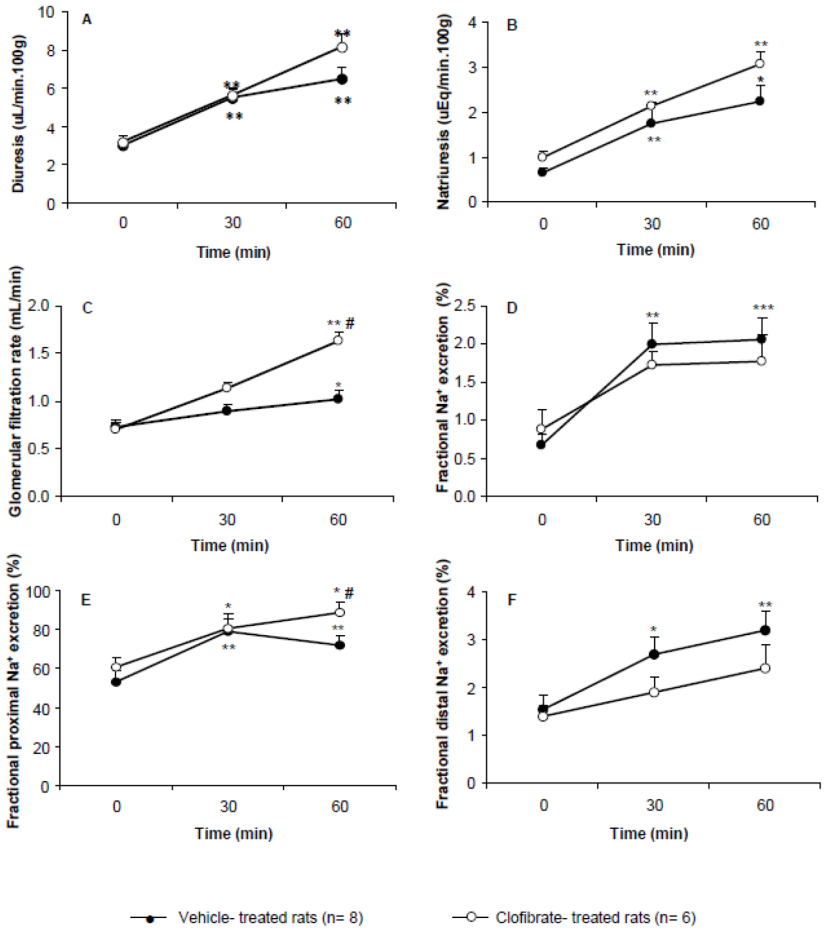

Figure 3.

Effects of the induction of the renal expression of CYP4A enzymes with clofibrate (400 mg/kg/day) on the natriuretic response to an i.v. infusion of dopamine (1.5 ug/kg.min). Numbers in parentheses indicate the number of animals studied per group. Values are means ± S.E.M. *, **, *** indicate $P<0.05$, 0.01 and 0.001 vs. the control value within the same group (0 min). # indicates $P<0.05$ vs. the corresponding value in the vehicle- treated rats.

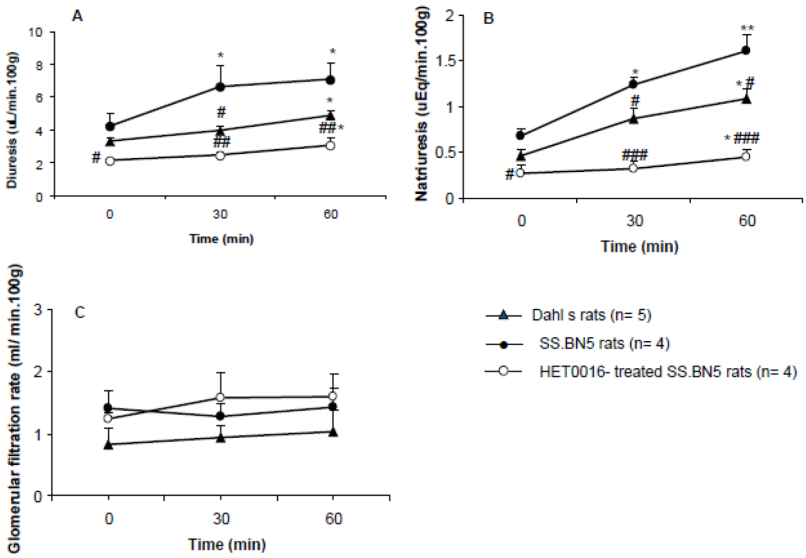

Figure 4.

Comparison of the natriuretic response to an i.v. infusion of dopamine (1.5 ug/kg.min) in Dahl salt sensitive (S), SS.BN5, and HET0016 (10 mg/kg/day, i.p.) treated SS.BN5 rats. Numbers in parentheses indicate the number of animals studied per group. Values are means ± S.E.M. *, ** indicate $P<0.05$ and 0.01 vs. the control value within the same group (0 min). #, ##, ### indicate $P<0.05$, 0.01, 0.001 vs. the corresponding value in the vehicle- treated rats.

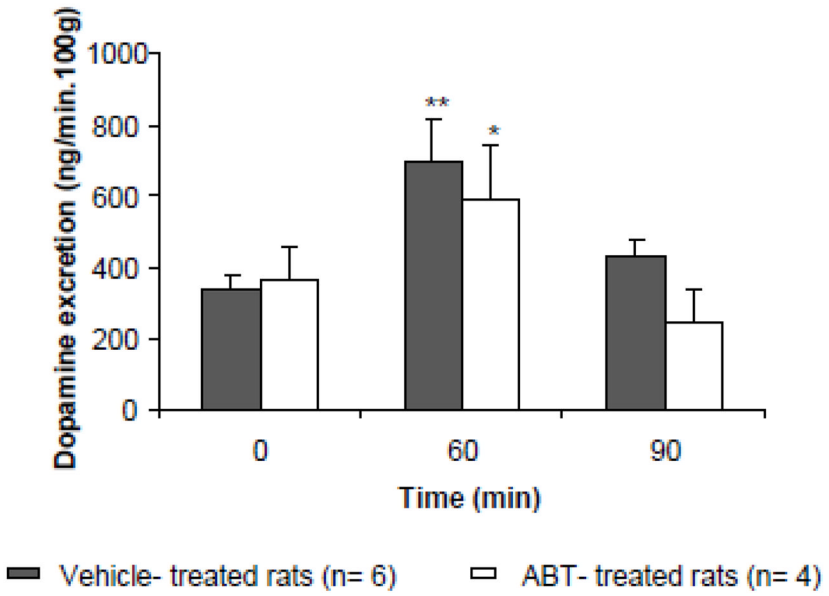

Figure 5.

Effects of blockade of the renal CYP4A with 1-aminobenzotriazol (ABT, 50 mg/kg, i.p.) on dopamine excretion in Sprague Dawley rats after 60 min of volume expansion (5% body weight) and after 30 min recuperation once the expansion was ceased (90 min). Numbers in parentheses indicate the number of animals studied per group. Values are means ± S.E.M. *, ** indicate $P<0.05$, and 0.01 vs. the control value within a group (0 min).

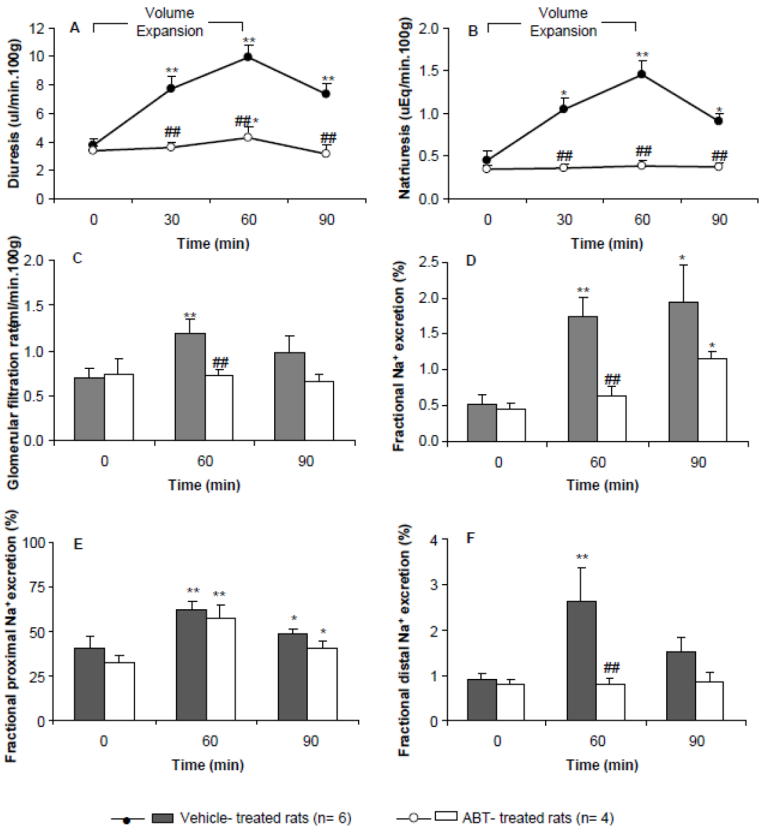

Figure 6.

Effects of blockade of the renal CYP4A with 1-aminobenzotriazol (ABT, 50 mg/kg, i.p).on the natriuretic response to acute volume expansion (5% body weight) with isotonic saline solution. Numbers in parentheses indicate the number of animals studied per group. Values are means ± S.E.M. *, ** indicate $P<0.05$, and 0.01 vs. the control value within a group (0 min). ## indicates $P< 0.01$ vs. the corresponding value in the vehicle- treated rats.