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Modulation of Vascular O₂ Responses by Cytochrome 450-4A ω -Hydroxylase Metabolites In Dahl Salt-Sensitive Rats

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Abstract

Objective—This study evaluated the role of the 20-HETE/cytochrome P450-4A ω -hydroxylase (CYP450-4A) system in microvascular regulation in the skeletal muscle circulation following short term (3 days) exposure to high salt (HS) diet in Dahl salt-sensitive (SS) rats.

Methods—The effects of inhibiting CYP450-4A on resting diameter, O₂-induced constriction, and vasodilator responses to acetylcholine (ACh) and the NO donor sodium nitroprusside (SNP) were evaluated in cremasteric arterioles of SS rats fed low salt (LS; 0.4% NaCl) or high salt (HS; 4% NaCl) diet for 3 days.

Results—HS diet up-regulated CYP450-4A mRNA expression and led to an enhanced constriction of arterioles in response to elevated PO₂ in SS rats, which could be blocked by inhibiting CYP450-4A enzymes with dibromododecyl methylsulfimide (DDMS). DDMS also inhibited resting tone significantly in SS rats fed HS diet, but not LS diet, despite similar resting diameters and active tone in the two groups. Arteriolar dilations in response to ACh and SNP were similar in SS rats fed LS vs. HS diet, and were unaffected by DDMS. These findings suggest that CYP450-4A enzymes contribute to resting tone and to an enhanced response to elevated PO₂ in arterioles of Dahl-SS rats fed HS diet.

Keywords

Dahl-SS rats; salt sensitive hypertension; 20-HETE; hypoxia; vasodilation; oxygen; cytochrome P450 ω -hydroxylase

INTRODUCTION

Vascular responses to changes in oxygen availability and to vasodilator stimuli are significantly altered in several different forms of hypertension including the Dahl rat model of salt-sensitive hypertension (25–28;33;36). For example, in SHR and reduced renal mass hypertension, arteriolar constriction in response to elevated PO₂ is enhanced very early in the development of hypertension (27;28), before responses to other vasoactive stimuli are altered (27). Of particular interest is the observation that consomic rats carrying the Brown Norway chromosome 13 (which reduces the salt-sensitivity of arterial blood pressure) (3) and congenic rats carrying the Dahl salt-resistant (Dahl R) renin gene (4) still show a salt-induced potentiation of arteriolar constriction in response to elevated superfusion solution PO₂, even though vasodilator responses to acetylcholine (ACh) are significantly improved by introgression of

either the BN renin allele in the SS.13^{BN} consomic rats (3;5–7) or the Dahl salt-resistant rennin allele in the RGRR congenic rats (4). Collectively, these findings suggest that alterations in the sensitivity of blood vessels to changes in PO₂ may be a common feature in many forms of hypertension, and that genetic factors may affect vascular responses to changes in PO₂, independent of salt-induced changes in blood pressure or altered responses to vasodilator stimuli.

Cytochrome P450-4A ω -hydroxylase (CYP450-4A) has been identified as a potential oxygen sensor, since this enzyme changes its production of the vasoconstrictor arachidonic acid metabolite 20-hydroxyeicosatetraenoic acid (20-HETE) in an O₂-dependent fashion (18). CYP450-4A is expressed in both the cremaster muscle microcirculation and the mesenteric circulation of normotensive Sprague-Dawley rats (21;37;38); and the expression of CYP450-4A isoforms can be altered by high salt diet (38). Blockers of 20-HETE synthesis also inhibit O₂-induced constriction of arterioles in the skeletal muscle microcirculation;(18; 21) and 20 HETE has been found to modulate hypoxic dilation of skeletal muscle resistance arteries of both normotensive Sprague-Dawley rats (12) and Dahl salt-sensitive rats. (15) In addition to their potential role in O₂ sensing, there is evidence that CYP450-4A enzymes may contribute to impaired responses to vasodilator stimuli in reduced renal mass (RRM) hypertension (11) and possibly during elevated dietary salt intake in normotensive rats (38).

At the present time, the mechanisms responsible for the changes in vascular O₂ sensitivity in multiple forms of hypertension (25–28;33;36), and the contribution of CYP450-4A enzymes to altered vascular oxygen responses and to impaired vasodilator responses in hypertension and during elevated dietary salt intake are poorly understood. The goal of the present study was to assess the role of the CYP450-4A/20-HETE system in contributing to early changes in the responses of cremasteric arterioles to elevated PO₂ and vasodilator agonists (acetylcholine and sodium nitroprusside) in Dahl salt-sensitive (SS) rats (a rodent genetic model of salt-sensitive hypertension in humans) fed a short term (3 days) high salt diet.

MATERIALS AND METHODS

Animal Preparation

Experiments were performed on 8–10 week-old male Dahl salt-sensitive (SS) rats (Dahl-SS/JrHsd/Mcwi), weighing 250–300 g. The rats were housed in the Biomedical Resource Center at the Medical College of Wisconsin (MCW), which is accredited by the American Association for the Accreditation of Laboratory Animal Care. All experimental protocols were approved by the MCW IACUC. The animals were fed a low salt (LS) (0.4% NaCl) AIN 76 diet (Dyets, Inc., Bethlehem, PA) with tap water *ad libitum*. Three days before the experiment, half of the animals were switched to a high salt (HS) diet containing 4% NaCl (Dyets Inc.). The average mean arterial pressure (MAP) ($\pm$ SEM) measured under anesthesia was 126 $\pm$ 2 mmHg in SS rats fed on LS diet (n = 21) and 128 $\pm$ 3 mmHg in SS rats fed a HS diet for 3 days (n = 21).

General Procedures

Rats were anesthetized with sodium pentobarbital (30 mg/kg. i.p) and the cremaster muscle was prepared for *in situ* visualization of the microcirculation using television microscopy, as described previously (26;28). A low dose of pentobarbital, sufficient to eliminate the pedal reflex, was used because SS rats are supersensitive to the effects of barbiturates (35). After preparing the cremaster muscle for *in vivo* microscopy, the tissue was continuously superfused with physiological salt solution (PSS) equilibrated with a gas mixture containing 0% O₂, 5% CO₂ and 95% N₂ for 30 minutes, to insure that O₂ delivery to the tissue was entirely via the microcirculation. The PSS used in these experiments had the following composition (in mM):

119 NaCl, 4.7 KCl, 1.17 MgSO₄, 1.6 CaCl₂, 1.18 NaH₂PO₄, and 24 NaHCO₃, and 0.03 disodium EDTA.

RT-PCR for CYP450-4A isoforms

For RT-PCR studies, intact cremasteric arterioles were isolated by microdissection and placed in cold (4°C) RNeasy lysis solution (Qiagen; Hilden, Germany). RNA was extracted using the single-step guanidiniumthiocyanate-phenol-chloroform method. RT and PCR reactions were performed using the Omniscript RT Kit (Qiagen; Hilden, Germany) and HotStarTaq Master Mix Kit (Qiagen) as described previously. (37;38) Reactions were conducted in the presence of $1.25 \cdot 10^{-6}$ L ($5 \cdot 10^{-7}$ mol/L) of specific oligonucleotide primers developed for the -4A1, -4A2, and -4A3 isoforms of CYP450-4A by Ito et al. (19) and for the CYP450-4A8 isoform of CYP450-4A by Gebremedhin et al. (16) Liver RNA was used as a positive control. Negative controls included experiments in which distilled water, rather than c-DNA, was added to the PCR reactions to exclude nonspecific contamination, or experiments in which the reverse transcriptase step was omitted before PCR to exclude any genomic DNA contamination. To check the quality of the extracted RNA sample and PCR reaction, forward and reverse primers for the housekeeping gene GAPDH (Operon Technologies) and the G3PDH control reagent (Clontech Laboratories; Palo Alto, CA) were included in each experiment.

Experimental Procedures on the Cremaster Muscle Microcirculation

After 30 minutes of equilibration, active tone in the arterioles was assessed by measuring the diameter increase during maximum dilation with 1 mM adenosine. After recovery from the adenosine, the O₂ concentration of the gas mixture in the PSS reservoir was changed from 0% O₂ to 21% O₂ (with 5% CO₂ and balance N₂ for each mixture) for 20 minutes. This leads to a significant increase in tissue PO₂, although not to the levels in the superfusion solution, e.g. O₂ microelectrode measurements have shown that tissue PO₂ approximately doubles in the hamster microcirculation during superfusion with 21% O₂ solution (8). Arteriolar diameter was measured with a video micrometer (28) before and during equilibration with the 21% O₂ gas mixture.

To determine the effect of 20-HETE on oxygen-induced constriction of cremasteric arterioles, dibromododecanyl methylsulfonamide (DDMS; 50 μM), a selective inhibitor of the formation of 20-HETE by CYP450-4A enzymes (34;40), was added to the superfusion solution for 30 minutes, as described previously (21). Changes in arteriolar diameter in response to the change from 0% O₂ superfusion to 21% O₂ superfusion were measured again during continued superfusion with PSS containing a maintenance concentration (10 μM) of DDMS.

Statistical analysis

Differences between individual groups was determined with a Student's t-test for comparison of two groups, or via ANOVA with a post hoc Newman-Keuls test for comparisons of more than two experimental groups. Statistical significance of the nonparametric variable of CYP450-4A isoform expression was determined using a Z test with a Yates correction, as described earlier (24). Statistical significance was taken as $P < 0.05$.

RESULTS

Effects of High Salt Diet and CYP450-4A Inhibition on Resting Tone and Cremasteric Arteriolar Responses to Elevated PO₂

In these experiments, HS diet had no effect on resting diameter or maximum diameter measured during adenosine (1 mM) application in arterioles of SS rats (Figure 1A). Resting tone during 0% O₂ superfusion was also similar in rats fed LS vs. HS diet (Figure 1B). However, blockade

of CYP450-4A enzymes during 0% O₂ superfusion (control) caused a significantly larger increase in arteriolar diameter in SS rats fed HS diet (Figure 2), indicating that the CYP450-4A/20-HETE system plays a more prominent role in regulating resting tone in arterioles of SS rats during elevated dietary salt intake.

In SS rats fed HS diet, arteriolar constriction in response to elevation of superfusion solution PO₂ was significantly larger than that in SS rats fed LS diet (Figure 3). Blocking 20-HETE formation with DDMS inhibited arteriolar constriction in response to elevated PO₂ in both groups of rats, and eliminated the difference in O₂-induced constriction of arterioles in the two groups.

Effects of High Salt Diet and CYP450-4A Inhibition on Vasodilator Responses to Acetylcholine (ACh) and Sodium Nitroprusside (SNP)

Arteriolar responses to ACh and sodium nitroprusside in the presence and absence of DDMS are summarized in Figure 4 and Figure 5, respectively. In these experiments, arteriolar responses to ACh and SNP were not significantly different in SS rats fed LS vs. HS diet for 3 days; and inhibition of 20-HETE formation with DDMS had no effect on arteriolar responses to ACh or SNP in either group.

Effects of High Salt Diet on CYP450-4A mRNA Expression

Figure 6 shows representative gels from experiments evaluating mRNA for CYP450-4A isoforms in cremasteric arterioles of SS rats fed LS or HS diet, with liver as a positive control; and Table 1 summarizes the overall results of the experiments. In positive control samples from the liver, mRNA for all isoforms of CYP450-4A ω -hydroxylase was detected in every sample, regardless of dietary salt intake (Table 1). Consistent with previous studies of arterioles and resistance arteries in normotensive Sprague-Dawley rats (37;38), mRNA for the CYP450-4A3 isoform was occasionally detected in cremasteric arterioles taken from SS rats fed LS diet, and the expression of mRNAs for the CYP450-4A3 and CYP450-4A8 isoforms was significantly up-regulated in arterioles from SS rats fed HS diet.

DISCUSSION

There are four main findings in the present study. First, short term (3 days) exposure to a HS diet potentiated arteriolar constriction in response to elevated PO₂ in the cremaster muscle of Dahl-salt sensitive (SS) rats and increased the expression of mRNA for the CYP450-4A3 and CYP450-4A8 isoforms of cytochrome P450-4A ω -hydroxylase in cremasteric arterioles of Dahl-salt sensitive (SS) rats. These enzymes could play a role in oxygen sensing in the microcirculation (18;21), as cytochrome P450-4A has been shown to produce the vasoconstrictor metabolite 20-HETE in an O₂-dependent manner over the range of PO₂'s likely be encountered *in vivo* (18). Second, blocking 20-HETE production by CYP450-4A enzymes inhibits O₂-induced constriction of cremasteric arterioles in SS rats, and abolishes the salt-induced potentiation of arteriolar constriction in response to elevated PO₂ in the cremaster muscle of SS rats fed HS diet. Third, inhibition of CYP450-4A with DDMS caused a greater increase in the resting diameter of arterioles in salt-fed SS rats compared to LS controls, despite similar resting and maximum vessel diameters in the two groups. Finally, inhibiting 20-HETE formation by CYP450-4A enzymes did not enhance vasodilator responses to ACh and SNP in arterioles of SS rats fed either LS or HS diet.

Expression of mRNA for CYP4504A Isoforms

Cytochrome P450 metabolites have been implicated in vascular oxygen sensing (18;21), myogenic responses in resistance arteries (13;14;16), autoregulation of blood flow (16;44), and in the development of vascular changes and elevated blood pressure in hypertension (39;

41). In the present study, expression of mRNA for the -4A3 and -4A8 isoforms of CYP450-4A was significantly up-regulated in arterioles from SS rats fed short term (3 days) HS diet. The upregulation of mRNAs for the 4A3 and 4A8 isoforms of CYP450-4A in SS rats fed HS diet are consistent with the results of earlier studies (38), where we found that high salt diet causes a significant upregulation of mRNA for the cytochrome CYP450-4A2 and CYP450-4A8 isoforms in mesenteric resistance arteries of Sprague-Dawley rats.

The changes in mRNA expression for cytochrome P450-4A enzymes in SS rats, the enhanced contribution of CYP450-4A enzymes to resting tone, and the contribution of cytochrome P450 metabolites to the enhanced response of arterioles to elevated PO_2 in these animals occur after only 3 days on high salt diet and are therefore present much earlier than the enhanced response to elevated PO_2 reported by Rafi and Boegehold (33), and prior to the major component of the salt-induced increase in arterial blood pressure that occurs in SS rats. This raises the possibility that changes in CYP450-4A enzyme expression and alterations in vascular responses to changes in PO_2 may contribute to the early development of the elevated vascular resistance and vascular dysfunction that exist in SS rats fed HS diet.

Vasoconstrictor Responses to Elevated PO_2

In the present study, arterioles of SS rats fed short term HS diet exhibited an enhanced constriction in response to elevated PO_2 , as previously reported by Rafi and Boegehold for SS rats with established (4 weeks) salt-sensitive hypertension (33) and by Frisbee et al. (11) in short-term (3 days) reduced renal mass (RRM) hypertensive rats. Inhibition of CYP450-4A enzymes with DDMS inhibited O_2 -induced constriction of arterioles in SS rats fed either a LS or HS diet, and eliminated the salt-induced potentiation of arteriolar constriction in response to elevated PO_2 in arterioles of SS rats fed HS diet. These findings are consistent with earlier reports indicating that 20-HETE plays an important role in mediating arteriolar constriction in response to elevated PO_2 in the skeletal muscle microcirculation (18;21); and with studies suggesting that elevated production of 20-HETE and/or increased sensitivity to the vasoconstrictor effects of 20-HETE contribute to the enhanced constriction of arterioles in response to elevated PO_2 in the skeletal muscle microcirculation of spontaneously hypertensive rats with established hypertension (22) and in rats with experimentally-induced reduced renal mass hypertension (11). By extension, these observations suggest that increases in CYP450-4A expression and/or 20-HETE production also may contribute to the salt-induced potentiation of arteriolar constriction in response to elevated PO_2 in the skeletal muscle microcirculation of Dahl SS rats [Figure 3 and Reference (33)].

The observation that HS diet potentiates arteriolar O_2 responses in SS rats fed HS diet differs from the report of Marvar et al. (31) in normotensive Sprague-Dawley rats, who showed that exposure to HS diet (4% NaCl) for 4–5 weeks actually reduced arteriolar constriction in response to elevated PO_2 in the spinotrapezius muscle. The ability of CYP450-4A inhibition to block the potentiated response to elevated PO_2 in SS rats fed HS diet indicates that upregulation of CYP450-4A enzyme expression, increased 20-HETE production, and/or an increased sensitivity of skeletal muscle arterioles to elevated PO_2 is present in SS rats fed HS diet (33), but not in normotensive Sprague-Dawley rats fed a HS diet (31).

The lack of a difference of arterial blood pressure in the present study presumably reflects the effects of anesthesia on the SS rats, as SS rats exhibit enhanced sensitivity to barbiturate anesthesia compared to other strains (35). In the present study, mean arterial pressures were not significantly different in SS rats fed low-salt versus high salt. However, studies employing chronic monitoring of blood pressure in conscious animals have shown that arterial pressure in SS rats increases significantly within three days when the animals are fed high salt diet (23), possibly due to their inability to reduce vascular resistance in response to volume expansion (17). Therefore, the measurements obtained in the present study could reflect either

pre-hypertensive alterations or changes in the early stages of salt-induced hypertension in SS rats.

Further investigation is needed to determine the mechanisms for the differences in the effect of high salt diet on the CYP450-4A component of O₂-induced constriction of arterioles in SS rats vs. normotensive Sprague-Dawley rats (31). Possible explanations include differences in the genetic regulation of some component of vasoconstrictor sensitivity rather than (or in addition to) differences in CYP450-4A enzyme expression and 20-HETE production, or vascular alterations associated with hypertension itself, which could contribute to an enhanced sensitivity of arterioles to 20-HETE, as previously reported in SHR rats with established (12–16 week old) but not early (4–6 week old) hypertension (22).

Finally, the greater increase in diameter during CYP450-4A inhibition in arterioles of rats fed HS diet in the face of identical resting and maximum diameters implies that CYP450-4A metabolites assume a greater role in regulating resting tone in SS rats that are fed HS diet compared to LS controls. The latter conclusion is consistent with the upregulation of mRNA for CYP450-4A3 and CYP450-4A8 isoforms in cremasteric arterioles of SS rats fed HS diet (Table 1).

Effect of HS Diet and DDMS on Vasodilator Responses to Acetylcholine (ACh) and Sodium Nitroprusside (SNP)

Previous studies in the literature (11;20), raise the possibility that the CYP450-4A system could play a role in the impaired responses of arterioles to vasodilator agonists. For example, Kerkhof et al. (20) reported that inhibition of CYP450-4A enzymes with 17-octadecynoic acid (17-ODYA) potentiated hypoxic dilation in isolated first order arterioles of the cremaster muscle, and that this response was mediated via NO, rather than the expected cyclooxygenase metabolites of arachidonic acid (9;10;29;32). Other studies (11) have shown that inhibition of CYP450-4A enzymes eliminates the impaired dilation of cremasteric arterioles to ACh in salt-fed RRM hypertensive rats, but had minimal effect on arteriolar dilation in response to ACh in normotensive rats fed either LS or HS diet.

In the present study, the absence of an effect of HS diet on arteriolar responses to ACh and SNP and the failure of CYP450-4A inhibition to potentiate vasodilator responses to ACh and SNP in the arterioles of SS rats fed either LS or, especially, HS diet was somewhat surprising. However previous studies have shown that vasodilator responses to ACh and reduced PO₂ are dramatically impaired not only during salt-induced hypertension in Dahl SS rats, but also in normotensive SS rats fed low salt diet (5–7). The impaired relaxation in response to ACh and reduced PO₂ are restored by substitution of the BN renin gene (SS.13^{BN} consomic rats) (5–7) or the functioning renin allele for the Dahl R rat (RGRR congenic rats) (4), suggesting that vascular relaxation mechanisms are impaired in normotensive SS rats fed LS diet as a result of chronic exposure to low levels of circulating ANG II.

Extensive evidence is accumulating that chronic exposure to low levels of ANG II contributes to impaired vasodilator responses in SS rats fed LS diet (5–7), and in normotensive Sprague-Dawley rats (30;42;43) or SS.13^{BN} consomic rats (6) fed high salt diet to suppress plasma ANG II levels. For example, in SS rats fed LS diet, vasodilator responses to ACh and reduced PO₂ can be restored by chronic i.v. infusion of a low dose of ANG II (6) or by introgression of the Brown Norway (BN) (3;5–7) or the Dahl salt-resistant (R) renin allele (4) into the SS genetic background, which restores normal regulation of plasma renin activity (PRA) and plasma ANG II levels (1;2).

In the present study, the most likely explanation of the lack of a difference in vasodilator responses to ACh and SNP in SS rats fed HS vs. LS diet and for the lack of an effect of DDMS

on vasodilator responses to these agonists is that the impaired vasodilator responses to ACh and SNP in SS rats is due to chronic exposure to low ANG II levels as a result of impaired regulation of the renin gene in the SS rat, but that alleles on other chromosomes are responsible for salt-induced potentiation of arteriolar responses to elevated PO₂. The latter interpretation is consistent with the earlier findings that arteriolar dilation in response to ACh is restored in the cremaster muscle of SS.13^{BN} consomic rats (3) and RGRR congenic rats (carrying the Dahl R renin allele) (4), while substitution of a functioning renin allele from either the BN rat or the Dahl R rat fails to eliminate the salt-induced potentiation of arteriolar constriction in response to elevated PO₂ that is present in cremasteric microcirculation of SS rats, SS.13^{BN} consomic rats, and RGRR congenic rats (3;4). Given the results of the present study, a likely candidate for the enhanced constriction of arterioles in response to elevated PO₂ in SS rats fed HS diet would be salt-induced alterations in CYP450-4A enzyme expression or 20-HETE production in the SS rats, as the genes for CYP450-4A enzyme isoforms are found on rat chromosome 5, rather than chromosome 13.

Summary and Conclusions

In summary, we found that vascular responses to elevated PO₂ are potentiated by short term exposure of SS rats to a HS diet, and that inhibiting CYP450-4A enzymes with DDMS eliminates the enhanced constriction to elevated PO₂ in vessels from SS rats fed HS diet. Inhibiting CYP450-4A enzymes with DDMS also led to a greater increase in resting diameter in SS rats fed short-term HS diet, despite nearly identical resting diameters, resting tone, and maximum diameters in arterioles of SS rats fed LS vs. HS diet. The latter observations suggest that CYP450-4A metabolites play an important role in the early alterations in vascular responses to elevated PO₂ and resting tone in Dahl salt-sensitive rats fed high salt diet. These alterations in vascular O₂ responses and in the modulation of vascular tone by CYP450-4A metabolites could play an important role in the early elevation of vascular resistance and in the inability of the vasculature to compensate for the volume expansion that occurs in response to high salt diet in this genetic model of salt-sensitive hypertension (17).

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A.

B.

Figure 1.

A. Resting diameter (left) and maximum diameter during 1 mM adenosine application (right) in cremasteric arterioles of SS rats fed low salt (open bars; n=23) vs. high salt (solid bars; n=23) diet. **B.** Resting tone in cremasteric arterioles of SS rats fed low salt (open bar; n=23) vs. high salt (solid bar; n=23) diet. Data are summarized as mean $\pm$ SEM.

Figure 2. Change in resting diameter ($\Delta \mu\text{m}$) from untreated control value in response to inhibition of CYP450- ω hydroxylase with DDMS during 0% O_2 control superfusion in SS rats fed low salt (open bar; $n=12$) vs. high salt (solid bar; $n=15$) diet. Data are summarized as mean $\pm$ SEM; *-- Significantly greater than response in SS rats fed LS diet.

Figure 3.

Effect of inhibiting CYP450-4A enzymes with DDMS on the constriction of *in situ* cremasteric arterioles in response to elevation of superfusion solution PO_2 in SS rats fed low salt (LS) (left; n=9) or high salt (HS) (right; n=7) diet. Data are summarized as mean $\pm$ SEM; *--Significantly greater than response in rats fed LS diet. †--Significantly less than control response in the absence of DDMS.

A.

B.

C.

Figure 4.

Response of cremasteric arterioles to acetylcholine (ACh) in the presence and absence of CYP450-4A inhibition with DDMS in SS rats fed LS (n=10–14) or HS diet (n=8–15). Data are summarized as mean ± SEM. There were no significant differences between the responses to ACh in any of the groups.

Figure 5.

Response of cremasteric arterioles to sodium nitroprusside (SNP) in the presence and absence of CYP450-4A inhibition with DDMS in SS rats fed LS (n=8–15) or HS diet (n=4–13). Data are summarized as mean $\pm$ SEM. There were no significant differences between the responses to SNP in any of the groups.

Figure 6. Representative gels evaluating mRNA for CYP450-4A isoforms in cremasteric arterioles of SS rats fed low salt (**left**) or high salt diet (**right**), with liver as a positive control. M-Molecular weight marker. Lanes for individual isoforms are labeled at the top of the gel.

Table 1

Expression of cytochrome P450-4A ω -hydroxylase isoforms in cremasteric arterioles of SS rats fed low salt (LS) and short term (3 days) high salt (HS) diet.

Dahl-LS	4A1	4A2	4A3	4A8
Liver	7/7 [*]	7/7 [*]	7/7 [*]	7/7 [*]
Cremasteric Arterioles	0/6	0/6	1/6	0/6
Dahl-HS	4A1	4A2	4A3	4A8
Liver	7/7 [*]	7/7 [*]	7/7 [*]	7/7 [*]
Cremasteric Arterioles	1/6	0/6	4/6 [*]	4/6 [*]

* Significant expression of the isoform (P <0.05).