Clofibrate Prevents the Development of Hypertension in Dahl Salt-Sensitive Rats

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We have reported that cytochrome P-450-dependent ω-hydroxylation of arachidonic acid is reduced in microsomes prepared from the renal outer medulla of Dahl salt-sensitive (SS/Jr) rats, but the functional significance of this observation is unknown. The present study examined whether long-term induction of renal fatty acid ω-hydroxylase with clofibrate would alter the development of hypertension in Dahl SS/Jr rats. Dahl SS/Jr rats were placed on a high salt diet (8.0% NaCl) and given either vehicle or clofibrate (80 mg/day) in their drinking water. After 4 weeks of a high salt diet, mean arterial pressure averaged 170±3 mm Hg in vehicle-treated (n=17) and 127±2 mm Hg in clofibrate-treated (n=19) SS/Jr rats. Clofibrate had no effect on arterial pressure in Dahl salt-resistant rats. The antihypertensive effect of clofibrate was reversible. Mean arterial pressure rose from 131±4 to 182±8 mm Hg in the first week after clofibrate treatment (n=6) was discontinued. Clofibrate had no effect on arterial pressure in SS/Jr rats (n=9) in which hypertension was already established by feeding the rats a high salt diet for 4 weeks before the study. In clofibrate-treated SS/Jr rats (n=12), the ω-hydroxylation of arachidonic and lauric acids by renal cortical and outer medullary microsomes was greater than that seen in vehicle-treated rats (n=9). These observations are consistent with the hypothesis that a deficiency in the ω-hydroxylation of fatty acids by P-450 in the outer medulla of the kidney may contribute to the resetting kidney function and the development of hypertension in Dahl SS/Jr rats. (Hypertension 1993;21:985-988)

KEY WORDS • cytochrome P-450 • eicosanoids • hypertension, renovascular • rats, inbred strains • hydroxyeicosatetraenoic acids • antilipemic agents

Renal transplantation studies indicate that an abnormality in kidney function underlies the development of hypertension in Dahl salt-sensitive (SS/Jr) rats, but the factors responsible for altering kidney function and the genetic basis of the disease have not been identified. Recently, we reported that the pressure-natriuretic relation is altered in SS/Jr rats such that they require an elevated arterial pressure to excrete sodium when challenged with a high salt intake. This abnormality has subsequently been shown to be a consequence of an elevated chloride reabsorption in the thick ascending loop of Henle and is associated with a reduction in the P-450-dependent metabolism of arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE) in renal outer medullary microsomes of Dahl SS/Jr rats. Because 20-HETE has been reported to be a locally formed inhibitor of chloride transport in the thick ascending limb, a deficiency in the production of this substance could contribute to the resetting renal function and the development of hypertension in Dahl SS/Jr rats. To test this hypothesis, we examined the effect of inducing renal P-450 fatty acid ω-hydroxylation activity with clofibrate on the development of hypertension in Dahl SS/Jr rats.

Methods

Experiments were performed on 36 inbred female Dahl SS/Jr and 13 salt-resistant (SR/Jr) rats purchased from Harlan Industries, Madison, Wis. The rats were housed in an animal care facility at the Medical College of Wisconsin approved by the American Association for Accreditation of Laboratory Animal Care and had free access to food and water throughout the study. All protocols involving animals were approved by the Animal Care Committee of the Medical College of Wisconsin. The rats were maintained on a low salt diet (0.3% NaCl) until they were 9 weeks old, then were switched to a high salt diet (8.0% NaCl), and vehicle (20 mM Na₂CO₃) or clofibrate (80 mg/day) was added to the drinking water. After 3 weeks on the high salt diet, the rats were anesthetized with ketamine (50 mg/kg) and xylazine (2 mg/kg), and a catheter was implanted in the femoral artery for measurement of arterial pressure. The catheter was exteriorized at the back of the neck and brought out through a stainless-steel spring and swivel device. After a 4-day recovery period, arterial pressure was directly measured with a pressure transducer and a computerized recording system for 3 hours per day on four consecutive days while the animal was conscious with free movement in its home cage. The signals were sampled at 30 Hz, and heart rate and systolic, diastolic, and mean arterial pressures were determined at 1-minute intervals and reduced to a mean value for the entire recording period. At the end of the experiment, the rats were anesthetized with pentobarbital (30 mg/kg i.p.), and a final blood sample
(1 mL) was collected for measurement of plasma lipids (cholesterol and triglycerides). In addition, the liver and kidneys were collected, rapidly frozen in liquid nitrogen, and stored at −80°C until assayed for the metabolism of arachidonic acid by P-450. Plasma cholesterol concentration was measured with a spectrophotometric enzymatic assay purchased from DMA Inc., Arlington, Tex. (catalog No. 2340). Plasma triglyceride levels were measured with a commercially available spectrophotometric assay based on the glycerol phosphate oxidase reaction (DMA catalog No. 2750).

**Measurement of Renal P-450 Fatty Acid ω-Hydroxylase Activity**

The cortex and the outer medulla of the kidney as well as the liver were homogenized in a 10 mM potassium phosphate buffer (pH 7.7) containing 250 mM sucrose, 1 mM EDTA, and 10 mM magnesium chloride, and microsomes were prepared by differential centrifugation. Microsomes (0.25 mg) were incubated for 30 minutes at 37°C in either 14C-labeled arachidonic or lauric acids (0.5 μCi/mL, 10 μM) in 0.5 mL of a 100 mM potassium phosphate buffer (pH 7.4) containing 5 mM MgCl2, 1 mM EDTA, 1 mM NADPH, and an NADPH-regenerating system consisting of 20 mM isocitrate and isocitrate dehydrogenase (0.4 units/mL). The reaction was terminated by acidification to pH 4 with formic acid and was extracted with ethyl acetate. The metabolites were separated using a 2 mm×25 cm C18 reversed-phase analytical high performance liquid chromatographic column and a linear elution gradient ranging from acetonitrile/water/acetic acid (50:50:0.1) to acetonitrile/acetate acid (100:1) over 40 minutes. The radioactive metabolites were monitored with a radioactive flow detector (model 120, Radiomatic Instrument Co., Tampa, Fla.) as we have previously described.10,11

**Statistics**

Data are presented as mean±1 SEM. Significance of differences in mean values within and between groups was determined with an analysis of variance for repeated measures with multiple independent factors followed by a Duncan's multiple-range test. A value of p<0.05 using a two-tailed test was considered significant.

**Results**

The effect of clofibrate on arterial pressures in Dahl SS/Jr and SR/Jr rats fed a high salt diet is presented in Figure 1. Mean arterial pressures averaged 171±4 mm Hg in Dahl salt-sensitive rats given vehicle and only 125±3 mm Hg in clofibrate-treated SS/Jr rats. Clofibrate had no effect on arterial pressure in SR/Jr rats (Figure 1). Systolic (141±3 versus 189±3 mm Hg) and diastolic (111±2 versus 152±3 mm Hg) pressures and heart rate (374±14 versus 426±6 beats per minute) were all significantly lower in rats given clofibrate than in vehicle-treated SS/Jr rats. Body weight (228±4 versus 220±3 g), hematocrit (0.41±0.01 versus 0.39±0.03), and plasma sodium and potassium concentrations were similar in vehicle- and clofibrate-treated SS/Jr rats. Plasma cholesterol levels were significantly reduced in SS/Jr rats given clofibrate (1.84±0.18 versus 2.59±0.21 mM), but plasma triglyceride levels were not significantly different and averaged 96±6 and 102±5 mg/dL in the clofibrate- and vehicle-treated rats, respectively.

The antihypertensive effect of clofibrate was reversible. Mean arterial pressure rose from 131±4 to 182±8 mm Hg during the first week after clofibrate treatment was discontinued (Figure 2). Moreover, clofibrate had no effect on arterial pressure in SS/Jr rats with established hypertension that was induced by feeding the rats a high salt diet for 4 weeks (Figure 2).

The effects of clofibrate on renal and hepatic P-450 fatty acid ω-hydroxylase activity are summarized in Figure 3. Clofibrate significantly increased the ω-hy-
Fatty acid \(\omega\)-hydroxylase activity in liver and kidney of Dahl salt-sensitive rats on a high salt (8.0% NaCl) diet. Renal and liver microsomes were incubated with \(14C\)-labeled arachidonic acid or lauric acid (0.5 \(\mu\)Ci, 10 \(\mu\)M) and the production of 20-hydroxyeicosatetraenoic acid (20-HETE) (top panel) or 12-hydroxylaurate (bottom panel) was determined. *Significant difference from corresponding value in vehicle-treated rats. N=number of rats in each group.

**FIGURE 3.** Bar graphs show effect of clofibrate on P-450 fatty acid \(\omega\)-hydroxylase activity in liver and kidney of Dahl salt-sensitive rats on a high salt (8.0% NaCl) diet. Renal and liver microsomes were incubated with \(14C\)-labeled arachidonic acid or lauric acid (0.5 \(\mu\)Ci, 10 \(\mu\)M) and the production of 20-hydroxyeicosatetraenoic acid (20-HETE) (top panel) or 12-hydroxylaurate (bottom panel) was determined. *Significant difference from corresponding value in vehicle-treated rats. N=number of rats in each group.

**Discussion**

The present study examined the effects of clofibrate, an inducer of renal P-450 fatty acid \(\omega\)-hydroxylase activity, on the development of hypertension in Dahl SS/Jr rats. Long-term treatment of Dahl SS/Jr rats with clofibrate prevented the development of hypertension when they were placed on a high salt diet. This effect was not due to a direct antihypertensive action of the drug or a nonspecific toxic effect, because clofibrate had no effect on arterial pressure in SS/Jr rats in which hypertension was already established by feeding the rats a high salt diet for 4 weeks before the study. In addition, clofibrate had no effect on arterial pressure in Dahl SR/Jr rats fed a high salt diet.

In the present study, the \(\omega\)-hydroxylation of arachidonic and lauric acids by P-450 was significantly induced in microsomes prepared from the renal cortex and outer medulla of clofibrate-treated SS/Jr rats. Clofibrate also increased the \(\omega\)-hydroxylation of arachidonic acid in hepatic microsomes. Unlike phenobarbital and aromatic hydrocarbons, which produce a generalized induction of P-450 enzymes, clofibrate is thought to be a specific inducer of the P-4504A gene family, which regulates the \(\omega\)-hydroxylation of fatty acids in the liver and the kidney.13 The present findings that the formation of epoxides by renal cortical microsomes incubated with arachidonic acid was not altered in clofibrate-treated rats support this view. Overall, these results are consistent with the hypothesis that an abnormality in the control of loop chloride reabsorption by P-450 metabolites of arachidonic acid may play a role in the development of hypertension in this model.13 However, additional experiments are needed to establish whether the antihypertensive action of clofibrate in SS/Jr rats is due to the induction of renal P-450 fatty acid \(\omega\)-hydroxylase activity and the renal actions of the metabolites formed or is due to some other action of clofibrate.

Although it has long been known that clofibrate induces the \(\omega\)-hydroxylation of fatty acids in the liver and kidney, the mechanism by which this occurs remains in question. Previous studies by Hardwick et al.14 established that this involves direct transcriptional activation of the P-4504A1 gene by clofibrate. On the other hand, some evidence indicates that the induction of P-450 \(\omega\)-hydroxylase activity may be secondary to the antilipidemic actions of clofibrate and the subsequent increase in the intracellular turnover of fatty acids.15 If this is true, other antilipidemic agents might also induce renal P-450 \(\omega\)-hydroxylase activity and lower arterial pressure in Dahl SS/Jr rats. In this regard, lovastatin has been reported to lower arterial pressure in Dahl SS/Jr rats.16,17 However, again it must be emphasized that the mechanism of the antihypertensive actions of antilipidemic agents remains unknown, and the link between these drugs, renal P-450 fatty acid \(\omega\)-hydroxylase activity, changes in renal function, and their antihypertensive effects has not been established.

Another concern is that it is difficult to relate the present findings, that induction of renal fatty acid \(\omega\)-hydroxylase with clofibrate lowers arterial pressure in Dahl SS/Jr rats, with previous results in spontaneously hypertensive rats (SHR). These studies indicating that renal P-450 activity is elevated18 and that the expression of the cytochrome P-4504A2 gene19 is enhanced in the kidneys of young SHR have led to the suggestion that an overproduction of 20-HETE in the kidney may play a role in the development of hypertension. This hypothesis is further supported by the observations that treatment of SHR with \(SnCl_2\)20,21 or heme arginate22 to reduce renal P-450 activity lowers arterial pressure. However, the relation between changes in renal P-450 activity, renal function, and the antihypertensive effects of these agents have not been established. In addition, it is difficult to understand how an elevated renal production of 20-HETE could contribute to the development of hypertension in SHR if it is a renal vasodilator substance23,24 that promotes sodium excretion.9,25 For
this reason, we have suggested that P-450 metabolites of arachidonic acid may contribute to the development of hypertension in SHR by altering tubular sodium reabsorption secondary to changes in renal medullary hemodynamics and renal interstitial pressure. However, the concept that 20-HETE is a renal vasocostrictor that promotes sodium retention is difficult to reconcile with the present observations that induction of the renal formation of this substance impaired the development of hypertension in Dahl SS/Jr rats. Clearly, more work is needed to sort out the role of P-450 metabolites of arachidonic acid in the control of renal tubular and vascular function and the mechanisms by which these compounds may contribute to the development of hypertension in different genetic models.

In summary, long-term treatment with clofibrate induced the \( \omega \)-hydroxylation of fatty acids by P-450 in the kidney and prevented the development of hypertension in Dahl SS/Jr rats. These observations are consistent with the hypothesis that an abnormality in the control of loop chloride reabsorption by P-450 metabolites of arachidonic acid may contribute to the resetting renal function and the development of hypertension in this model. However, the mechanism of the antihypertensive action of clofibrate in Dahl SS/Jr rats and the role of P-450 eicosanoids in the long-term control of renal function and arterial pressure need to be further clarified.

References

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