Research Article

Effects of Clofibrate on Salt Loading-Induced Hypertension in Rats

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1. Introduction

Fibrates are synthetic agonists of peroxisome proliferator-activated receptor-α (PPARα), a subfamily of the nuclear receptor superfamily naturally activated by ligands such as free fatty acids and eicosanoids [1]. PPARα is expressed in the liver and in tissues with highly active fatty acid metabolism, such as the heart, kidney, endothelium, and vascular smooth muscle, all primarily related to blood pressure (BP) control. Fibrates have in clinical use as hypolipidemic agents for several decades. More recently, they have been reported to have beneficial effects on cardiovascular function [1–3] and elevated BP [4, 5]. Fibrates also exert antithyroid effects. PPAR agonists interact with thyroid receptors (TRs) in part by sharing binding sites and heterodimeric partners such as RXRs [6–8]. PPARs and TRs also share coactivators. In addition, the activation of response elements by TRs and PPARs is modulated by PPAR agonists [8]. The molecular mechanisms of the negative interaction between thyroid hormone and PPARs include an increase in thyroid hormone deactivation [9] and reductions in the gene expression of their transporters [10–12] and in the activity of deiodinases [10] and action [6, 8, 10–12]. Thus, clofibrate treatment markedly reduced plasma thyroid hormone levels and increased tissue activity of phenol-UGT, an enzyme that deactivates thyroid hormones in chronically treated hyperthyroid rats [13].

The antihypertensive effects of clofibrate-induced PPARα activation may include the following: higher production of endothelial [14] and renal [15] nitric oxide, which plays an important homeostatic role in the response to an increased saline intake [16]; lower production of reactive oxygen species and reduced NAD(P)H oxidase activity [3], which are increased in saline drinking rats [17]; lower production of ET-1 [3] also elevated in saline models of experimental hypertension [3, 18]. Moreover, the mechanism responsible for the protective effects of clofibrate on the development of hypertension may also be linked to its antithyroid actions,
since antithyroid drugs have prevented BP elevation in all experimental rat models of hypertension studied to date [19]. In this regard, we recently reported [13] that chronic clofibrate treatment prevented and reversed the characteristic hemodynamic manifestations, increased temperature of hyperthyroidism in rats, and reduced their plasma thyroid hormone levels.

All of the above data indicate that PPARα activation can have important protective effects on cardiovascular function and interfere with the prohypertensive effects of increased saline intake, but the precise mechanisms involved have not been elucidated. In this study, we analyzed the putative role of several factors, focusing the investigation on the antithyroid effects of clofibrate.

2. Methods

2.1. Animals. Thirty-two male Wistar rats born and raised in the experimental animal service of the University of Granada were used. The experiment was performed according to European Union guidelines for the ethical care of animals. Rats initially weighing 280 ± 4 g were randomly assigned to the different groups. Each experimental group comprised eight animals. All rats had free access to food and tap water. Clofibrate (240 mg/kg/day) was given by gavage because of the low solubility of this compound. The dose of clofibrate was in accordance with previously published protocols used in experimental hypertension in rats [3, 13].

2.2. Experimental Protocol. The groups were: control, clofibrate-treated, salt-loaded (2% NaCl via drinking water), and clofibrate plus salt-treated rats. Treatments were administered for five weeks. Body weight (BW), tail systolic BP (SBP), and heart rate (HR) were measured once a week. Tail SBP and HR were measured with the use of tail-cuff plethysmography in unanesthetized rats (LE 5001-Pressure Meter, Letica SA, Barcelona, Spain).

When the experiment was completed, all rats were then housed in metabolic cages (Panlab, Barcelona, Spain) with free access to food and water for a four-day period (two days for adaptation + two experimental days), during which food and water intakes were measured, and urine samples were collected. Twenty-four-hour urine volume, proteinuria, creatinine, isoprostanes, nitrate-nitrites, endothelin (ET), vasopressin (VP), and total sodium, potassium, and calcium excretion were measured. Mean values of all intake and urinary variables obtained during the two experimental days were used for statistical analyses among groups.

After completion of the metabolic study, the rats were anesthetized with ethyl ether. A polyethylene catheter (PE-50) containing 100 units of heparin in isotonic sterile NaCl solution was inserted into the femoral artery to measure intra-arterial BP and HR and pulse pressure (PP) in conscious rats and to extract blood samples. Intra-arterial BP was measured at 24 h after implantation of femoral catheter. Direct BP and HR were recorded continuously for 60 min with a sampling frequency of 400/s (McLab, AD Instruments, Hastings, UK); BP and HR values obtained during the last 30 min were averaged for intergroup comparisons. Subsequently, blood samples taken with the femoral catheter were used to determine plasma variables. The plasma variables measured were: urea, creatinine, total proteins, electrolytes (sodium and potassium), thyroid hormones (FT3 and FT4), and thyroid stimulating hormone (TSH).

Finally, the rats were killed by exsanguination, and the thyroid, liver, kidneys, and ventricles were removed and weighed. The heart was divided into right ventricle and left ventricle plus septum and the kidney was dissected to separate cortex and medulla. Tissue FT3 and FT4 levels were measured in liver and renal cortex and medulla.

2.3. Analytical Procedures. Proteinuria was measured by the method of Bradford [20]. Plasma and urinary electrolytes and creatinine were measured in an autoanalyzer (Hitachi-912, Roche, Spain). Plasma and tissue levels of thyroid hormones (free circulating T3 and T4) were determined using rat radioimmunoassay kits according to the manufacturer’s instructions (Diagnostic Products Corporation, Los Angeles, CA, USA). An enzyme immunoassay kit (8-isoprostane Ela Kit, Cayman Ann Arbor, MI, USA) was used to measure urinary 8-isoprostane levels, and samples were previously purified using the Affinity purification kit (Cayman). Immunoreactive urinary ET and VP levels were measured with a radioimmunoassay kit purchased from Assay designs, (Ann Arbor, MI, USA). Urine NO2 and NO3 (NOx) concentrations were measured using nitrate reductase and Griess reaction [21]. Rat plasma TSH was measured by a solid phase competitive chemiluminescent enzyme immunoassay using the IMMULITE 2000 Analyzer (EURO/DPC, Llanberis, Gwynedd, UK).

2.4. Preparation of the Tissue Homogenates. For thyroid hormone measurements, the liver and the dissected renal cortex and medulla were homogenized with a glass homogenizer in ice-cold HEPES buffer containing (mmol/L) sodium HEPES 25, EDTA 1, phenylmethylsulfonyl fluoride 0.1, and PBS. After centrifugation of the homogenate at 6000 g for 5 min at 4 °C, the supernatant containing membrane and cytosolic components, termed homogenate, was separated into aliquots, frozen in liquid N2, and stored at −80 °C until use.

2.5. Statistical Analysis. One-way ANOVA was used to compare each variable at the end of the experiment. When the overall ANOVA was significant, pairwise comparisons were performed using Bonferroni’s methods. P < .05 was considered significant.

3. Results

3.1. Blood Pressure and Heart Rate. BP and HR values are summarized in Figure 1. The left-hand graph in Figure 1 shows the final SBP and the right-hand graph shows the final HR measured by direct recording in the experimental groups. Saline loading produced an increase in SBP and
HR and PP in comparison to control rats. Clofibrate administration to normal rats at the dose and time used in this experiment produced a modest but significant decrease in BP (4.8 ± 0.6 mmHg) and HR (16 ± 5.7 beats/min). Clofibrate administration to saline-loaded rats reduced SBP, HR (14 ± 0.7 mmHg and 50 ± 5.7 beats/min, respectively, \( *P < .01 \) versus saline-drinking rats for both variables), and PP values. Hence, the salt + clofibrate group showed similar final SBP, HR, and PP values to those of control rats. PP values in the groups were: control, 36.2 ± 1.2; clofibrate, 31.2 ± 2.2; salt, 45.3 ± 1.2\(^*\); salt+clofibrate, 37.3 ± 1.5\(^+\) (\( *P < .01 \) versus controls; \( +P < .01 \) versus saline group).

3.2. Morphological Variables. Body weight at the end of the five-week study period was significantly lower in the salt and clofibrate + salt groups than in controls. Absolute kidney weight was significantly increased and the absolute left ventricular weight was reduced in the salt + clofibrate group. Kidney-to-body weight ratio was significantly increased in the clofibrate and salt groups and was markedly increased in the clofibrate + salt group. Left ventricular-to-body weight and left ventricular-to-right ventricular ratios, both indexes of cardiac hypertrophy, were not significantly modified by the treatments. The thyroid weight-to-body weight ratio was not significantly modified in the groups. The liver-to-body weight ratio was significantly increased in the clofibrate group but showed only a nonsignificant increase in the salt + clofibrate group (Table 1).

3.3. Plasma Variables and Thyroid Hormone Levels. Plasma sodium and potassium levels were similar among the control, clofibrate, and salt groups, but plasma sodium was higher and potassium lower in the clofibrate + salt group. Plasma urea and creatinine were similar in all groups, and plasma protein, an index of plasma volume, was also similar in all groups (Table 2).

FT\(_3\) values were significantly decreased in the clofibrate + salt group but did not differ among the other groups. However, FT\(_4\) levels were significantly reduced in the clofibrate and salt groups, especially in the salt + clofibrate group. TSH values were increased and decreased in the clofibrate and salt groups, respectively, and were not significantly modified in the salt + clofibrate group (Table 2).

3.3.1. Metabolic and Urinary Variables. Metabolic studies at the end of treatment showed increased food and fluid intake (g/100 g body weight) in the salt-treated group in comparison with controls. Clofibrate treatment did not affect food and fluid intake in normal rats but reduced the food intake in saline-loaded rats (Table 3). Water and sodium balances were increased in the salt group. Clofibrate reduced water and sodium balances in saline-loaded rats (Table 3).

Table 4 lists the results for the urinary variables. The salt group showed increased diuresis, natriuresis, kaliuresis, and calcium excretion. Clofibrate did not significantly modify these variables in control rats but increased the diuresis, natriuresis, and kaliuresis and reduced the calcium excretion in saline-loaded rats. Total creatinine excretion and creatinine clearance were similar in all groups.

Proteinuria was increased in the salt group, and clofibrate reduced this variable in control and saline-drinking rats. Total isoprostane and NO\(_x\) excretion were increased in the salt group, and clofibrate did not significantly modify these variables in control or salt-loaded rats. Total immunoreactive ET and VP excretion were increased in the salt group, and clofibrate did not modify these variables in control and salt-loaded rats.

3.4. Tissue Thyroid Hormone Levels. These results are summarized in Figure 2. Except for FT\(_3\) in the clofibrate group, FT\(_4\) and FT\(_3\) values were significantly reduced in the liver in all experimental groups with respect to controls, observing the greatest reduction in the salt + clofibrate group. In the renal cortex, a significant reduction in FT\(_4\) was only observed in the salt + clofibrate group, while FT\(_3\) was decreased in all groups, especially in the salt + clofibrate group. In the renal medulla, FT\(_4\) and FT\(_3\) were reduced in the salt-loaded group and more markedly reduced in the salt + clofibrate group. The salt + clofibrate and salt groups significantly differed in FT\(_4\) and FT\(_3\) values in all tissues.

4. Discussion

The main findings of this study were that chronic clofibrate administration to salt-loaded rats prevented the BP increase in these animals and that this effect may be mediated by the antithyroid action of fibrates. Clofibrate treatment markedly reduced plasma and tissue thyroid hormone levels in saline-treated rats. These findings are in agreement with previous observations by our group in hyperthyroid rats [13]. A fibrate-induced reduction in thyroid hormone levels may be protective against hypertension in saline-loaded rats, since antithyroid drugs are known to prevent the development of hypertension in rats [19]. We also found that clofibrate reduced the water and sodium balance in saline-loaded rats with respect to saline-drinking rats, which may also contribute to its antihypertensive effect. However, clofibrate did not significantly change nitrate/nitrite, isoprostane, ET, or VP levels, suggesting that these variables do not play a role in the prevention of saline load hypertension induced by this agent.

An increase in BP in response to dietary sodium (salt sensitivity) is considered an important factor in the pathogenesis of hypertension in humans [22]. In the present study, chronic 2% NaCl loading via drinking water produced a moderate BP increase (15 mmHg) in male Wistar rats, in agreement with several reports in Sprague-Dawley rats [3, 18–24]. However, other studies found no significant change in BP with salt loading in Sprague-Dawley rats [25, 26]. These discrepancies may reflect differences in the duration of saline loading or in the administration route (with food or fluid intake).

The antihypertensive effect of clofibrate in the saline-loaded rats is consistent with previous reports of the attenuation by fibrates of BP elevation in genetic models of hypertension [5] and in nitric oxide-deficient and DOCA salt-treated hypertensive mice and rats [3].

In this study, clofibrate produced a modest but significant BP reduction in untreated rats, which was also associated
Figure 1: Final systolic blood pressure (SBP) and heart rate (HR) measured by direct recording (femoral artery) in conscious rats at the end of the experimental period (five weeks). Data are means ± SEM. *P < .05; **P < .01 compared with controls; †P < .01 compared with the salt group.

Table 1: Morphologic variables in the experimental groups.

| Groups        | Control     | Clofibrate  | Salt        | Salt + clofibrate |
|---------------|-------------|-------------|-------------|-------------------|
| FBW, g        | 381 ± 9     | 368 ± 7     | 325 ± 5*    | +284 ± 4*         |
| KW, g         | 1.19 ± 0.03 | 1.22 ± 0.03 | 1.26 ± 0.06 | 1.33 ± 0.02*      |
| LVW, g        | 0.75 ± 0.03 | 0.72 ± 0.03 | 0.69 ± 0.02 | 0.60 ± 0.03*      |
| KW/BW, mg/g   | 2.93 ± 0.03 | 3.31 ± 0.09*| 3.92 ± 0.24*| **5.04 ± 0.22**   |
| LVW/RVW       | 3.71 ± 0.41 | 4.24 ± 0.52 | 4.66 ± 0.79 | 3.44 ± 0.55       |
| LiW/BW, mg/g  | 29.97 ± 0.75| 37.52 ± 1.01*| 30.86 ± 1.39| 33.34 ± 1.58      |
| TW/BW, mg/g   | 1.82 ± 0.07 | 2.05 ± 0.14 | 1.60 ± 0.09 | 1.69 ± 0.06       |

Data expressed as means ± s.e.m. FBW, final body weight; KW, kidney weight; LVW, left ventricular weight; KW/BW, kidney weight versus body weight ratio; LVW/BW, left ventricular weight versus body weight ratio; LVW/RVW, left ventricular weight versus right ventricular weight ratio; LiW/BW, liver weight versus body weight ratio; TW/BW, thyroid weight versus body weight ratio. *P < .05; **P < .01 versus the control group; +P < .05, ++P < .01 versus the salt group.

Table 2: Plasma variables in the experimental groups.

| Groups        | Control     | Clofibrate  | Salt        | Salt + clofibrate |
|---------------|-------------|-------------|-------------|-------------------|
| Na, mEq/L     | 143.00 ± 0.87| 141.43 ± 0.69| 144.83 ± 1.62| **155.00 ± 3.72** |
| K, mEq/L      | 4.23 ± 0.08 | 4.43 ± 0.10 | 4.12 ± 0.19 | **3.35 ± 0.18**   |
| Urea, mg/dL   | 44.29 ± 1.54| 49.54 ± 2.00| 54.05 ± 9.34| 47.92 ± 3.05      |
| Creatinine, mg/dL | 0.54 ± 0.02 | 0.51 ± 0.01 | 0.44 ± 0.04 | 0.53 ± 0.02       |
| Total Proteins, g/dL | 6.12 ± 0.08 | 5.81 ± 0.11 | 5.80 ± 0.24 | 5.81 ± 0.44       |
| FT₃, pg/mL    | 2.78 ± 0.08 | 2.73 ± 0.06 | 2.85 ± 0.17 | **2.06 ± 0.13**   |
| FT₄, ng/dL    | 3.23 ± 0.16 | 1.67 ± 0.11*| 2.22 ± 0.19*| **1.29 ± 0.21**   |
| TSH, mg/dL    | 3.20 ± 0.30 | 4.10 ± 0.12*| 2.1 ± 0.10* | 3.7 ± 0.4         |

Data expressed as means ± s.e.m. FT₃, free triiodothyronine; FT₄, free thyroxine; TSH thyroid-stimulating hormone. *P < .05; **P < .01 versus the control group; +P < .05, ++P < .01 versus the salt group.
Figure 2: Tissue levels of free T4 and free T3 (FT4 and FT3) in the experimental groups after five weeks of treatment. Data are means ± s.e.m.

(a) Liver
(b) Renal cortex
(c) Renal medulla

FT4 (pg/g) FT4 (pg/g) FT4 (pg/g)

FT3 (pg/g) FT3 (pg/g) FT3 (pg/g)

Control Salt Salt + clofibrate
Control Clofibrate Salt Salt + clofibrate
Control Salt Salt + clofibrate

Figure 2: Tissue levels of free T4 and free T3 (FT4 and FT3) in the experimental groups after five weeks of treatment. Data are means ± SEM. *P < .05 compared with controls. **P < .01 compared with control group; +P < .05, ++ P < .01 versus salt group.
with a reduction in plasma FT₄ levels and a significant decrease in tissue thyroid hormone levels. These data contrast with the normal BP and plasma thyroid hormone levels observed in normal rats treated with clofibrate at the same dose for three weeks [13] but are in agreement with previous reports that fibrates reduce thyroid hormone levels in several species [10, 27, 28]. The discrepancy with our previous reports that fibrates reduce thyroid hormone levels at the same dose for three weeks [13] but in agreement with the normal BP and plasma thyroid hormone levels observed in normal rats treated with clofibrate at the dose used in our study.

The saline-treated rats showed reduced plasma and tissue levels of thyroid hormones, consonant with the decrease in thyroid hormone levels reported in saline-loaded rats with low-renin hypertension [29, 30]. This reduced thyroid activity reported in low-renin hypertension is believed to be mediated by the action of an unidentified substance referred to as the “thyroid-depressing factor” by Threatte et al. [31]. This substance reduces the uptake and binding of ¹³¹I by the thyroid gland and blocks the ¹³¹I uptake stimulated by TSH [32], and it was found to be increased in the blood of hypertensive versus normotensive rats [32]. More recently, several authors reported that low plasma thyroid hormone levels are the expression of an inflammatory state in patients with chronic renal disease [33] and that plasma and liver thyroid hormone levels are reduced in uremic rats with reduced renal mass [34]. This thyroid abnormality is referred to as “the euthyroid sick syndrome”, which can also be produced by elevated saline intake, as indicated by the present data.

Nitric oxide (NO) plays an important role in renal function and sodium excretion and regulates the homeostatic response to an increased sodium intake [16]. Thus, Shultz and Tolsin [16] showed that high salt intake in rats for 2 weeks resulted in increased serum concentration and urinary excretion of the NO decomposition products (NOx) and more recently, salt loading has been used even as an activator of NO production [15]. In consonance with this, the present study confirms that increased renal NOx production is seen following high dietary salt intake. Clofibrate has also been reported to increase renal NO production as measured by the urinary excretion of nitrite [15]. In the present study, however, clofibrate alone did not increase NOx production, and the increase in nitrite excretion found with the combination of clofibrate and NaCl was not greater than that produced by salt alone, while Na excretion was further increased in comparison to the NaCl group. The markedly increased natriuresis of the clofibrate-salt group is consistent with the decreased sodium and water balance of these rats and can be produced by clofibrate-stimulated 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs) with natriuretic properties [35]. This is because fibrates act as inducers of cytochrome P-450 enzymes [35], which catalyze arachidonic acid for the formation of 20-HETE and 5- and 6-EET, among others.

Interestingly, the salt + clofibrate-treated rats showed increased natriuresis and kaliuresis and reduced water and sodium balance, with higher plasma sodium and lower potassium levels. All of these observations resemble the “exaggerated natriuresis” and electrolytic plasma abnormities observed in DOCA-salt treated rats, suggesting that clofibrate may produce a positive cross-talk pattern between mineralocorticoid receptors and PPARs at renal level. Thus, PPARs belong to a large superfamily of nuclear hormone receptors that include retinoic acid (RXR), steroids, thyroid hormones, and vitamin D receptors [7, 8]. Steroid and other nuclear hormone receptors can modulate each other’s transcriptional activities. This cross-talk may result in inhibition of mineralocorticoid activity, as observed with thyroid hormones, or in its potentiation, as suggested by the alterations in the clofibrate salt-treated rats.

Our finding of increased urinary excretion of isoprostanes in the saline-loaded group is in agreement with reports that a higher saline intake increases NAD(P)H oxidase activity [17]. Moreover, experimental studies have demonstrated a PPARα activator-mediated reduction in oxidative stress [3]. Thus, bezafibrate reduced L-NAME-induced increases in plasma 8-isoprostane levels, and clofibrate diminished the increased NAD(P)H oxidase activity in DOCA-salt rats [3]. However, clofibrate treatment was unable to reduce the isoprostane levels in our saline-loaded rats, suggesting that clofibrate lacks antioxidant properties under these conditions, probably because the oxidative stress is lower than observed in L-NAME or DOCA-salt hypertension.

Our data show that saline-loaded rats have increased levels of immunoreactive ET and VP. It has been suggested that ET and VP are stimulated in a compensatory manner when the renin-angiotensin system is blunted [36]. Moreover, a role has been proposed for both ET and VP in the development and maintenance of high blood pressure and renal damage in low-renin models of hypertension [37], and a positive interaction between them has also been reported [38]. Evidence of the involvement of renal ET in salt excretion regulation includes findings of a positive correlation between changes in natriuresis and urine ET produced by a salt load [39]. Our data and these observations support participation of this peptide in the regulation of salt balance. Moreover, Newaz et al. [3] reported that clofibrate reduced plasma ET in DOCA/salt hypertensive rats [3]. However, our data show that PPARα activation was unable to modify the urinary levels of ET and of VP in saline load hypertension.

In summary, the present study shows that chronic clofibrate treatment prevents the increased blood pressure of saline-loaded rats. This effect was associated with a marked reduction in plasma and tissue thyroid hormone levels and with a reduced water and sodium balance. However, clofibrate treatment did not affect variables related to nitric oxide, oxidative stress, or ET or VP production. Moreover, clofibrate did not modify the cardiac mass but reduced the proteinuria of these animals.

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