Differential effect of tetradecythioacetic acid on the renin-angiotensin system and blood pressure in SHR and 2-kidney, 1-clip hypertension

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HYPERTENSION IS CLOSELY related to an increase in cardiovascular death and represents a major challenge in health care all over the world. The use of animal models has been of major importance in examining the mechanisms involved in the development and maintenance of high blood pressure and is widely used in studies of new antihypertensive drugs to explore the mechanisms involved in blood pressure reduction.

Several rat models are used. The spontaneously hypertensive rat (SHR) is a genetic model of hypertension and is looked upon as a model for essential hypertension in humans (32). In this model, the activity of the renin-angiotensin system is normal or low (27). Despite this, the commonly used ACE inhibitor and AT1 receptor blockers lower blood pressure in SHR (16, 29).

The 2-kidney, 1-clip (2K1C) is a renovascular model of hypertension with increased activity of renin-angiotensin system that plays a key role in the development and maintenance of high blood pressure (17, 21). The plasma renin and ANG II concentrations are high for several weeks after clipping, a finding that may be due to increased ANG II production and/or decreased ANG II degradation (17, 19). The renin concentration, as well as the levels of ANG I and II, is high in the clipped kidney, in contrast to findings in the nonclipped kidney where renin concentration is low, whereas ANG I and II levels are unchanged or increased (8). The increased ANG II levels in the nonclipped kidney seem to be dependant on the ANG II-AT1 receptor (1a, 7). Exogenous ANG II is taken up from plasma probably through a receptor-mediated mechanism, and the level of ANG II in clipped and nonclipped kidney may be different (20).

Tetradecythioacetic acid (TTA) is a novel agent that is presently tested in phase two studies in humans with hypertension. The drug is a fatty acid analog with sulphur substituted for carbon in the third position of the carbon chain counted from the COOH end of the normal saturated fatty acid. TTA does not undergo -oxidation and that makes this fatty acid different from the oxidizable fatty acids (1, 3). TTA is known to exhibit pleiotropic effects mediated both through all of the three described types of peroxisome proliferator-activated receptors (PPAR) and through PPAR-independent mechanisms (4, 30). PPAR activation has been shown to interfere with the renin-angiotensin system (11, 31). Although the effect of PPAR activation on systemic blood pressure is controversial, some authors have found a reduction of systemic blood pressure under PPAR activation (6, 12, 28), while others found either no relationship or an increased systemic blood pressure (13).

We recently showed that TTA lowers blood pressure in 2K1C hypertension (15). To further pursue the mechanisms involved in reduction of blood pressure, we compared the blood-lowering effect in 2K1C hypertension with the effect in SHR. In our previous study, we have data indicating TTA lowered plasma renin activity (PRA), although the effect on plasma ANG II was not significant. Furthermore, control studies were omitted and the present paper intends to describe the effects also in normotensive controls.

Our working hypothesis is that TTA interferes with the renin-angiotensin synthesis and reduces PRA, normalizes ANG I and ANG II levels in plasma and renal tissue followed by normalization of renal vascular reactivity and attenuation of systemic blood pressure. We predict that TTA has an effect on blood pressure in 2K1C with high activity of the renin-angiotensin system but may have minor effect in SHR and normtensive controls where the renal levels are normal or low.
MATERIALS AND METHODS

Animals. The study was performed on 51 male Wistar rats, 20 SHR, and 20 Wistar-Kyoto rats (WKY) from Møllegaard Breeding Colony (Skensved, Denmark) with a body weight of 180–200 g at the start of the study. The experiments were performed in accordance with, and under the approval of the Norwegian State Board for Biological Experiments, the Guide for the Care and Use of Laboratory Animals and the Guidelines of the Animal Welfare Act.

The rats were housed in cages with constant temperature (25°C) and humidity and were exposed to a 12:12-h light-dark cycle. They had unrestricted access to tap water and food intake.

Induction of 2K-1C hypertension. The right kidney was exposed through a lumbar incision and the right renal artery was carefully dissected and clipped by placing a rigid U-shaped silver clip with an internal opening of 0.25 mm. After the clip was attached, the lumbar muscles and skin were sutured with resorbable wires. The animals were anesthetized with isoflurane (Forene-Abbott) using an Ohmeda Iso Tec 3 anesthesia utility (BOC-Health Care). The rats were followed for the next 6 wk before the renal hemodynamic studies and plasma and tissue collections were performed.

Measurement of systolic blood pressure and body weight. Systolic blood pressure (SBP) was measured using the tail-cuff method (UGO BASILE) before surgical procedure of clipping or start of treatment. Thereafter, SBP and body weight were measured weekly during the development of hypertension in all studied groups. The rats were prewarmed to 35°C for 10 min in a cupboard.

Study groups. The animals were randomized in two types of experiments, consisting of eight groups: two WKY groups (n = 10 in each group, one TTA treated and one nontreated) used as controls for SHR, two SHR groups (n = 10 in each group, one TTA treated, one nontreated), two Wistar groups (n = 6 in each group, one TTA treated, one nontreated) used as controls for 2K1C, and two groups with 2K1C hypertension (n = 6 in each group, one TTA treated, one nontreated). Twenty-seven Wistar and 2K1C rats were used for the hemodynamic study.

The nontreated controls and hypertensive groups were fed by standard rat chow diet (0.25 g% Na, 14.7% proteins), whereas the TTA-treated controls and hypertensive groups were given standard rat chow mixed with TTA dissolved in acetone (9 g TTA in 1 L acetone) and mixed with 2.7 kg food pellets 3 days before administration to facilitate acetone evaporation. To control that treatment of pellets with acetone and TTA did not affect the rat’s food intake, the amount of pellet consumed was measured.

Collection of plasma. The 2K1C rats were killed after 6 wk of TTA treatment, whereas the WKY and SHR rats were killed after 16 wk under anaesthesia. Blood samples were collected in EDTA-coated collection tubes on ice. Samples were gentle rocked for 30 s and then centrifuged at 4,000 rpm for 15 min at 4°C. The supernatant was kept at −80°C until analyzed.

Collection of renal cortex from 2K1C and their controls. Left kidney from control groups and both clipped and nclipped kidneys were collected from anesthetized rats and immediately frozen by immersion in liquid nitrogen. The cortex was isolated and homogenized in lysis buffer (5 ml/g tissue; 10 mM Tris-HCl, pH 7.4) containing protease inhibitors according to the protocols for the assay used. The homogenized samples were centrifuged and the supernatant was kept at −80°C until analyzed.

Measurements of PRA, plasma and renal cortex ANG I and ANG II concentrations. PRA was measured by a radioimmunassay (RIA; Diasorin, CA-1553). ANG I and II were measured also by a RIA assay (Phoenix Pharmaceutical, RK-002-01 and RK-002-12). The protein extraction was made with C18 SEP-COLUMNs, 200 mg (Phoenix Pharmaceutical, RK-SEP-COL1), 1% trifluoroacetic acid (TFA) in H2O (Phoenix Pharmaceutical, RK-BA), and 60% acetonitrile in 1% TFA (Phoenix Pharmaceutical, RK-BB) according to the producer’s protocol. Each sample was assayed in duplicate and incubated 20 h at 4°C with primary antibody. Thereafter, the 125I peptides were added and the samples were incubated for 20 h at 4°C. The secondary antibody was added and the samples were incubated for 90 min at room temperature. Then, samples were centrifuged at 1,700 g for 20 min at 4°C and the supernatant was rapidly aspirated. A γ-counter was used to count the CPM of the pellet. The results were standardized after a titration curve with known amounts of peptide.

Surgical preparation for hemodynamic studies from 2K1C and their controls. The left, nonclipped kidney was used for measurement of renal blood flow (RBF) response to ANG II injections into the left renal artery. Simultaneously, the mean arterial pressure (MAP) was also recorded and renal vascular resistance (RVR) was calculated based on RBF and MAP, using BOLUS program, customized software for the automated analysis of data. The experimental procedure has been described before (5). In short, anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt) and the animals were placed on a servo-controlled heating table to keep the body temperature at 38°C. Tracheostomy was performed to facilitate free breathing.

The right femoral artery was cannulated with a PE-25 catheter to monitor arterial pressure using a Statham P 23 XL pressure transducer. Another PE-25 catheter was introduced into the right femoral vein for infusion of albumin solution and supplementary doses of pentobarbital sodium. Bovine serum albumin (Sigma), dissolved in isotonic saline at a concentration of 4.7 g/dl, was infused initially at a rate of 50 µl/min to replace losses associated with surgery (1.25 ml/100 g body wt), and then at 10 µl/min during the experiment.

The abdominal aorta and left kidney were exposed through a midline incision. For the injection of ANG II, a PE-10 catheter was introduced through the internal iliac artery and then advanced until its tip was positioned ~2–3 mm inside the left renal artery without affecting the RBF.

Measurement of RBF from 2K1C and their controls. The left renal artery was localized and dissected free. The adjacent fat tissue was carefully removed for proper acoustical coupling. An ultrasonic V Transonic System flow probe with an internal diameter of 0.8 mm was placed on the renal artery. The Dane-Gel E2 (Rohde Produits) aqueous transmission was used as an acoustic couplant. The flow probe was interfaced to the data-acquisition system using a Transonic T 206 electromagnetic flowmeter and a Gould TA 5000 recorder and installed on a PC compatible computer with a 12-bit analog-to-digital converter. A Gould TA 5000 Hewlett Packard carrier amplifier was used for the pressure transducer sensor interface. The outputs of the transducer monitoring arterial pressure and RBF were sampled at a rate of 1 sample/s. Each recording started 15 s before injection of vasoconstrictor in left renal artery. RBF values were normalized and expressed as a percentage of baseline values.

After completion of the surgical preparation, animals were allowed to stabilize for 1 h before starting the records of MAP and RBF response to ANG II injections in left renal artery. The records were made during injections of 1 and 2.5 ng ANG II in two consecutive measurements for each dose. A Cheminert injection valve with a 10-µl injection volume and 2-s delivery time was used.

At the end of experiments, the rats were killed by an extra dose of pentobarbital sodium and kidneys were extracted and weighed.

Chemicals. ANG II and bovine serum albumin were obtained from Sigma. Heparin (100 IE/ml) was obtained from Leo-Pharma.

Statistical methods. Data are reported as means ± SE. Statistical analyses was done using SPSS 8.0 for Windows. Differences between groups were assessed by one- or two-way repeated-measures ANOVA, followed by Student’s t-tests with Bonferroni or Scheffé’s correction. P values <0.05 were considered statistically significant.
RESULTS

Kidney weight. The clipped kidney in 2K1C groups was 0.85 ± 0.08 g in the TTA-treated and 0.73 ± 0.16 g in nontreated groups. The nonclipped kidney was 1.67 ± 0.09 g in the TTA-treated and 1.81 ± 0.06 g in nontreated groups. These results indicate a similar degree of clipping in both the TTA-treated and nontreated groups. In the control groups, the right kidney weight was 1.31 ± 0.1 g and the left was 1.52 ± 0.2 g. In TTA-treated control rats, the kidney weights were 1.37 ± 0.1 g for the right kidney and 1.51 ± 0.1 g for the left.

Body weight. TTA reduced body weight in WKY from 406 ± 7 g in the untreated animals to 367 ± 6 g (P < 0.001). Similarly, body weight was reduced in SHR during TTA treatment (402 ± 7 vs. 357 ± 8 g, P < 0.001). TTA reduced body weight after 6 wk of treatment in both control and 2K1C group: 380 ± 10 vs. 333 ± 10 g, P = 0.007 and 337 ± 20 vs. 288 ± 8 g, P = 0.03.

Food intake. The intake of food was similar in all groups. It was 88.9 ± 1 g·kg⁻¹·day⁻¹ in control nontreated, 84.9 ± 1 g·kg⁻¹·day⁻¹ in control TTA-treated, 85.4 ± 1 g·kg⁻¹·day⁻¹ in 2K1C-nontreated, and 85.2 ± 2 g·kg⁻¹·day⁻¹ in 2K1C TTA-treated groups.

SBP in SHR and WKY. The effect of TTA on SBP was investigated in rats with genetic hypertension starting at an age of 2 wk and the animals were studied until an age of 16 wk. WKY rats were used as controls. In young SHR developing hypertension, the blood pressure increase was delayed in TTA-treated rats and was lower than the untreated SHR from week 2 to 8 after TTA was introduced in the diet. The maximal difference was seen after 5 wk of treatment where the SBP was reduced to 125 ± 4 vs. 141 ± 6 mmHg in the untreated rats (P < 0.01; Fig. 1A). At higher ages, there were no differences in SBP in untreated and TTA-treated SHR (Fig. 1A). The SBP in WKY increased more than expected after 6–8 wk of age, and declined thereafter. TTA did not change the blood pressure in WKY (Fig. 1B).

SBP in 2K1C and their controls. As shown before, the SBP increased to 173 ± 4 mmHg 6 wk after clipping compared with 122 ± 3 mmHg before clipping (P < 0.001). In the controls SBP was 125 ± 4 mmHg before and 120 ± 3 mmHg after 6 wk, lower than in 2K1C-hypertensive rats (P < 0.01). In the 2K1C-treated group, TTA attenuated the development of high blood pressure, which was 123 ± 4 mmHg before clipping and 138 ± 3 mmHg 6 wk after clipping, lower compared with 173 ± 4 mmHg in untreated 2K1C (P < 0.001). TTA did not affect the blood pressure in control rats (Fig. 1C).

PRA. PRA was examined at the end of the study in all groups. There was no difference in PRA between nontreated WKY and SHR and TTA had no effect on PRA in the two strains. PRA was 4.2 ± 0.8 in untreated and 4.8 ± 1.1 ng·ml⁻¹·h⁻¹ in treated WKY, and the corresponding values in SHR were 3.3 ± 1.2 vs. 4.1 ± 1.1 ng·ml⁻¹·h⁻¹. PRA was higher in the nontreated 2K1C-hypertensive group compared with control (22.9 ± 1.3 vs. 12.2 ± 2.5 ng·ml⁻¹·h⁻¹, P = 0.001). TTA treatment of the 2K1C-hypertensive group reduced the PRA to 16.2 ± 2.2 ng·ml⁻¹·h⁻¹ (P = 0.02) compared with nontreated 2K1C-hypertensive rats which was still higher than in controls (P = 0.01). PRA was similar in control animals independent of TTA treatment.

Plasma ANG II concentrations in 2K1C and their controls. TTA treatment did not change plasma ANG II concentrations in controls. It was 55 ± 6 fmol/ml in nontreated controls and 68 ± 3 fmol/ml in TTA-treated controls. In the nontreated 2K1C group, ANG II plasma concentrations increased to 101 ± 3 fmol/ml compared with controls (P = 0.001). TTA treatment reduced ANG II plasma concentrations in 2K1C animals to 81 ± 5 fmol/ml (P = 0.005 compared with non-
Both untreated and treated 2K1C animals had higher ANG II plasma levels than controls ($P < 0.001$; Fig. 2).

Renal cortex ANG I concentrations in 2K1C and their controls. The ANG I concentrations in the renal cortex were similar in controls, independent of TTA treatment (469 ± 8 fmol/g tissue in nontreated animals and 467 ± 15 fmol/g tissue in TTA-treated control animals; Fig. 3A). In the nontreated 2K1C-hypertensive group, the renal cortex ANG I concentration in the clipped kidney was 933 ± 68 fmol/g tissue ($P < 0.001$ compared with controls). In the TTA-treated 2K1C group, the ANG I concentrations in the renal cortex of the clipped kidney were normalized to control values (518 ± 60 fmol/g tissue, $P < 0.001$; Fig. 3B). In the nonclipped kidney of the 2K1C groups, the renal cortex ANG I concentration was 370 ± 16 fmol/g tissue and TTA had no effect on the concentration of ANG I (425 ± 46 fmol/g tissue; Fig. 3C).

Renal cortex ANG II concentrations in 2K1C and their controls. The renal cortex ANG II concentration was the same in controls independent of TTA treatment (Fig. 4A). It was 175 ± 10 fmol/g tissue in nontreated control group and 158 ± 10 fmol/g tissue in the TTA-treated control group. In the nontreated 2K1C group, the renal cortex ANG II concentration in the clipped kidney was 527 ± 38 fmol/g tissue ($P < 0.001$ compared with control). In TTA-treated 2K1C group, the ANG II concentration in renal cortex of the clipped kidney was reduced (149 ± 21 fmol/g tissue, $P < 0.001$; Fig. 4B, compared with the nontreated 2K1C). In nontreated 2K1C rats, ANG II concentration in the nonclipped kidney was 290 ± 26 fmol/g tissue ($P = 0.002$, compared with controls). TTA treatment reduced ANG II concentration in the nonclipped kidney, but not to control values (229 ± 56 fmol/g tissue, $P = 0.05$ compared with control; Fig. 4C).

RBF response to exogenous ANG II injection into the renal artery in 2K1C and their controls. TTA treatment did not change RBF in the nonclipped kidney of 2K1C-hypertensive rats. It was 6.3 ± 0.5 ml·min⁻¹·g⁻¹ on untreated controls and 6.2 ± 0.4 ml·min⁻¹·g⁻¹ in TTA-treated rats. The corresponding values in 2K1C were 6.4 ± 0.2 and 6.5 ± 0.2 ml·min⁻¹·g⁻¹.

The ANG II injection elicited a dose-dependent response as shown in Fig. 5. To simplify the presentation, the results are given only after injection of 2.5 ng ANG II, and the results are expressed as percentage reduction from the baseline line. In control groups, the responses were not changed by TTA treatment (23 ± 4% in nontreated control, Fig. 6A and 23 ± 4% in the TTA-treated control group, Fig. 6B). In 2K1C group, the RBF decrease was 10 ± 2%, which was lower than in the control group ($P < 0.001$; Fig. 6C). TTA treatment increased RBF response to ANG II in the 2K1C group to 20 ± 2% ($P < 0.001$ compared with nontreated 2K1C; Fig. 6D).

RVR in 2K1C and their controls. The RVR was calculated based on MAP and RBF values in nontreated and TTA-treated animals. The RVR in control groups was similar independent of TTA treatment (15 ± 2 mmHg·ml⁻¹·min⁻¹·g⁻¹ in the non-
DISCUSSION

The main finding in the present study is that TTA attenuated the blood pressure increase in 2K1C, had a minor effect on SBP in SHR, and no effect on blood pressure in WKY controls. While we confirm that TTA has an effect in renovascular hypertension (15), we provide significant new information about the mechanisms involved in the blood pressure-lowering effect in this model of hypertension. The intrarenal content of ANG I and II was reduced to control values in the clipped kidney of 2K1C after TTA treatment. In the nonclipped kidney, TTA has no effect on ANG I levels, while there was a minor effect on ANG II. The vascular reactivity to ANG II was also normalized by TTA treatment in the nonclipped kidney. TTA has no effect on PRA in Wistar and WKY control animals. PRA was also unchanged in SHR after TTA treatment, while in 2K1C, TTA treatment reduced PRA.

The normalization of the renin-angiotensin system in the 2K1C-hypertensive rats supports our hypothesis that TTA interferes with the renin-angiotensin system and the blood pressure increase is attenuated due to the lowering of the ANG II concentration. The effect on blood pressure in SHR was very small in young animals and showed a delay of ~1–2 wk in blood pressure development in the SHR during TTA treatment, but thereafter the blood pressure developed with the same speed as in the untreated SHR. There was no effect on blood pressure in adult SHR animals. There was no effect on PRA in SHR, the activity was very low and not different from the normotensive controls.

These findings indicate substantial differences between this new drug, TTA, and other drugs interfering with the renin-angiotensin system like ACE inhibitors and AT1 receptor blockers (16, 29). It is well known that both these drugs have a blood pressure-lowering effect both in SHR and 2K1C hypertension. The acute effect of ACE inhibition in SHR is known to be dependent on the renin-angiotensin system as salt retention in DOCA-salt-treated SHR reduces the response to ACE (26). These results are confirmed by other studies indicating that the activity of the renin-angiotensin system is of major importance as long as ACE inhibition can be organ-specific.

Fig. 5. Renal blood flow (RBF) response after injection of 1 and 2.5 ng ANG II into the nonclipped kidney of 2K1C, showing normalization of the response in TTA-treated 2K1C rats. *P < 0.001 indicates the reduced vasoconstriction to 1 ng ANG II injected into the renal artery of nonclipped kidney in 2K1C-untreated group compared with controls and 2K1C TTA-treated group. **P < 0.001 indicates the reduced vasoconstriction to 2.5 ng ANG II injected into the renal artery of nonclipped kidney in 2K1C-untreated group compared with controls and 2K1C TTA-treated group.
A possible theory that could explain the difference between 2K1C and SHR is that TTA may affect a transcriptional mechanisms specific to renin-producing granular cells. Indeed, results from cells expressing different parts of the renin promoter indicate that certain parts cause a strong activation, overriding other segments. This gives an on/off effect such as seen in granular cells while other segments of the promoter elicit more graded changes in expression (18). Further studies should be provided to clarify whether these mechanisms are involved during TTA treatment.

A striking finding is that TTA reduces ANG I and ANG II in the clipped kidney while the effect in the nonclipped kidney is very small. The renin concentration in the clipped kidney is high and the production of ANG I and II is upregulated. In contrast, renin concentration is low in the nonclipped kidney, although ANG I and ANG II are high. There is reason to believe that the mechanisms behind the increased level of ANG I and II are different in the two kidneys of 2K1C (20, 22) and that TTA only influences one of these mechanisms. TTA seems to reduce ANG II level in the clipped kidney to control values, but the effect of this drug on ANG II level in the nonclipped kidney was relatively small. The result that ANG II levels in the nonclipped kidney are higher than plasma is consistent with earlier data (20). The increased tissue concentration of ANG II may be due to intrarenal formation of ANG II but may also depend on plasma uptake, a mechanism described in ANG II-infused rats (14, 33). Infusion of Val5-ANG II intravenously showed that Val5-ANG II accumulated in the kidney and that a major part of the ANG II content of the kidney is taken up from the circulation (33). Our investigations showed that the effect of TTA on ANG II in the nonclipped kidney is small and this finding indicates that TTA does not interfere with the mechanisms involved in transport of ANG II from plasma to the renal tissue. In the clipped kidney, the levels of both ANG I and II are reduced during TTA treatment indicating that the synthesis of these peptides is reduced. This is also supported by the earlier findings that mRNA for renin is reduced in the clipped kidney and unchanged in the nonclipped kidney where mRNA for renin was very low (15).

When the integrated response to ANG II was examined by injection of ANG II into the artery of the nonclipped kidney, the renal vascular response to exogenous ANG II was normalized after TTA treatment. Reduced renal vascular response to
ANG II in the nonclipped kidney of 2K1C-hypertensive has been shown before (5). It might be due to increased endogenous ANG II concentration with reduced number of available AT1 receptors and attenuated renal vascular response as a result. Normalization of the ANG II level should make more AT1 receptors available for exogenous ANG II and consequently elicit a stronger response. TTA is known to exert various effects, mediated through activation of PPARs of all three described types of receptors (α, β, or Δ, and γ) (2, 30), but also through PPAR-independent pathways (2, 30). The mechanism behind TTAs activation of PPAR in the kidney is uncertain, but the renin promoter includes a PPAR-binding motif which may suggest a possible pathway (18, 25). The slight effect we found in young SHR may be due to higher activity of the renin-angiotensin system in these young rats for a short period of early life, as has been described before (27). Despite the lack of antihypertensive effect in SHR, the effect on body weight was similar as in 2K1C-hypertensive rats suggesting that TTA has several effects.

In conclusion, results of the present study indicate that TTA interferes with the production of ANG I and ANG II in a way that seems to be specific to the 2K1C model. TTA does not affect the basal activity to the renin-angiotensin system neither in normotensive rats nor in the genetically hypertensive SHR.

REFERENCES