Effect of Cystamine on Blood Pressure and Vascular Characteristics in Spontaneously Hypertensive Rats

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Introduction

Essential hypertension is associated with alterations of the resistance vessels [1]. Narrowing of the lumen occurs, causing a reduced lumen diameter and increased media-lumen ratio, but with no increase in the media cross-sectional area. These morphological changes are termed inward eutrophic remodelling and have been demonstrated to be a consequence of rearrangement of the existing extracellular matrix (ECM) in essential hypertension and not by cellular growth as observed in secondary forms of hypertension [2]. Such findings have been reported by numerous studies during the last decades in animal as well as human studies, and it has recently become clear that inward remodelling of the microvasculature causes an increased risk of cardiovascular events [3, 4]. Both low flow conditions [5] and chronic vasoconstriction [6] of resistance arteries are known to induce inward remodelling, but the underlying mechanisms are still to a great extent unknown. Recent findings suggest an important role of the enzyme tissue transglutaminase (t-TG) in rats [7]. t-TG is a bifunctional enzyme with both GTP-hydrolyzing activities and protein cross-linking functions [8]. The enzyme is Ca2⁺ dependent and ubiquitously expressed within cells and in the ECM [9]. t-TG is known to crosslink ECM proteins, such as fibrinogen and cell surface integrins, via mechanically resistant Nε-(γ-glutamyl)lysine cross-linkings [10], and it is known to be involved in multiple physiological functions including
cell-cell interaction, cell adhesion, matrix reorganization and wound healing [11]. A role of t-TG in remodelling of resistance vessels is supported by the finding that type 2 Tgase null mice have a delayed development of inward remodelling in response to reduced blood flow, compared to wild-type (WT) mice [12]. Furthermore, small arteries show inward remodelling after exposure to exogenous transglutaminase in vitro [7].

Since inward remodelling of resistance vessels is observed in hypertension, and since t-TG activity depends on pressure [7], there is a possible link between the structural and functional alterations observed in hypertension. Two in vivo studies have been conducted, concerning the role of t-TG in inward remodelling of small arteries, from animals with induced hypertension. In one mouse study, the authors induced L-NAME hypertension and found a reduced wall-to-lumen ratio in t-TG knockout mice compared to WT, with no effect on blood pressure [13]. In the second study, authors induced chronic vasoconstriction with phenylephrine and found that cystamine, an organic disulfide with multiple modes of action that includes competitive inhibition of t-TG, attenuates small artery remodelling in normotensive rats [14].

To our knowledge, no studies have determined the role of t-TG in animals genetically disposed to hypertension. We have therefore determined whether cystamine administration can cause a reduction in blood pressure of spontaneously hypertensive rats (SHR) and if so to what extent this is mediated through small arteries. In vitro myograph experiments were conducted to assess the role of cystamine on small artery morphology and function, and cystamine was administered in vivo to study unaesthetized SHR in relation to hemodynamic parameters, obtained with telemetric transmitters.

Materials and Methods

Experiments were approved by the Animal Ethics Committee and conducted according to Danish legislation. SHR (Charles River Laboratories Inc.) were housed in pairs in the faculty animal facility with a 12-hour light/dark cycle and provided with free access to food and drinking water. Animals were acclimatized for 1 week prior to surgery or euthanization.

**In vitro**

Twelve-week-old male SHR were euthanized by cervical dislocation and the mesenteric bed was harvested and immediately immersed in 4°C cold physiological saline solution (PSS) of the following composition (10−3 M): 119 NaCl, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4, 1.5 CaCl2, 24.9 NaHCO3, 0.026 EDTA and 5.5 glucose (pH 7.5). Mesenteric second-order branches (diameter approximately 250 μm) were dissected out under clean conditions and carefully cleaned of connective and fat tissue and used for further investigation.

**In vivo**

Fourteen-week-old male SHR rats (n = 24, 293 ± 2.5 g) were used to study the role of t-TG in inward remodelling and the reversion of remodelling in relation to blood pressure and artery structure in hypertensive rats. Animals were divided into 3 groups and received treatment with either cystamine 80 mg/kg/day (Sig-
ma-Aldrich), amlodipine 10 mg/kg/day (Pfizer) or 40% sterile 0.9% saline + 60% PEG-400 (vehicle) for 3 weeks. At the end of the treatment period, animals were euthanized by cervical dislocation and tissue harvested for further analysis.

Telemetric Measurements of Arterial Pressure
Animals were anaesthetized with isoflurane (1.5–4%) and a telemetry transmitter (TA11-PAC40; Data Sciences International) was implanted according to the manufacturer as described by others [17]. Body temperature was kept constant at 37°C with a heating plate during surgery. Prior to implantation of transmitters, accuracy was verified with the Dataquest Acquisition program. Under sterile conditions, a skin incision was made in the femoral region and the femoral artery was exposed. A small incision was made in the artery and the transmitter catheter introduced into the aorta through the femoral artery and secured in place with a suture. The transmitter body was implanted intraperitoneally in the abdominal cavity through a midline skin incision and firmly secured to the abdominal wall along the incision with 3-0 (Ethibond Excel) sutures. Skin incisions were closed with 9-mm staples (Autoclips®) that were removed after healing of incisions. A single injection of benzyl penicillin (Panpharma) was given following surgery as prophylaxis against infection. Post-surgical pain was treated with 0.04 mg buprenorphine (Temgesic® 0.3 mg/ml). Animals were allowed 8 days to recover from surgery in their normal housing before sampling of 24-hour baseline recordings. Sampling was performed with Dataquest ART™ Acquisition System (DSI) connected to DSI APR-1 (Ambient Pressure Reference) and data analysis with Dataquest ART Analysis System (DSI). During treatment, 24-hour recordings of systolic and diastolic arterial pressure (sample frequency of 1 every 30 s), heart rate and locomotor activity were performed once every week.

Administration and Formulation of the t-TG Inhibitor Cystamine and Amlodipine
Following baseline recordings of arterial pressure, treatment was initiated with implantation of osmotic minipumps (Alzet®, model 2ML2). One day prior to implantation, pumps were filled with drugs and primed in sterile 0.9% saline at 37°C. Subcutaneous implantation of pumps was done in the subcapsular region through a small skin incision under anaesthesia with isoflurane. After 12 days of treatment, pumps were renewed by repeating the described procedure. Cystamine was dissolved in sterile 0.9% saline and amlodipine was dissolved in 60% PEG400 and 40% sterile water for pump delivery of drugs. Vehicles were filled with 60% PEG400 and 40% sterile water.

Vessel Morphology
Following 3 weeks of treatment, animals were euthanized and small mesenteric arteries harvested and immediately placed in cold (4°C) PSS. Three mid-segment second-branch vessels from each animal were selected and dissected by a person blinded to group of treatment. Segments were cannulated and mounted in a pressure myograph (DMT) with the blind-sack method [18]. The inner medium was composed of calcium-free PSS. Investigator-blinded passive pressure-diameter relationships were determined (0–120 mm Hg) at 37°C after incubation with papaverine 10⁻⁴ M for 15 min in a solution of calcium-free PSS in myograph chamber. Mean values for the 3 vessels were used for data analysis [14].

Blood Sample Collection
After 10 days of treatment, blood was collected in microtubes (Sarstedt) containing 1.6 mg EDTA/ml blood, by penetrating the retro-orbital plexus with a glass capillary tube, and immediately placed on ice. Plasma was extracted after centrifugation at 3,000 rpm for 8 min at 6°C and immediately frozen at –80°C.

Determination of Cystamine and Other Thiols in Rat Plasma
Cystamine is the disulphide of cysteamine, but can also exist in plasma as other oxidized forms of cysteamine. We used a liquid chromatography set-up to determine total cysteamine in plasma and calculated the concentration of cystamine. The liquid chromatography equipment (Hewlett-Packard; 1100 series system) consisted of a quaternary pump, autosampler, thermostated column compartment, vacuum degasser and diode-array detector and was controlled by HP ChemStation software. Water was purified (Milli-Q RG system; Millipore).

Analytical Procedures for Determination of Cysteamine and the Other Thiols
The method for determination of reduced and total thiols is based on derivatization with 2-chloro-1-methylquinolinium tetrafluoroborate (CMQT) [19] to stable 2-S-quinoilinium derivatives, and separation and quantitation by ion-pairing reversed-phase liquid chromatography [20]. The method is based on the fact that disulfides are converted to their thiol counterparts by reductive cleavage with tris(2-carboxyethyl)phosphine.

Determination of Reduced Thiols, Procedure 1
One hundred microliter of plasma was mixed with 50 µl of 0.2 × 10⁻⁷ M phosphate buffer (pH 7.6) containing 2 × 10⁻³ M EDTA, and 10 µl of 10⁻³ M CMQT [19]. After 5 min derivatization at room temperature, 15 µl of 50% perchloric acid solution was added, the mixture was vortex mixed and precipitated protein separated by centrifugation for 10 min at 12,000 g. The supernatant was transferred to a vial, followed by injection (20 µl) into the HPLC system.

Determination of Total Thiols, Procedure 2
One hundred microliter of plasma was mixed with 50 µl of 0.2 M phosphate buffer (pH 7.6) containing 2 × 10⁻³ M EDTA, and 10 µl of 0.25 M tris(2-carboxyethyl)phosphine. After 15 min reduction at room temperature, 10 µl of 0.1 M CMQT were added, vortex mixed and kept at room temperature for 5 min, followed by addition of 15 µl of 50% perchloric acid solution. Precipitated protein was then removed by centrifugation for 10 min at 12,000 g, supernatant was transferred to a vial and 20 µl was injected into the HPLC system.

HPLC Analysis
Final analytical solutions (20 µl) were injected into the Zorbax SB-C18 (150 × 4.6 mm, 5 µm) column (Agilent Technologies). For separation of 2-S-quinoilinum derivatives of thiols from each other and from reagent excess chromatographic condition described earlier [20] was used. Briefly, the elution profile was as follows: 0–4 min 12% B; 4–7 min 12–40%; 7–8 min 40% B; 8–10 min 40–12% B. Elution solvent was (A) 5 × 10⁻² M trichloroacetic acid buffer (pH 3, 15 prepared from 5 × 10⁻² M TCA and 5 × 10⁻³ M LiOH) and (B) acetonitrile. The temperature was 25°C, the flow rate 1 ml/min and the detector wavelength 355 nm. Peaks
identification was based on comparison of retention times and diode-array spectra, taken at real time of analysis, with a corresponding set of data obtained for authentic compounds.

**Statistical Analysis**

Data are expressed as means ± SE. Results were analyzed with Student’s t test, unless otherwise stated. p < 0.05 was considered significant.

**Results**

**Structural Adaptations during in vitro Organ Culture**

Small arteries mounted in organ culture developed spontaneous tone. Artery segments were activated with ET-1 for 20 h and the passive pressure diameter relationships were determined by comparison of area under curve (AUC) before and after incubation. Resistance arteries from SHR developed a significant reduction in passive lumen diameter after 20 h of activation with ET-1. AUC was reduced by 7.2 ± 1.9% (day 0 vs. day 1, p < 0.05, n = 5), indicating that activation induces inward remodelling in SHR and thereby a reduced lumen diameter for the same level of pressure (fig. 1). Concomitant incubation with cystamine, which among other actions inhibits t-TG, attenuated the inward remodelling induced by activation. In the presence of cystamine, there was no significant change in AUC after activation (1.2 ± 0.9%, day 0 vs. day 1, p > 0.05, n = 5) and thereby no significant inward remodelling was induced. This indicates that cystamine attenuates inward remodelling in SHR and supports a role of t-TG in remodelling of SHR small arteries. Control arteries showed no significant change in AUC following 20 h of culture (1.6 ± 0.9%, day 0 vs. day 1, p > 0.05, n = 5).

All activated artery segments maintained vasoconstriction during the entire culture period and no change in the initial vasoconstriction induced by ET-1 was observed in the presence of cystamine (fig. 1).

**Functional Effects of Cystamine in Small Arteries Obtained from SHR**

The functional effects of cystamine were investigated in the wire myograph. SHR small mesenteric arteries contracted concentration-dependently as response to stimulation with phenylephrine and concentration-response relationships were then determined in the presence of cystamine or amlodpine in increasing concentrations. Cystamine and amlodpine caused concentration-dependent antagonism of phenylephrine-induced vasoconstriction. 10⁻⁵ M cystamine caused a 22.4 ± 5.9% reduction in the contractile response to phenylephrine, suggesting a potential vasodilator capacity of cystamine (fig. 2).

**Chronic in vivo Administration of Cystamine in SHR**

SHR received 3 weeks treatment with cystamine (80 mg/kg/day) to study if inhibition of t-TG could reduce blood pressure and reverse remodelling of small arteries in hypertensive rats. Left ventricular weight was significantly (p < 0.05, n = 8) lower in SHR treated with amlodipine than found in SHR receiving treatment with cystamine (table 1). There was a trend for a lower gain of weight in SHR treated with amlodipine than observed in cystamine or vehicle treated animals, but it did not reach statistical significance. Measurements of systemic haemodynamics were performed with telemetry in conscious SHR during administration of cystamine or amlodipine. Chronic treatment with cystamine caused a significant reduction in mean arterial pressure (MAP) of 9 mm Hg after 2 weeks treatment (fig. 3; table 2). This drop in MAP was larger than the change in MAP observed in vehicle-treated animals. The blood pressure-lowering effect of cystamine was primarily observed in the diastolic blood pressure, supporting that it could be caused by an increase in the lumen diameter of the small arteries. Amlodipine caused a 22-mm Hg reduction in MAP.

The experimental procedure caused a 20 ± 4% reduction in mean locomotor activity level during the experimental time period; however, treatment had no significant effect on mean activity, either with cystamine or amlodipine (p > 0.05, 2-way ANOVA) compared to control. The reduction was thus presumably due to the telemetric transducers and osmotic minipumps.

**Determination of Thiols in SHR Plasma**

The plasma concentration of cystamine, together with other thiols, was determined in plasma samples obtained during in vivo administration of cystamine or amlodipine (table 3). Plasma level of cystamine was significantly increased in SHR receiving continuous subcutaneous administration of cystamine in osmotic pumps for 10 days (80 mg/kg/day), confirming appropriate delivery of cystamine. Cystamine could not be detected in SHR receiving vehicle or amlodipine treatment. Plasma level of total-glutathione was significantly higher, while reduced-cysteine was significantly lower in SHR receiving continuous subcutaneous administration of amlodipine (10 mg/kg/day) than in vehicles. We observed an insignificant trend for a higher level of plasma homocysteine in SHR receiving cystamine and amlodipine than in vehicles (table 3).
We observed a tendency for an increased vessel diameter, following 3 weeks treatment with cystamine or amlodipine, but it did not reach level of statistical significance (fig. 4; table 4). No significant change in wall to lumen ratio was found, after treatment with cystamine or amlodipine for 3 weeks (p > 0.05, n = 8, ANOVA). Functional studies of small arteries harvested during follow-up, showed no difference in maximal tension development to 10 μM phenylephrine, between vehicle- (3.9 ± 0.4 N/m), cystamine- (4.3 ± 0.3 N/m) or amlodipine- (3.7

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**Fig. 1.** a Pressure-diameter relationships of maximally dilated small mesenteric arteries from SHR. Activation with 10−8 M ET-1 induced significant inward remodelling of small artery segments in organ culture (⁎ p < 0.05, n = 5). No significant remodelling was induced when activation was performed with ET-1 in combination with 10−4 M cystamine (p > 0.05, n = 5). Control arteries showed no significant change in diameter during culture period (p > 0.05, n = 5). b Change in AUC from day 0 to day 1. ET-1 caused significant inward remodelling which was attenuated by concomitant incubation with cystamine. Data were divided by 120 mm Hg to express change in AUC as μm (⁎⁎ p < 0.01, ⁊⁎⁎ p < 0.001, one-way ANOVA with post-Tukey test, n = 5). c Diameter of vessel segment during organ culture. Passive diameter was obtained with papaverine at 80 mm Hg. Arteriole segments developed spontaneous tone after removal of papaverine. Segments were either incubated with ET-1 alone or with ET-1 in combination with cystamine. ET-1 induced vasoconstriction that was sustained during organ culture. Passive (0) = Passive diameter on day 0; tone (0) = spontaneous tone on day 0 after removal of papaverine; ET-1 (0) = vessel diameter after activation with 10−8 M ET-1 on day 0; ET-1 (1) = vessel diameter after 20-hour incubation with ET-1.
To investigate if chronic administration of cystamine causes interference with the Rho/ROCK pathway and its intracellular functions, wire-mounted small arteries were exposed to the selective inhibitor of Rho-associated kinases Y27632. In cystamine-treated rats, we found (data not shown) that 1 μM Y27632 caused a –35 ± 8% reduction in contractile response to phenylephrine, which was not significantly different from the change observed with vehicle or amlodipine (–21 ± 6, –40 ± 8%, p > 0.05, n = 8, ANOVA). Taken together the data indicate that no morphological or functional changes are induced in SHR small arteries after 3 weeks treatment with cystamine or amlodipine.

### Discussion

The primary findings in this paper were that in vitro activation of resistance arteries induces inward remodelling of SHR small arteries and that cystamine (a competitive inhibitor of t-TG) attenuates inward remodelling in hypertensive rats in vitro. We also found that 3-week in vivo administration of cystamine causes modest reduc-

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**Table 1. Summary of body weight and left ventricular weight**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight, g</th>
<th>LVW, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>follow-up</td>
</tr>
<tr>
<td>Vehicle</td>
<td>286.6 ± 6.7</td>
<td>327.4 ± 5.4</td>
</tr>
<tr>
<td>Cystamine</td>
<td>281.3 ± 7.3</td>
<td>325.6 ± 3.3</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>277.6 ± 1.7</td>
<td>305.3 ± 4.9</td>
</tr>
</tbody>
</table>

Data are means ± SEM. Twenty-two SHR received treatment with vehicle, cystamine or amlodipine. LVW = Left ventricular weight. * p < 0.05, amlodipine vs. cystamine, ANOVA.

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**Fig. 2.** In vitro wire myograph experiments. Small arteries from SHR (n = 6) were mounted for each experiment. Cystamine (CYS) and amlodipine (AM) showed antagonism to phenylephrine-induced concentration-response curves. Data represent mean values ± SEM. Statistics were performed as change in AUC compared to control (* p < 0.05, *** p < 0.001, repeated-measures ANOVA with post-Tukey test).

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**Fig. 3.** MAP during 3 weeks treatment with vehicle, cystamine (CYS) or amlodipine (AM) in osmotic minipumps. Cystamine caused a significant reduction in MAP after 2 and 3 weeks administration. Amlodipine also caused significant lowering of MAP. Osmotic minipumps were renewed 2 weeks after implantation, which may explain the fluctuation observed in MAP in animals receiving amlodipine. Details of data are shown in table 2, which also shows the statistical analysis (** p < 0.01, *** p < 0.001).

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± 0.4 N/m) treated animals (p > 0.05, n = 8, ANOVA). To investigate if chronic administration of cystamine causes interference with the Rho/ROCK pathway and its intracellular functions, wire-mounted small arteries were exposed to the selective inhibitor of Rho-associated kinases Y27632. In cystamine-treated rats, we found (data not shown) that 1 μM Y27632 caused a –35 ± 8% reduction in contractile response to phenylephrine, which was not significantly different from the change observed with vehicle or amlodipine (–21 ± 16, –40 ± 8%, p > 0.05, n = 8, ANOVA). Taken together the data indicate that no morphological or functional changes are induced in SHR small arteries after 3 weeks treatment with cystamine or amlodipine.
Table 2. Cardiovascular responses to chronic administration of cystamine or amlodipine in SHR

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 22)</th>
<th>Vehicle (n = 7)</th>
<th>Cystamine (n = 7)</th>
<th>Amlodipine (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart rate, bpm</td>
<td>331 ± 3</td>
<td>335 ± 4*</td>
<td>352 ± 5</td>
</tr>
<tr>
<td></td>
<td>MAP, mm Hg</td>
<td>141 ± 1</td>
<td>142 ± 3</td>
<td>137 ± 3</td>
</tr>
<tr>
<td></td>
<td>Systolic BP, mm Hg</td>
<td>168 ± 1</td>
<td>171 ± 3</td>
<td>164 ± 3</td>
</tr>
<tr>
<td></td>
<td>Diastolic BP, mm Hg</td>
<td>113 ± 1</td>
<td>113 ± 3</td>
<td>111 ± 3</td>
</tr>
<tr>
<td></td>
<td>Pulse pressure, mm Hg</td>
<td>55 ± 1</td>
<td>59 ± 1</td>
<td>53 ± 3</td>
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</table>

Data are means ± SEM obtained with telemetry (DSI) in SHR that underwent chronic administration with either vehicle, cystamine or amlodipine. Data analyzed by two-way ANOVA. In all cases there was significant interaction and data were then compared to baseline using Bonferroni post-test. BP = Blood pressure.

* p < 0.05, ** p < 0.01, *** p < 0.001.

Table 3. SHR plasma thiols during treatment with cystamine and amlodipine

<table>
<thead>
<tr>
<th>Thiols, nmol/ml</th>
<th>Group</th>
<th>Cystamine (n = 7)</th>
<th>Amlodipine (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vehicle</td>
<td>cystamine</td>
<td>amlodipine</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Cystamine</td>
<td>n.m.</td>
<td>2.47 ± 0.36***</td>
<td>n.m.</td>
</tr>
<tr>
<td>Total glutathione</td>
<td>31.14 ± 1.85</td>
<td>32.78 ± 1.50</td>
<td>37.41 ± 1.19*</td>
</tr>
<tr>
<td>Total homocystine</td>
<td>2.36 ± 0.23</td>
<td>2.94 ± 0.18</td>
<td>3.19 ± 0.32</td>
</tr>
<tr>
<td>Total cysteine</td>
<td>232.7 ± 21.0</td>
<td>214.4 ± 32.9</td>
<td>273.1 ± 32.2</td>
</tr>
<tr>
<td>Reduced cysteine</td>
<td>21.91 ± 2.26</td>
<td>19.48 ± 1.65</td>
<td>12.31 ± 1.96**</td>
</tr>
</tbody>
</table>

Data are means ± SEM obtained in 22 SHR after 10 days of treatment with cystamine (80 mg/kg/day) or amlodipine (10 mg/kg/day).

n.m. = Not measurable. * p < 0.05, ** p < 0.01, *** p < 0.001 vs. vehicle by ANOVA.

Table 4. Normalized small artery structure from SHR treated with cystamine or amlodipine

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Cystamine</th>
<th>Amlodipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen diameter, μm</td>
<td>253.7 ± 4.2</td>
<td>279.2 ± 16.1</td>
<td>269.5 ± 9.3</td>
</tr>
<tr>
<td>Wall thickness, μm</td>
<td>72.58 ± 2.6</td>
<td>72.56 ± 2.4</td>
<td>67.64 ± 3.3</td>
</tr>
<tr>
<td>W:L ratio, %</td>
<td>14.40 ± 0.59</td>
<td>14.14 ± 0.59</td>
<td>13.19 ± 1.0</td>
</tr>
<tr>
<td>WCSA, μm²</td>
<td>33,213 ± 3,340</td>
<td>33,633 ± 3,340</td>
<td>32,132 ± 1,370</td>
</tr>
</tbody>
</table>

Data are means ± SEM. Small mesenteric arteries from 24 SHR (n = 8) were mounted in pressure myographs and normalized to 60 mm Hg. No significant differences were found in small artery structure between groups by ANOVA; however, a trend for increased lumen diameter following treatment with amlodipine or cystamine was observed. WCSA = Wall cross-sectional area; W:L ratio = wall to lumen ratio.

We found inhibition of T-TG attenuates inward remodelling in SHR. Certain limitations exist with this method since vessel segments are not in their habitual environment but mounted in vitro and kept in organ culture medium, which may influence their functional and morphological characteristics. However, vessels maintained viability during the culture period as previously shown [21]. Furthermore, our finding that induction of inward remodelling occurs as a response to long-term vasoconstriction in SHR is consistent with previous findings in Wistar rats [6]. T-TG activity has been shown to be dependent on pressure and recent findings demonstrate that ET-1 increases T-TG mRNA and protein expression in a concentration-dependent manner via the ET_A receptor in rat cardiomyocytes [7, 22], suggesting two potential mechanisms for the induction of inward remodelling by ET-1. Increased tone in small artery smooth muscle cells causes activation of T-TG, which facilitates rearrangement and formation of cross-linking in the ECM, around a narrowed lumen. Addition of selective T-TG inhibitors, including cystamine, attenuates the development of inward remodelling by blocking of T-TG’s active site and thereby blocking of the cross-link formation in the ECM [7]. This was supported by Eftekhari et al. [14] who showed that in vivo activation of resistance arteries induces inward remodelling in normotensive rats, and that this could be inhibited by inhibition with cystamine. The action of cystamine (β-mercaptoethanolamine, MEA, disulfide) is thought to be through its reduction to MEA within the cells. MEA acts as a T-TG substrate and...
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...demonstrated to attenuate phenylephrine-induced inward remodelling in rats, independent of blood pressure [14]. Moreover, a recent study shows that cystamine causes significant attenuation of inward remodelling in pulmonary arteries from rats with chronic hypoxia-induced pulmonary hypertension, with no change in pulmonary arterial pressure [22]. This finding suggests that the role of t-TG in remodelling is not restricted to systemic hypertension but also involves pulmonary hypertension.

We aimed to study if reduction in vascular smooth muscle t-TG activity, following chronic administration of cystamine, could reduce blood pressure. We performed measurements of haemodynamics in conscious SHR with telemetry, which is currently considered the ‘gold standard’ of hemodynamic measurements in laboratory animals, and we observed significant reduction in MAP after treatment with cystamine. Pisteà et al. [13] found no difference in blood pressure between t-TG knockout mice and WT with l-NAME hypertension, despite a more pronounced development of inward remodelling in WT, indicating that in that model blood pressure is unaffected by the alterations mediated by t-TG in the vessel wall. We used a cystamine concentration of 80 mg/kg/day which was higher than that used in the previously mentioned studies of resistance artery structure (10–40 mg/kg/day), but significantly lower than the doses used in neurological research of Huntington’s disease (100–400 mg/kg/day) [29]. We found mean plasma cystamine concentration to be about 2.5 nmol/ml (table 2), and more research is needed to answer whether the reduction in MAP caused by cystamine was due to attenuation of t-TG cross-linking activity or by its vasodilator activity (fig. 2), possibly by interference with t-TG’s role as a G protein in transmembrane signalling [30].

The observed reduction in MAP with cystamine was not associated with change in vascular structure. The lack of improvement in the vascular structure may be because longer treatment is needed or because it was too small to detect. Furthermore, the cross-links mediated by t-TG in ECM have high mechanical and chemical stability which would tend to prevent remodelling, as shown previously in organ culture [14]. The dose of amlodipine used in the study (10 mg/kg/day) did not cause significant reversion of remodelling either, despite its vasodilator effect and previously demonstrated ability to regress structural remodelling in small arteries from SHR [31]; however, there the treatment period was longer (12 weeks) and the dose higher (20 mg/kg/day).

In conclusion, to the extent that cystamine is an inhibitor of t-TG, our results support a role of t-TG in vas-
cular remodelling since cystamine attenuated ET-1-induced remodelling in SHR. Furthermore, mean arterial pressure was slightly reduced following administration of cystamine, but without functional or structural changes in small arteries.

Acknowledgements

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References


