Simultaneous actions of angiotensin (Ang) II to enhance proximal tubular reabsorption and to increase tubuloglomerular feedback (TGF) responses have been proposed to form a potent sodium-retaining mechanism. Angiotensin-converting enzyme (ACE) inhibitors exert their actions on the kidney by inhibiting the effects of Ang II, which includes attenuation of TGF responses. In contrast to acute ACE inhibitor (ACE-I) treatment, we have found normal TGF responses during chronic ACE-I treatment in Fawn-Hooded (FH) rats. However, this study was not designed to specifically investigate TGF responsiveness during chronic ACE-I. Furthermore, FH rats display impaired autoregulation and remarkably low afferent-to-efferent arteriolar resistance ratios. The present study investigated in normal rats whether prolonged administration of ACE-I and AT1-receptor antagonist conditions the TGF system so that responsiveness is restored.

We also studied the mechanism of the normal TGF responses during continued ACE-I. Two options were considered. First was that the attenuation of TGF responsiveness after acute ACE-I concerns the direct effect of inhibition of Ang II formation. In that case, return of TGF responsiveness could be explained by restoration of intrarenal Ang II levels despite continued ACE-I. Return of Ang II activity can be tested by addition of AT1-receptor antagonist during chronic ACE-I. Support for this hypothesis can also be obtained by prolonged administration of AT1-receptor antagonist. Alternatively, attenuation of TGF responsiveness is a consequence of stimulation of intrarenal nitric oxide (NO), caused by ACE-I–induced stimulation of bradykinin action. In the case that the actions of acute stimulation of NO synthesis wane and adapt to the lower Ang II levels gradually, we would expect that NO synthesis (NOS) inhibition would strongly elevate TGF responses during acute ACE-I but have little effect during chronic ACE-I.
Therefore, TGF responses were studied in untreated rats and in rats acutely and chronically treated with ACE-I. To address whether TGF responses are normal as the result of residual AT1-receptor activity, a high dose of the AT1-receptor antagonist losartan (LOS) was infused intrarenally in rats chronically treated with ACE-I. Finally, TGF responsiveness was assessed during chronic treatment with LOS. To address whether TGF responses were normal during chronic ACE-I administration as the result of decreased actions of NO on the TGF system, TGF responses were assessed before and during proximal tubular infusion of the NOS inhibitor nitro-l-arginine (L-NNA) in all groups.

Methods

Animals
Male Sprague-Dawley rats (200 to 350 g; Harlan-Olac) received normal rat chow (Hope Farms) and had free access to tap water. Sentinel animals were monitored regularly for infection by nema-todes and pathogenic bacteria as well as for antibodies for a large number of rodent viral pathogens and were consistently negative throughout the course of the experiment. The Utrecht University Board for Studies in Experimental Animals approved the studies.

Surgical Procedure and Infusions
On the day of the experiment, the animals were anesthetized with Inactin (thiobutabarbital, Research Biochemicals International) (120 mg/kg body wt IP) and placed on servo-controlled surgical table that maintained rectal temperature at 37°C. After intubation of the trachea, a catheter was placed into the left jugular vein (PE50) for infusion of solutions. Through the carotid artery, a catheter was placed into the renal artery (tapered PE10) for the intrarenal infusion of solutions. The catheter was advanced into the renal artery under visual inspection. The femoral artery was cannulated (PE50) to measure arterial pressure and to collect blood samples. The left kidney was approached by a flank incision, freed from surrounding tissue, and placed into a plastic holder. The left ureter was cannulated with PE10 tubing. A 1RB ultrasonic transit-time flow probe was placed around the left renal artery and connected to a transit-time blood flowmeter (model T206; Transonics). An agar wall was made around the kidney to form a saline well.

All animals received an intravenous infusion of a 150 mmol/L NaCl solution containing 6% BSA (Sigma Chemical Co) at a rate of 100 μL/min. After 20 minutes, the infusion was switched to a 150-mmol/L NaCl solution with 1% BSA at 30 μL/min. This infusion was maintained throughout the experiment. An intrarenal infusion of a 150-mmol/L NaCl solution was maintained throughout the experiment at a rate of 10 μL/min. Experimental compounds were added to these standard solutions. After surgery, a 60-minute equilibration period was observed before the start of the measurements. At the end of each experiment, the kidneys were removed, blotted dry, and weighed.

Experimental Design
Measurements were obtained in 5 different groups. In control rats (control, n=6), the intravenous and intrarenal infusions were continued throughout the experiment, as indicated above. After a control maximum TGF response with artificial tubular fluid (ATF), the response was repeated by late proximal perfusion with ATF containing I mmol/L L-NNA in the same nephron for 10 minutes. The late proximal perfusion was continued until stop-flow pressure (SFP) had stabilized for ≥2 minutes. Time control responses were obtained in different nephrons. In these nephrons, a control TGF response with ATF was followed by a second response with ATF. Arterial pressure and renal blood flow (RBF) were measured continuously, and blood samples were taken at 60-minute intervals. To test the adequacy of ACE-I treatment, 25 pmol Ang I was injected intravenously at the end of the experiment.

In group 2 (acute ACE-I, n=6), baseline measurements of mean arterial pressure (MAP) and RBF were followed by a bolus injection (0.2 mg/kg body wt IV) and infusion (3 μg/min IV) of enalaprilat (Merck Sharpe & Dohme). After RBF was stable, assessment of TGF responses, plasma sampling, and the Ang I challenge were all performed similar as in the control group.

In group 3 (chronic ACE-I, n=7), rats were administered 100 mg/L enalapril in 17±2 days in drinking water. To maintain ACE inhibition, this group received the same dosage of enalaprilat during the experiment as the acute ACE-I group. The remainder of the experiment was performed similarly as in the acute ACE-I group.

In group 4 (chronic ACE-I+LOS, n=6), chronically treated enalapril rats (19±1 days) were infused with enalaprilat during the experiment as described above. After baseline measurements, an intrarenal infusion of the AT1-receptor antagonist LOS (50 mg/kg body wt in 20 minutes; Merck Sharpe & Dohme) was started. When RBF was stable, TGF responses were assessed, and plasma samples were collected. Before the LOS infusion, 25 pmol Ang I was injected intravenously to check the adequacy of ACE-I. Before, 30 minutes after LOS infusion, and at the end of the experiment, 100 pmol Ang II was injected intravenously to check the adequacy of AT1-receptor blockade. In pilot experiments, we found that lower dosages of AT1-receptor antagonist only partially attenuated the TGF responses.

In group 5 (chronic LOS), rats were treated with 400 mg/L LOS for 17±2 days in drinking water. During the experiment, no additional LOS was given. After equilibration, TGF responses were assessed and plasma samples were collected. At the end of the experiment, 100 pmol Ang II was injected intravenously to check the adequacy of AT1-receptor blockade.

Analyses, Calculations, and Statistics
Plasma protein concentrations were determined by the Bradford method. Renal vascular resistance (RVR) was calculated as MAP divided by RBF. Colloid osmotic pressure (πc), was calculated from plasma protein content, by the equation from Navar and Navar.11 Maximum TGF-mediated decreases in SFP were obtained as described previously.4 Glomerular capillary pressure (PGC) under stop-flow condition was calculated from the equation $\text{PGC} = \frac{\pi_c}{1 - \frac{5}{6} \pi_c}$, in which $\pi_c$ in femoral artery plasma samples is presumed to equal afferent arteriolar colloid osmotic pressure. Data are expressed as mean±SEM. Clearance data and responses to Ang I were compared by 1-way ANOVA. If a variance ratio reached statistical significance, the Dunnett’s method was performed as a post hoc test. TGF data were compared by 2-way ANOVA for repeated measurements. If a variance ratio reached statistical significance, the Student-Newman-Keuls test was performed as a post hoc test. Since the chronic LOS group was added later, data were compared with the control and chronic ACE-I group only. Pressure and flow systems were connected to a PC by an analog-to-digital converter with 10-Hz sample frequency.

Results

Studies in Control Rats and in Rats With Acute ACE-I Treatment
MAP during acute ACE-I treatment was not significantly lower as compared with that in control rats (Table 1); however, a significant decrease was observed as compared with the MAP in the baseline period of this group (123±2 mm Hg; P<0.05, 1-way repeated-measures ANOVA). RBF was not different but tended to increase during acute ACE-I. RVR and PGC were not different between control and acute ACE-I. The adequacy of ACE inhibition was confirmed with bolus injections of Ang I. As can be appreciated from Table 2, responses to bolus injections of Ang I responses on MAP and RBF were strongly diminished during acute ACE-I but were still detectable.
Repeated measurements of maximum responses during ATF infusion revealed no time-dependent changes. Maximum TGF responses during intraluminal L-NNA infusion in the chronic group are shown in Table 1. Baseline SFP in the control rats was 47.1±2.3 mm Hg (14 nephrons; 6 rats). The maximum SFP response during ATF infusion was 6.5±0.8 mm Hg. Intraluminal infusion of 1 mmol/L L-NNA increased maximum TGF responses to 16.7±1.9 mm Hg (P<0.05 versus ATF).

Baseline SFP in the acute ACE-I rats was 43.6±1.6 mm Hg (12 nephrons; 6 rats; Figure 1). Acute ACE-I virtually abolished maximum TGF responses as compared with the control group (P<0.01 versus control, Figure 1). Addition of L-NNA slightly increased TGF responses; however, the responses were significantly lower than in control rats during L-NNA (P<0.01).

Studies in Rats With Chronic ACE-I Treatment
Chronic ACE-I significantly decreased MAP as compared with control rats (P<0.05; Table 1). RBF and RVR were not different between control and chronic ACE-I. PGC values were lower than in the control group (P<0.05). Similar as in the acutely treated animals, MAP and RBF responses to bolus injections of Ang I were strongly diminished but were still detectable. Again, repeated measurements of maximum responses during ATF infusion revealed no time-dependent changes (Table 2). In chronic ACE-I, TGF responses were normal and not significantly different from responses observed in untreated animals (16 nephrons, 7 rats) and proximal tubular infusion of L-NNA enhanced TGF responses in chronic ACE-I to a similar extent as in the control group (Figure 2A).

Studies in Rats With Chronic ACE-I+Acute Intrarenal LOS Treatment
Chronic ACE-I+LOS significantly decreased MAP as compared with control rats (P<0.01; Table 1). MAP in the chronic ACE-I+LOS group during LOS infusion was significantly lower than before the LOS infusion (98±6 versus 111±8 mm Hg; P<0.05, 1-way repeated-measures ANOVA). RBF and RVR were not different between control and chronic ACE-I+LOS and before and during LOS treatment. The PGC was decreased as compared with the control group (P<0.05). During chronic ACE-I, before LOS infusion, MAP and RBF responses to the Ang I challenge were again strongly diminished (Table 2). Adequate AT1-receptor blockade by LOS was confirmed with bolus injections of Ang II. RBF decreased by 67.5±9.4% before and by 0.1±0.9% 30 minutes after administration of LOS and by 0.0±0.5% at the end of the experiment.

Repeated measurements of maximum TGF responses during ATF infusion revealed no time-dependent changes (Table 3). In chronic ACE-I+LOS–treated animals, the maximum TGF responses were strongly attenuated (12 nephrons, 5 rats; P<0.01 versus control and P<0.05 versus chronic ACE-I; Figure 2A). Intraluminal L-NNA administration now only resulted in a small increase in maximum TGF response to 3.1±0.6 mm Hg (NS versus ATF in the same animals).

Studies in Rats Chronically Treated With LOS
Chronic treatment with LOS had a lower MAP as compared with that in control rats (109±5 mm Hg, P<0.05). MAP was not different compared with chronic ACE-I treatment. RBF was 9.4±1.3 mL/min and RVR was 12.8±2.0 mm Hg·mL⁻¹·min⁻¹ and were not different compared with the other groups. PGC was lower as compared with the control group (60.6±1.1 mm Hg, P<0.05). The Ang II bolus decreased RBF 32.0±7.4% at the end of the experiment. In animals chronically treated with LOS, TGF responses were significantly attenuated during ATF infusion (2.6±0.8 mm Hg; 13 nephrons/5 rats; P<0.05 versus control and chronic ACE-I; Figure 2B). Intraluminal L-NNA administration significantly increased maximum TGF responses to 12.1±1.6 mm Hg (P<0.05).

Discussion
In the present study, TGF responses were investigated during acute and chronic ACE-I administration. Acute ACE-I infusion strongly diminished TGF responses, whereas TGF responses were normal in rats administered ACE-I for 2 to 3

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**Table 1. Systemic Hemodynamic and Clearance Data of the Left Kidney of the Control, Acute ACE-I, Chronic ACE-I, and Chronic ACE-I+LOS Groups**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acute ACE-I</th>
<th>Chronic ACE-I</th>
<th>Chronic ACE-I+LOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>253±4</td>
<td>248±11</td>
<td>251±8</td>
<td>247±10</td>
</tr>
<tr>
<td>Left kidney, g</td>
<td>0.97±0.02</td>
<td>0.94±0.04</td>
<td>0.93±0.05</td>
<td>0.92±0.03</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>126±5</td>
<td>110±6</td>
<td>107±5*</td>
<td>98±6†</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>7.7±0.9</td>
<td>8.5±1.5</td>
<td>8.2±0.4</td>
<td>8.6±1.8</td>
</tr>
<tr>
<td>RVR, mm Hg·mL⁻¹·min⁻¹</td>
<td>17.3±1.9</td>
<td>14.9±2.2</td>
<td>13.5±1.1</td>
<td>13.6±2.5</td>
</tr>
<tr>
<td>PGC, mm Hg</td>
<td>65.4±2.2</td>
<td>61.6±2.2</td>
<td>59.5±1.4*</td>
<td>57.9±2.7*</td>
</tr>
</tbody>
</table>

*P<0.05 vs control; †P<0.01 vs control.

**Table 2. Responses to Bolus Injection of Ang I at End of Experiment in the Control, Acute ACE-I, and Chronic ACE-I Groups and Before Starting LOS Infusion in the Chronic ACE-I+LOS Group**

<table>
<thead>
<tr>
<th></th>
<th>MAP Increase, %</th>
<th>RBF Decrease, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.4±4.3</td>
<td>60.7±9.5</td>
</tr>
<tr>
<td>Acute ACE-I</td>
<td>11.0±3.2†</td>
<td>24.4±7.3†</td>
</tr>
<tr>
<td>Chronic ACE-I</td>
<td>11.0±1.9*</td>
<td>28.8±4.6†</td>
</tr>
<tr>
<td>Chronic ACE-I+LOS</td>
<td>10.6±3.2†</td>
<td>32.7±3.3*</td>
</tr>
</tbody>
</table>

*P<0.05 vs control; †P<0.01 vs control.
weeks. To ensure maintained levels of enalaprilat during the experiment in rats chronically treated with ACE-I, the rats received the identical dose of enalaprilat, as used in the group treated acutely with ACE-I. Despite this acute infusion, TGF responses were normal in the chronically treated rats. Acute intrarenal infusion of LOS in rats treated chronically with ACE-I resulted in strong attenuation of TGF responses. In contrast, rats administered LOS for 2 to 3 weeks had attenuated TGF responses, whereas systemic MAP and RVR were comparable to values observed in the chronic ACE-I group. Intraluminal application of L-NNA enhanced TGF responses in nontreated rats and in rats chronically treated with ACE-I. In contrast, this enhancement was diminished in rats acutely treated with ACE-I and chronically treated with ACE-I and acutely with LOS. Remarkably, TGF responses were enhanced by intraluminal L-NNA administration in the rats that had received prolonged treatment with LOS.

In a previous study from our laboratory, TGF responsiveness was studied during prolonged ACE-I treatment with lisinopril in FH rats. These rats have spontaneous development of hypertension and renal failure; they display impaired RBF autoregulation and have a very low afferent-to-efferent arteriolar resistance ratio. TGF responses in these FH rats were completely normal after administration of lisinopril for 4 weeks. The study, however, was not specifically designed to evaluate TGF resetting, and acute effects of ACE-I on the TGF system were not tested. Lisinopril treatment lowered MAP and PGC and completely prevented proteinuria and glomerular lesions in these rats. The dosage of enalapril used in the present study has been shown by others to prevent renal damage in several rat models. In both the rats that only received enalaprilat acutely and in the rats that had been treated for 3 weeks with enalapril, the degree of attenuation of the response to an intravenous Ang I bolus on MAP and RBF
was similar, suggesting comparable activity of ACE in both groups. Meanwhile, there were large differences between the TGF responses in those two groups. It should be stressed that in the rats chronically treated with ACE-I, enalaprilat was also infused acutely. Altogether, the present study shows that rats subjected to chronic ACE-I treatment display normal TGF responsiveness, whereas MAP and PGC reflected continued inhibition of Ang II formation.

Studies from our own laboratory and studies by Mitchell and Navar have shown that the enhancement of TGF responses by Ang II is not dependent on systemic hemodynamics, as local peritubular infusion of Ang II also increases TGF responses. Conversely, Mitchell and Navar have reported that local peritubular infusion of Ang II also leads to attenuation of TGF responses. These studies make it appear very unlikely that the normal TGF responses after prolonged ACE-I treatment was mediated by systemic hemodynamic factors.

Under normal conditions, TGF responses are under strong influence of NO. As reviewed recently, there are many studies showing a strong positive interaction between Ang II and NO on afferent arteriolar function and TGF responses. Acute ACE inhibition may well cause a dysbalance between the Ang II and NO systems, resulting in a relatively high NO activity and low Ang II activity. During chronic ACE-I administration, however, the balance may be restored, resulting in a low Ang II activity and a low NOS activity. Besides the direct interactions between the NO system and the renin-angiotensin system, bradykinin accumulation in response to acute ACE-I treatment could also account for a dysbalance between NO and Ang II activities as bradykinin stimulates the release of NO. A nonspecific kallikrein antagonist, has been demonstrated to increase TGF responses and to prevent the attenuation of TGF responses of acute captopril treatment.

This study of Schnermann et al. indicated that during acute ACE-I, the influence of NO on the TGF system might be increased. In the present study, acute ACE-I strongly attenuated the NO dependency of the TGF system, and enhancement of TGF responses during chronic ACE-I treatment by L-NNA was entirely normal. These observations argue against the hypothesis that enhanced influence of NO (partially) mediates the attenuation of TGF responses by acute ACE-I and that the conditioning influence of chronic ACE-I administration on TGF responses is due to a decreased influence of NO on the TGF system. The observation that rats treated chronically with LOS had low TGF responses yet were very sensitive to local inhibition NO points to enhanced local influence of NO on the TGF system. A possible explanation for this finding is that NO formation was stimulated by increased activity of AT2 receptors in the setting of AT1-receptor antagonism.

Further experiments were performed to investigate whether the normal TGF response in the rats chronically treated with ACE-I could be due to restored influence of Ang II on the TGF system. In the group treated chronically with ACE-I, TGF responses were strongly attenuated during concomitant infusion of high intrarenal dosages of the AT1-receptor antagonist LOS. Furthermore, chronic treatment with the AT1-receptor antagonist LOS was associated with attenuated TGF responses. This suggests that normal TGF responsiveness during chronic treatment with ACE-I is due to restored influence of Ang II. The findings on Ang I injections are compatible with a sustained inhibition of Ang II formation. Although LOS was infused intrarenally in the rats treated chronically with ACE-I with LOS superimposed acutely, the dose was so high that considerable spillover occurred into the systemic circulation: Blood pressure responses to Ang II injections were strongly inhibited. Nevertheless, the rats treated with ACE-I for 3 weeks and administered LOS acutely had similar blood pressure, RBF, and thus RVR as compared with the rats only treated chronically with ACE-I. In other words, acute administration of LOS intrarenally did not unmask actions of systemic Ang II activity. Meanwhile, TGF responses were different in the two groups, indicating that the actions of AT1-receptor activation on the juxtaglomerular apparatus (JGA) were present.

In view of this seemingly normal local AT1-receptor activation, several potential mechanisms deserve attention. One option is that Ang II levels are restored locally, which could be established in several ways. The JGA or structures very close to the JGA may have the potential to form Ang II out of angiotensinogen. Ang II could also be trapped in the cell and evoke intracellular actions, a process about which only minimal information is currently available. Furthermore, in a chronic situation, the local metabolism of enalaprilat may have been altered. Finally, degradation of Ang II may be locally diminished. In view of the high degree of compartmentalization of components of the renin-angiotensin system, measurement of renal tissue levels of angiotensin peptides will not be informative to resolve this issue.

### Table 3. Zero-Flow SFP and Maximum TGF-Mediated Decreases in SFP in Response to Late Proximal Perfusion With Artificial Tubular Fluid During 2 Responses in the Same Nephron in the Control, ACE-I, Chronic ACE-I, and in Chronic ACE-I+LOS Groups

<table>
<thead>
<tr>
<th>Nephrons/Rats</th>
<th>1st Response</th>
<th>2nd Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SFP&lt;sub&gt;0&lt;/sub&gt;</td>
<td>ΔSFP&lt;sub&gt;MAX&lt;/sub&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>12/6</td>
<td>46.9±2.0</td>
</tr>
<tr>
<td>Acute ACE-I</td>
<td>12/6</td>
<td>43.7±2.2</td>
</tr>
<tr>
<td>Chron ACE-I</td>
<td>16/7</td>
<td>43.8±1.3</td>
</tr>
<tr>
<td>Chron ACE-I+LOS</td>
<td>13/6</td>
<td>39.9±3.2</td>
</tr>
</tbody>
</table>

*P<0.01 vs control; †P<0.01 vs acute ACE-I; ‡P<0.01 vs chronic ACE-I; §P<0.05 vs control.
Another option is that low Ang II concentrations elicit a normal AT1-receptor activity. Potential mechanisms are numerous and include increased AT1-receptor expression and modulation of postreceptor pathways. The presence of AT1 receptors on macula densa cells has been recently demonstrated through the use of immunohistochemistry. No differences in AT1-receptor density in intrarenal arteries and glomeruli could be demonstrated between captopril-treated rats and untreated control rats. This observation is in contrast with the observations that Ang II downregulates and sodium loading increases glomerular and mesangial AT1 receptors, as summarized in a recent review on AT1-receptor regulation. Recently, Peti-Peterdi and Bell reported that Ang II may influence macula densa sodium transport and regulate cell alkalization through the apical Na+/H+ exchanger, through the AT1 receptor. Thus, Ang II may modulate the TGF transmission, at least in part, through a direct effect on macula densa cell function. Thus, normal TGF responsiveness during chronic ACE-I is due to restored influence of Ang II on the JGA and could be caused by increased local AT1-receptor expression and residual Ang II concentrations.

The study also points to an interesting difference between the systemic vasculature and the activity of the TGF system in that the JGA seems to be able to restore the influence of Ang II, in contrast to the vasculature. In a different setup, Thomson et al observed that the TGF system desensitizes with sustained increases in proximal tubular flow by acute administration of benzolamide, a carbonic anhydrase inhibitor. Their explanation was that when TGF resets in response to systemic events, this resetting is actually mediated by a prior sustained alteration in tubular flow such that TGF efficiency is optimized by alignment of the TGF function with ambient flow in each nephron. It could be speculated that a similar phenomenon acts to reset the maximum responses of the TGF system during chronic treatment with ACE-I. Regardless the exact mechanism, the TGF system can exert normal control on renal hemodynamics during chronic treatment with ACE-I.

The present study demonstrates that chronic ACE-I treatment did not affect the responsiveness of the TGF system, in contrast to acute ACE-I treatment. Acute high dosages of AT1-receptor antagonist in rats chronically treated with ACE-I and prolonged administration of AT1-receptor antagonist was associated with strongly attenuated TGF responses. This indicates that during chronic ACE-I treatment, in a dose that had strong antihypertensive effects, TGF responses are normal as the result of a restored influence of Ang II on the TGF system. Numerous options remain toward the potential mechanism, and further study may lead to more information about the function of the highly specialized cells of the macula densa.

Acknowledgments

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References

Normal TGF Responsiveness During Chronic Treatment With Angiotensin-Converting Enzyme Inhibition: Role of AT1 Receptors
Erika Turkstra, Branko Braam and Hein A. Koomans

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