Enalapril Prevents Imminent and Reduces Manifest Cerebral Edema in Stroke-Prone Hypertensive Rats

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Background and Purpose—Stroke-prone spontaneously hypertensive rats (SHRSP), subjected to high NaCl intake, show severe hypertension, organ damage, and early death. Preventive treatment with an angiotensin-converting enzyme (ACE) inhibitor is known to reduce mortality. Previously we found that proteinuria always precedes cerebral edema in SHRSP. Hence, in this study ACE inhibition was started later, ie, directly after manifestation of either proteinuria or cerebral edema.

Methods—SHRSP were subjected to 1% NaCl intake. Group 1 served as a control. In group 2 early-onset treatment with the ACE inhibitor enalapril was initiated after proteinuria was >40 mg/d. In group 3 late-onset ACE inhibition was started after the first observation of cerebral edema with T2-weighted MRI. Cerebral edema was expressed as the percentage of pixels with an intensity above a defined threshold.

Results—In controls median survival was 54 days (range, 32 to 80 days) after start of salt loading. The terminal level of cerebral edema was 19.0±3.0%. Under early-onset enalapril, median survival increased to 320 days (range, 134 to 368 days; P<0.01 versus group 1). Cerebral edema was prevented in all but 1 rat. Systolic blood pressure was slightly and transiently reduced at day 14. Proteinuria was markedly reduced (52±7 versus 190±46 mg/d in group 1 at day 7; P<0.05). Under late-onset enalapril, median survival was 264 days (range, 154 to 319 days; P<0.01 versus group 1). Cerebral edema decreased to baseline levels (9.6±2.9 at day 0 to 3.4±0.5% at day 3; P<0.05). Ultimately cerebral edema recurred in 6 of the 8 rats. SBP decreased slightly at day 7 only. Proteinuria decreased from 283±27 at day 0 to 116±22 mg/d at day 7 (P<0.05). Complete remission of the original locus of cerebral edema was confirmed histologically.

Conclusions—In SHRSP with proteinuria, treatment with an ACE inhibitor both prevented the development of cerebral edema and reduced manifest cerebral edema and proteinuria. Survival was markedly prolonged. These findings support the use of ACE inhibition for treatment in hypertensive encephalopathy. (Stroke. 1998;29:1671-1678.)

Key Words: angiotensin-converting enzyme inhibitors ■ cerebral edema ■ magnetic resonance imaging ■ proteinuria ■ rats

Hypertensive encephalopathy is a dangerous sequel to severe hypertension. The male stroke-prone spontaneously hypertensive rat (SHRSP) is an experimental model of severe hypertension and of the subsequent development of hypertensive encephalopathy as well as renal glomerulopathy.1 By replacing drinking water with a 1% NaCl solution at an early age, the appearance of both cerebral edema and proteinuria is accelerated.2 Many studies in salt-loaded SHRSP have shown that long-term administration of angiotensin-converting enzyme (ACE) inhibitors, when initiated simultaneously with the start of salt loading and thus long before the development of proteinuria and cerebral edema, prevents the development of renal and cerebral damage and reduces mortality in this model.3-12 However, no information is available on the effects of treatment with an ACE inhibitor on survival and on the evolution of cerebral damage in salt-loaded SHRSP at later, and clinically more relevant, stages, ie, after manifestation of either proteinuria or cerebral edema.

We previously used T2-weighted (T2W) MRI to follow the process of appearance and progression of cerebral edema quantitatively in salt-loaded SHRSP.13 A proteinuria level >40 mg/d invariably preceded the occurrence of cerebral edema by 3 to 15 days, and in 70% of the rats cerebral edema could be detected by T2W MRI before the occurrence of neurological symptoms. Thus, proteinuria as well as T2W MRI of the brain appears to be a useful noninvasive tool to facilitate the choice of onset of treatment for long-term studies in this model.

In the present study T2W MRI was applied to examine whether ACE inhibition prevents or delays the occurrence of cerebral edema when treatment is initiated after proteinuria is...
Materials and Methods

Animals
Male SHRSP (n=23), aged 6 weeks, were obtained from IFFA Credo, France. They were housed in constant environmental conditions (12-hour light/dark cycle; humidity 55%; temperature 22°C) and were given free access to a standard rat chow (RMH-TM rat chow; protein 22.2%; fat 4.8%; potassium 0.85%; sodium 0.40%; Hope Farms) and allowed water ad libitum. The protocol was approved by the Utrecht University Committee for study in experimental animals.

Protocol
Baseline measurements were done in all rats at 7 weeks of age. Subsequently, at the age of 8 weeks, all rats were switched to a high salt intake by adding 1% NaCl to the drinking water (170 mmol/L). Salt intake was continuously and randomized into 3 groups. Group 1 served as a control (n=6). In group 2 (n=8), enalapril (100 mg/L) was added to the drinking water after proteinuria was >40 mg/d (early-onset treatment, day 0EARLY). This resulted in an intake of enalapril of 24 mg/kg per day, which is slightly higher than the dose that has been shown to prevent stroke and kidney dysfunction in this model.16 In group 3 (n=9), enalapril was added to the drinking water at the same concentration after the first observation of a focus of cerebral edema with T2W MRI (late-onset treatment, day 0LATE). Because water intake was higher at this time point, the intake of enalapril also increased (see Results). Groups 1 and 3 were subjected to T2W MRI every 3 to 4 days after proteinuria was >40 mg/d, until detection of the first cerebral abnormalities. Groups 2 and 3 were subjected to T2W MRI at days 3 or 4, 7, 56, and subsequently once every 56 days after the start of treatment. In group 1 the rats were subjected to T2W MRI every 3 to 4 days until the experiment was terminated when an animal was very debilitated or died spontaneously.

Proteinuria and Blood Pressure
In all groups, 24-hour urine was collected weekly until proteinuria was >40 mg/d. In group 1, urine collection was continued weekly until the end of the experiment. In groups 2 and 3, urine was collected weekly until 70 days after the start of enalapril treatment, after which it was collected monthly. Before the urine collection the rats were weighed and then housed individually in metabolic cages for 24 hours. During this period water intake was measured, and urine was collected. Urine volume and protein concentrations were determined. Urinary protein was determined with the Bradford method. Systolic blood pressure (SBP) was measured with tail-cuff plethysmography (ITC) weekly in the conscious rats after the rats were prewarmed at an ambient temperature of 37°C.

T2-Weighted MRI
After anesthesia was induced with 1% halothane in N2O/O2 (70%/30%), rats were intubated and mechanically ventilated during the MRI session with the same mixture. Expiratory CO2 was monitored, and the body temperature was maintained at 37°C with a heated water pad. The animals were fixed in a stereotaxic holder to prevent movement and positioned in a 4.7-T SIS Co 200–400 NMR spectrometer. A 120-mm Helmholtz coil was used for both transmission and signal reception. After a sagittal scout image, coronal multislice spin-echo T2 MRI, covering the whole brain (25 slices of 1 mm; echo time, 60 ms; repetition time, 3000 ms; matrix, 128×128; field of view, 40×40 mm; two transitions) was performed.

Calculation of Cerebral Edema
The amount of cerebral edema was determined according to methods described previously.14 In short, the examination was performed as follows: A standard individual image set (SIIS) was collected before salt loading. The four slices caudal of the cerebellum/germinal line in the SIIS were defined as reference area (REF), where edema never occurred. The remaining part of the brain rostral of this line until the last slice with a cortical area was analyzed for the appearance of brain edema (13 to 14 slices). From both areas the mean intensity (mi) of the pixels was computed (pBRAIN REF and pBRAIN SIIS, respectively). The standard deviation (σ) of the baseline pBRAIN SIIS was also calculated. The standardized threshold (pBRAIN standardized threshold) of edema in the brain is unique for an individual animal and is defined as follows:

\[ p_{\text{BRAIN standardized threshold}} = p_{\text{BRAIN SIIS}} + 2 \times \sigma_{p_{\text{BRAIN SIIS}}} \]

In every succeeding MRI experiment the mean pixel intensity in the reference area, pREF, was calculated and multiplied by pBRAIN standardized threshold to give the threshold of the experiment, pBRAIN exp threshold. Every individual pixel with an intensity above pBRAIN exp threshold was considered to indicate edema. Previously we confirmed that T2 prolongation is associated with cerebral edema.13 For spatial evaluation of the progression/regression of edema after day 0, we identified the slice with the primary lesion site from which edema would eventually progress or regress. The percentage of edematous pixels was evaluated in 9 slices: 4 caudal and 4 rostral to the central slice.

Histology
Directly after the last MRI session, the anesthetized animals were thoracotomized, and a cannula was inserted into the left ventricle for cerebral perfusion. A washout with isotonic heparinized (270 IU/kg) saline was performed (2 to 3 minutes), which was immediately followed by perfusion fixation with 4% formaldehyde in 0.1 mmol/L phosphate buffer at a pressure of two thirds times the last SBP measured by tail-cuff plethysmography. Brains of rats that had died spontaneously were collected in formaldehyde for histology. After paraffin embedding, 10-μm serial sections (10 sections at 500-μm intervals) were cut. Staining was performed with hematoxylin-eosin (HE).

Statistical Analysis
Data were evaluated by two-way ANOVA for repeated measurements, followed by a pairwise multiple comparison procedure (Student-Newman-Keuls method). One rat in each of the enalapril treatment groups accidentally died during the anesthesia required for the MRI measurement. Data from these rats were not included in the repeated-measures analysis. Data are presented as mean±SEM. Survival was evaluated with Kruskal-Wallis one-way ANOVA on ranks. Survival data are presented as median and range. P<0.05 was considered statistically significant.

Results

Development of Cerebral Edema and Proteinuria in Controls (Group 1)
All rats in group 1 showed a progressive decrease in food intake and body weight (Table 1), developed cerebral edema, defined as areas with high signal intensity on T2W MRI, at a median of 43 days (range, 25 to 59 days), and died at 54 days (range, 32 to 80 days) after the start of salt loading (Table 2). Edema was determined in each rat in relation to an individual threshold, defined as the mean pixel intensity plus twice the standard deviation at baseline (see Materials and Methods).
TABLE 1. Body Weight, Food, Water, Sodium, and Enalapril Intake in Controls (Group 1) and During ACE Inhibition With Enalapril Initiated After Proteinuria >40 mg/d (Group 2) or After the First Detection of Cerebral Edema (Group 3)

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>Day 0</td>
<td>Day +7</td>
<td>Terminal</td>
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<td>Day +7</td>
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<td>Group 1</td>
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<td>194±6†</td>
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<td>9±1†</td>
<td>17±1*</td>
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<td>Water intake, mL/d</td>
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<td>77±7</td>
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<td>Sodium intake, mmol/d</td>
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<td>Enalapril intake, mg/(kg·d)</td>
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<td>Group 3</td>
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<td>...</td>
<td>43±4†</td>
<td>26±3</td>
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Sodium intake was calculated from both food and water intake.

*P<0.05 vs group 1; †P<0.05 vs group 2.

By definition, 2.5% of the pixels lie above this threshold and are therefore “edematous” at baseline. Cerebral edema in the analyzed slices (13 to 14 slices, rostral of the line cerebrum/cerebellum) increased to a maximum of 19.0±3.0% of the pixels (Table 2). SBP and proteinuria increased to terminal levels of 270±11 mm Hg and 316±53 mg/d, respectively (Table 2).

Effect of Early-Onset Enalapril Treatment at Proteinuria >40 mg/d (Group 2 Versus Group 1)

Enalapril treatment was initiated after proteinuria reached >40 mg/d (defined as day 0EALRY) in 7 animals. At day +7, enalapril intake was 24±2 mg/kg per day and remained practically constant thereafter (Table 1).

After initiation of enalapril, rats in group 2 maintained food intake and showed a progressive increase in body weight, reaching a terminal body weight that was 175% of that observed in group 1 (Table 1). Enalapril did not affect water or sodium intake. Comparison of survival (Table 2) clearly shows the effectiveness of enalapril. Compared with group 1, survival was increased 6-fold in group 2, to 320 days after the start of salt loading (range, 134 to 368 days). After proteinuria was >40 mg/d (day 0EALRY), a locus of cerebral edema appeared in group 1 within a median of 14 days (range, 2 to 27 days), and the percentage of pixels indicating edema increased rapidly (Figure 1a). In contrast, in the rats in group 2 that were treated with enalapril from day 0EALRY, no loci of cerebral edema were observed. The percentage of pixels considered to represent edema only increased very slightly and gradually, from 3.1±0.1% at day 0EALRY to 6.5±1.2% at the last measurement before natural death occurred (P<0.05 versus group 1; Table 2). Only 1 of 7 animals in group 2 eventually showed a locus of cerebral edema during enalapril treatment. In this case, observed postmortem in a rat that had

TABLE 2. Survival After Start of Salt Loading and Terminal Values of Cerebral Edema, SBP, and Proteinuria in Controls (Group 1) and During ACE Inhibition With Enalapril Initiated After Proteinuria >40 mg/d (Group 2) or After the First Detection of Cerebral Edema (Group 3)

<table>
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<tr>
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<th>Median Survival, d</th>
<th>Cerebral Edema, %</th>
<th>SBP, mm Hg</th>
<th>Proteinuria, mg/d</th>
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</thead>
<tbody>
<tr>
<td>Group 1 (n=6)</td>
<td>54 (32–80)</td>
<td>19.0±3.0</td>
<td>270±11</td>
<td>316±53</td>
</tr>
<tr>
<td>Group 2 (n=7)</td>
<td>320 (134–368)†</td>
<td>6.5±1.2*</td>
<td>239±11</td>
<td>229±43</td>
</tr>
<tr>
<td>Group 3 (n=8)</td>
<td>264 (154–319)†</td>
<td>14.8±3.2†</td>
<td>257±7</td>
<td>418±44†</td>
</tr>
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</table>

Values in parentheses are ranges.

*P<0.05; †P<0.01 vs group 1; ‡P<0.05 vs group 2.
Effect of Late-Onset Enalapril Treatment After the First Appearance of Cerebral Edema (Group 3 Versus Group 1)

In this group enalapril treatment was initiated after the first appearance of cerebral edema (defined as day 0\text{LATE}) in 9 rats. At day +7, enalapril intake was $42\pm4$ mg/kg per day, and net intake remained practically constant thereafter, although intake corrected for body weight decreased slightly (Table 1).

Food intake increased rapidly after institution of enalapril in group 3, and body weight showed a progressive increase, reaching a terminal value similar to that observed in group 2 (Table 1). Enalapril increased sodium intake in group 3 because of increases in both food and water intake. As in group 2, comparison of survival (Table 2) clearly shows the effectiveness of enalapril treatment in group 3. Compared with group 1, survival was increased 5-fold in group 3, to 264 days after starting salt loading (range, 154 to 319 days). When related to the first appearance of cerebral edema, all rats in group 1 died within 4 weeks (median, 11 days; range, 3 to 28 days). At day 0\text{LATE} all rats in groups 1 and 3 showed a locus of cerebral edema and a percentage of edematous pixels significantly ($P<0.05$) above baseline as well as day −3, ie, 6.6±1.6% in group 1 and 9.6±2.9% in group 3 (Figure 2a). In group 1 cerebral edema increased to 19.0±3.0% as described above. However, in group 3, as a result of treatment with enalapril, the percentage of cerebral edema returned to levels close to baseline (3.4±0.5% at day +3 to +4) and remained low until a new locus of cerebral edema appeared shortly before natural death at a median of 210 days (range, 70 to 257 days) after 0\text{LATE} in 6 of the 8 rats. The mean level of edema at the last measurement before natural death was 14.8±3.2%, a value that was twice as high as that observed in group 2 ($P<0.05$; Table 2). Figure 3a shows typical consecutive T2W MRI in 1 rat, illustrating development of cerebral edema, its rapid regression due to enalapril treatment, and eventually its reappearance despite continued enalapril treatment.

Previously we observed that cerebral edema in this model invariably starts at a single locus, from which it spreads to adjacent tissue. This phenomenon facilitates quantitative illustration of the dramatic effect of enalapril on cerebral edema. The percentage of edematous pixels in the slice that showed the primary lesion site on day 0 and that in the surrounding slices is shown in Figure 4. It is apparent that at day 0, all slices show an increase in the percentage of edematous pixels per slice. Cerebral edema in the central slice (slice 0) was significantly increased compared with day −3 in both groups ($P<0.05$). However, in group 1 the percentage of edematous pixels in the central slice and the 8 surrounding slices increased slightly at day +3 and substantially at day +7, whereas in group 3 the percent cerebral edema per slice decreased dramatically to prelesional levels at day +3 to +4 and day +7.

As in group 2, enalapril treatment initially caused a slight decrease in SBP. After 7 days, SBP had decreased from 274±2 to 244±7 mm Hg ($P<0.05$; Figure 2b). However, terminal SBP in group 3 (257±7 mm Hg) was intermediate between groups 1 and 2 (Table 2). Enalapril initially caused proteinuria to decrease rapidly from 283±27 to 116±22

been treated with enalapril for 151 days, cerebral edema was accompanied by hemorrhage. The remaining 6 animals did not develop distinct cerebral lesions that could be identified by MRI.

Enalapril treatment initially caused a slight decrease in SBP. After 14 days, SBP had decreased from 265±5 to 247±2 mm Hg, significantly different from the findings in control rats (from 263±5 to 274±8 mm Hg; Figure 1b). However, terminal SBP during enalapril (270±11 mm Hg) was not lower than in the control group (239±6 mm Hg; Table 2). Enalapril initially stabilized proteinuria, whereas this increased progressively in untreated rats. For example, after 14 days proteinuria had decreased slightly from 76±20 to 61±12 mg/dl, whereas it had increased in the untreated group from 94±21 to 331±47 mg/dl (Figure 1c). Eventually, proteinuria also started to increase in the enalapril-treated animals and reached a terminal value similar to that found in untreated rats (Table 2). At this point food intake was also depressed (Table 1).
mg/d within 7 days after initiation of treatment (Figure 2c). In group 1, proteinuria (319 ± 44 mg/d at day 0 LATE) remained high or fell with failing renal function in the final measurement. As in group 2, proteinuria also started to increase in group 3 despite enalapril treatment. Group 3 had a terminal excretion of urinary protein that was numerically higher than that found in the control rats (P ≤ 0.05) and nearly twice as high as that found in the rats in group 2, where enalapril was initiated when proteinuria was less severe (P < 0.05; Table 2).

At this point food intake was markedly depressed (Table 1). It was notable that cerebral edema in the enalapril-treated rats appeared in regions that were not affected previously. This allowed histological evaluation of the originally affected areas, which appeared completely free of edema (Figure 3b).

**Discussion**

In salt-loaded SHRSP with proteinuria we found that oral treatment with the ACE inhibitor enalapril not only prevented the development of imminent cerebral edema but also resulted in the complete dissipation of manifest cerebral edema. These effects were achieved without a sustained fall in blood pressure.

Previous studies have shown that long-term administration of ACE inhibitors, when initiated simultaneously with the start of salt loading and thus months before the development of cerebral damage, can prevent the formation of cerebral edema and reduce mortality in salt-loaded SHRSP.3,4,8,9,11,12 No information is available on the effects of treatment with an ACE inhibitor in this model at a later and clinically more interesting stage, ie, after the manifestation of proteinuria, either when cerebral edema has appeared or at a stage of imminent cerebral edema. In our previous study we demonstrated with MRI that T2 prolongation identified cerebral edema in this model, and we were able to quantify the appearance and progression of cerebral edema. The magnitude of proteinuria and cerebral edema were correlated. Moreover, proteinuria >40 mg/d predicted the imminent appearance of cerebral edema.13 We now applied this experience to explore whether ACE inhibition could prevent imminent cerebral edema, ie, edema expected within 2 weeks...
after proteinuria was >40 mg/d, and whether ACE inhibition could induce regression of manifest cerebral edema when started directly after its initial detection. We reasoned that if ACE inhibition would not be able to resolve existing cerebral edema, it might at least be able to prevent or delay its imminent appearance. Indeed, ACE inhibition could almost completely prevent the occurrence of cerebral edema in the group with proteinuria (group 2). Remarkably, ACE inhibition also had an antiedemic effect when applied directly after the appearance of cerebral edema. In fact, quantitative MRI revealed a complete dissipation of cerebral edema in the original locus and in all surrounding slices.

As a result of ACE inhibition, a small decrease in SBP occurred temporarily for 1 to 2 weeks. Similar small decreases have been reported previously during ACE inhibition in this model. It cannot be excluded that this mild antihypertensive effect contributed to the prevention or resolution of cerebral edema. Moreover, the effect of ACE inhibition on diastolic blood pressure, which cannot be measured with the tail-cuff method, may have been slightly larger. However, because blood pressure was only decreased for 14 days at most after initiation of therapy, it is likely that ACE inhibition contributed to amelioration of cerebral edema. Furthermore, angiotensin can increase permeability of the blood-brain barrier under certain pathological conditions. A reduced angiotensin II level, as a result of ACE inhibition, could therefore reverse the elevated permeability of the blood-brain barrier. Indeed, Takahashi et al showed that disruptions in the blood-brain barrier resolved after treatment with an ACE inhibitor.

Intuitively one would assume that the effectiveness of treatment decreases when started later. In other words, we expected less effect of treatment in group 3 than in group 2. In the kidneys this supposition was confirmed. In group 3 proteinuria was significantly higher than in group 2 at the start of ACE inhibition (283±27 versus 76±20 mg/d; P<0.0001). Despite a marked decrease within 1 week after initiation of ACE inhibition, proteinuria remained much higher (P<0.01) in group 3 (∼120 mg/d; Figure 2) than in group 2 (∼55 mg/d; Figure 1). Eventually proteinuria rose to substantially higher levels in group 3. These data suggest that the protection against further renal damage offered by ACE inhibition is limited by the degree of glomerular injury already present at the start of treatment. Stier et al started treatment with either enalapril or captopril at a baseline protein loss of ∼10 mg/d and succeeded in stabilizing it at this level. Late-onset treatment is thus relatively more effective, but the absolute posttreatment proteinuria is higher than in early-onset treatment (group 2) or than in true preventive treatment, confirming observations in other models. The novel finding of our study is that this was also the case for cerebral edema, which appeared in 75% (6/8) of the rats in which it had been observed previously (group 3) compared with 14% (1/7) in group 2, in which cerebral edema had not been present previously (Z test, P=0.067).

Fortuitously, cerebral edema did not appear at the original focus but in areas that had not been edematous before. This circumstance permitted histological inspection of the original site. The initial blood-brain barrier disruption appeared to have been effectively repaired by ACE inhibition. Nevertheless, some generalized initial vascular damage does seem to have occurred in group 3 because, with one exception, cerebral edema never developed in group 2. Probably the cerebral arteries were damaged slightly in the period (∼14 days) between proteinuria >40 mg/d and the disruption of the blood-brain barrier and development of a distinct locus of edema. The initiation of ACE inhibition was able either to restore the blood-brain barrier or to limit extravasation to such an extent that this was balanced by removal through the cerebral spinal fluid. Eventually edema tended to reoccur. The fact that this was not observed at the original site may be due to the relatively small number of animals in this group. However, it is also possible that the edema did not recur in the same locus because barrier repair resulted in more collagen and a stronger basement membrane.

We monitored the rats until natural death because survival studies after ACE inhibition in SHRSP are rare. In the present study the time of initiation of ACE inhibition did not have a
significantly different effect on mortality, even though terminal values of cerebral edema and proteinuria were both significantly higher in group 3 than in group 2. However, there was a numerical difference, survival being prolonged by ≈20% by the earlier onset of treatment in group 2, in which the median survival was 320 days after initiation of salt loading compared with 264 days in group 3.

In conclusion, we have shown that in salt-loaded SHRSP with proteinuria, long-term treatment with the ACE inhibitor enalapril prevents the development of cerebral edema and causes manifest cerebral edema to disappear. Severe proteinuria was also markedly diminished. The effects of ACE inhibition were achieved with only a slight and temporary fall in blood pressure. These findings support the use of ACE inhibition for treatment in hypertensive encephalopathy.

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References

Editorial Comment

SHRSP, treated with high salt in their drinking water, develop severe hypertension that is associated with proteinuria and cerebral edema. The present study suggests that treatment with enalapril, an ACE inhibitor, is protective and prolongs survival in this model. The ACE inhibitor was effective, even though it was administered after the onset of cerebral edema.

How would an inhibitor of ACE be protective in SHRSP on a high-salt diet? Several mechanisms seem possible. First, ACE inhibitors may be protective because of their antihypertensive effect on blood pressure. Second, ACE inhibitors may be protective because of their antiproteinuric effect. Third, ACE inhibitors may be protective because of their antihypertensive effect on cerebral arterioles. Finally, ACE inhibitors may be protective because of their antihypertensive effect on cerebral arterioles.
tensive effects. This does not seem to be the predominant mechanism, however, since the protective effect of enalapril was not associated with a sustained reduction in arterial pressure. Second, ACE inhibitors may be beneficial by reducing formation of angiotensin II. Angiotensin II could have multiple detrimental effects. For example, in some species, angiotensin II produces constriction of cerebral vessels.\textsuperscript{1,2} In addition, recent evidence suggests that angiotensin II may be an important stimulus for production of superoxide and peroxynitrite (formed by the reaction of nitric oxide and superoxide anion) in blood vessels.\textsuperscript{3,4} One could speculate that treatment of SHRSP with a high-salt diet may increase formation of angiotensin II, which increases formation of superoxide anion, resulting in cytotoxic effects, including increases in permeability of the blood-brain barrier. Oxygen-derived free radicals are known to increase permeability of the blood-brain barrier.\textsuperscript{5} Thus, ACE inhibitors may protect the blood-brain barrier by reducing formation of reactive oxygen species and peroxynitrite in SHRSP on a high-salt diet.

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