Protective effects of delapril, indapamide and their combination chronically administered to stroke-prone spontaneously hypertensive rats fed a high-sodium diet

Giuseppe BIAGINI†*, Michele ZOLI†*, Carla TORRI†, Sabina BOSCHI‡*, Giuseppe VANTAGGIATO‡*, Marco BALLESTRI§, Alberto BARALDI§ and Luigi F. AGNATI†‡
*Department of Biomedical Sciences, Section of Physiology, University of Modena, via Campi, 287, 41100 Modena, Italy, †Interuniversity Centre for the Study of Aging in Brain and Endocrine System, Universities of Milan, Modena and Brescia, via Vanvitelli 32, 20129 Milano, Italy ‡Centre of Clinical Nutrition and Metabolic Diseases, University Hospital, via del Pozzo 71, 41100 Modena, Italy, and §Division of Nephrology, University Hospital, via del Pozzo 71, 41100 Modena, Italy

(Received 15 April/25 June 1997; accepted 14 July 1997)

1. Stroke-prone spontaneously hypertensive rats (SHRsp) have been used widely to test agents putatively capable of vascular protection. These animals present an accelerated time course of hypertension and a reduced life-span. When fed a high-sodium diet from the eighth week of life, a further acceleration in blood pressure increase is obtained, and rats start to die after 5 weeks of diet as a consequence of cerebral haemorrhage. In this model, angiotensin-converting enzyme (ACE) inhibitors were repeatedly proved to prevent vascular lesions and death. Notably, this effect was independent of any hypotensive effect. On the contrary, diuretics were shown not to be equally effective. A combination of ACE inhibitors and diuretics, although known to have synergistic effects in the therapy of hypertension, has never previously been tested.

2. Our aim was to study the effects of long-term treatment with the ACE inhibitor delapril (12 mg day⁻¹ kg⁻¹), the thiazide-like diuretic indapamide (1 mg day⁻¹ kg⁻¹), and their combination (12 and 1 mg day⁻¹ kg⁻¹ respectively), on the survival of SHRsp rats fed a high-sodium diet from the eighth week of life onwards. The effects of the treatments on blood pressure, body weight, food and fluid intake, diuresis, proteinuria and the appearance of lesion signs and death were assessed weekly. When control rats reached 50% mortality, they were killed, together with some drug-treated rats, to compare lesions in brain and kidney. The other drug-treated rats continued treatments until 50% mortality was reached in two treatment groups.

3. All drug treatments were able to delay death significantly when compared with control rats, which reached 50% mortality after 6 weeks of salt loading. This event was preceded by a highly significant increase in proteinuria, diuresis and fluid intake that took place 3 weeks after the increase in blood pressure over the initial range. In delapril- or indapamide-treated SHRsp these changes were never seen, even when animals started to die. In the combination-treated group, a significant increase (P<0.01) in fluid intake and diuresis, but not proteinuria, was observed from the third week of treatment onwards.

4. Treatment with delapril or indapamide did not block the progressive increase in blood pressure as observed in control animals. However, the increase in blood pressure was markedly retarded with respect to control rats. At variance with this, in combination-treated animals blood pressure levels were maintained until the end of the experiment within the 99% confidence interval initially observed in control animals.

5. Infarctual and haemorrhagic cerebral lesions were observed in 38% of control rats; no lesions were noted in brains of age-matched rats receiving a drug treatment. Kidneys from control animals presented major degenerative lesions of glomeruli and arteries, characterized by fibrinoid necrosis. This condition was absent in drug-treated animals, which presented minor signs of ischaemic lesion. Heart hypertrophy, when heart weight was expressed as a percentage of body weight, was similar in saline-, delapril- or indapamide-treated rats. At variance with this, in combination-treated animals the heart weight to body weight ratio was significantly (P<0.01) lower than in the other groups.

6. In conclusion, the diuretic indapamide showed...
similar protective effects as the ACE inhibitor delapril on acute vascular lesions and survival of SHRsp. Moreover, their combination synergized in preventing heart hypertrophy consequent to long-term hypertension. This result is probably related to the enhanced diuresis and the better control of blood pressure levels selectively found in combination-treated animals.

INTRODUCTION

Spontaneously hypertensive rats (SHR) represent a widely accepted model for studying the pathophysiology of hypertension [1]. Several substrains have been developed from SHR, based on the rate and severity of blood pressure increase, the kind of vascular lesions and the mean life-span [4]. Among them, the stroke-prone substrain (SHRsp) is characterized by more quickly reaching higher values of arterial blood pressure and by premature death as a consequence of cerebral haemorrhage [2]. Moreover, it is possible to accelerate the development of hypertension and to shorten the life-span by increasing sodium content in the diet [3]. For these reasons, the SHRsp model has been found to be suitable by many authors for testing pharmacological agents that are putatively capable of cerebrovascular protection.

Although several antihypertensive agents have been shown to be able to prolong life-span in SHRsp [4, 5], it appeared clear that hypertension is not the only factor involved in development of strokes [3]. In fact, the increase in blood pressure levels did not correlate with mortality in SHRsp chronically treated with dexamethasone [6]. Eventually, the angiotensin I-converting enzyme (ACE) inhibitors were shown to have protective effects, independently of any effect on blood pressure increase [7, 8]. Much less effort was made to investigate similar effects for diuretics, which represent another major class of antihypertensive drugs [9]. In fact, diuretics are considered first-line drugs for treatment of hypertension and, when combined with ACE inhibitors, can provide further advantages in case of resistance to single drug administration [10]. Therefore, it is of interest to investigate the effects of diuretics and their combination with ACE inhibitors in the pathophysiology of experimental hypertension.

With this aim, we studied the consequence of chronic treatment with delapril \{N-[N-(3S)-1-ethoxy carbonyl-3-phenylpropyl]-L-alanyl]-N-(indan-2-yl)glycine hydrochloride\}, indapamide \{1-(4-chloro-3-sulphamylbenzamido)-2-methyl-indoline\} and their combination on salt-loaded SHRsp. Delapril is a highly lipophilic ACE inhibitor which efficiently inhibits the activity of the renin–angiotensin system at tissue level [11]. Indapamide is an orally active sulphonamide diuretic agent which also presents a direct vascular activity [12]. Based on a previous study [13], delapril and indapamide were combined at doses shown to induce an antihypertensive effect in SHR.

MATERIAL AND METHODS

Male pathogen-free SHRsp \(n = 88\), 5–6 weeks old, were purchased from Iffa Credo (l’Arbresle, France). They were housed, two per cage, under standard temperature \((24 ± 1°C)\), humidity \((40–60\%)\) and lighting \((\text{light on 7.00 hours–} 19.00\text{ hours})\) conditions. After 3 weeks, they were divided into four treatment groups, receiving, respectively, delapril \((12 \text{ mg day}^{-1} \text{ kg}^{-1})\), indapamide \((1 \text{ mg day}^{-1} \text{ kg}^{-1})\), their combination \((12 \text{ and } 1 \text{ mg day}^{-1} \text{ kg}^{-1} \text{ respectively})\) or saline (control rats) by mouth. Drugs were dissolved in the saline drinking solution \(1\% \text{ NaCl}\) that was administered in order to accelerate the development of strokes. Thus, each drug was taken by the animals continuously over a 24 h period, as described by Smeda and Tkachenko [9]. In order to allow adjustments of drug administration to changes in body weight and fluid intake, drug concentration in the saline drinking solution was re-calculated five times a week. Together with the saline solution, SHRsp were fed pellets resembling in composition the Japanese-style diet (Mucedola s.r.l, Settimo Milanese, Italy), which is characterized by increased sodium \(0.36\%, \text{w/w}\) and reduced potassium \(0.72\%, \text{w/w}\) and protein \(18\%, \text{w/w}\) content [14]. All the procedures used were in accordance with institutional guidelines for animal care (D.L. 116/92) and were approved by the appropriate Italian Ministry (Ministero della Sanità, authorization no. 8/96-B).

In a first experiment (control rats, \(n = 16\); drug-treated rats, \(n = 12\) per treatment group), body weight, food and fluid intake, and diuresis were measured twice a week over a 24 h period, as previously described [15]. Proteinuria was measured once a week using the dye-binding method (Bio-Rad Laboratories GmbH, München, Germany) and BSA as standard [16]. In order to avoid contamination of urinary samples, the animals were housed in metabolic cages for a period of 24 h. Blood pressure measurements were performed three times a week using the tail cuff method (BP recorder, Ugo Basile, Comerio, Italy) as described by Razzetti et al. [13], and the values obtained for each week were averaged. When 50% mortality was reached for each treatment group (with the exception of indapamide-treated rats, which were killed together with the delapril-treated group), surviving animals were killed in order to study chronic lesions in heart, kidney and brain. In this paper, only results concerning heart hypertrophy are presented.

In a second experiment (control rats, \(n = 10\); drug-treated rats, \(n = 8\) per treatment group), acute vascular lesions were assessed in brains and kidneys. The animals were placed on high-sodium diet at
8 weeks of age and killed 8 weeks later when control SHRsp reached 50% mortality. At this time they were anaesthetized by ketamine hydrochloride (Ketalar, Parke-Davis, 100 mg/kg intraperitoneally) and transcardially perfused with 100 ml of saline solution followed by 100 ml of ice-cold Zamboni’s fixative [17]. Brains were dissected out, post-fixed overnight in the same fixative, rinsed in 15% and then 30% sucrose–phosphate buffer solutions to assure cryo-protection, and cut at a cryotome (section thickness = 50 μm). The extent of lesion was evaluated in adjacent sections stained respectively, by means of the Nissl technique or immunocytochemistry, with a polyclonal antibody against glial fibrillary acidic protein (GFAP; Dakopatts Italia, Milan, Italy) [18]. An arbitrary score was assigned to lesions according to the following scale: 0 = absence of lesion, 1 = initial lesion (rarefaction of normally-stained neurons in consequence of extracellular oedema, focal increase of GFAP immunoreactivity without necrosis); 2 = full lesion (chromatolysis or shrinkage of neurons, loss of neurons, red blood cell extravasation, loss of GFAP-positive astrocytes into necrotic areas). Kidneys were dissected out, post-fixed in Zamboni’s fixative, preserved in 70% ethanol, paraffin embedded and then cut at a microtome (section thickness = 2–3 μm). Kidney sections were stained using the Masson’s trichrome and haematoxylin–eosin techniques to assess the presence of vascular and glomerular lesions, as described by Stier et al. [19]. Briefly, lesions found in blood vessels were classified as acute when presenting fibrinoid necrosis, haemorrhagic extravasation, or concentric or nodular proliferation of swollen myointimal cells surrounded by interstitial inflammatory reaction. Chronic vascular lesions were defined by thickening of the vessel wall and concentric myointimal fibroplasia. Glomerular lesions were studied qualitatively, to assess the presence of fibrinoid necrosis, mesangial expansion and sclerosis, and quantitatively by means of computer-assisted morphometry. In particular, glomerular and flocular areas were manually selected and measured; nuclei located in floculi were discriminated by gray tone analysis and were counted automatically. At least 50 randomly selected glomeruli, in which the hilum was clearly detectable, were sampled on two different sections per animal. The analysis was conducted by means of the SIS software (SIS; Münster, Germany) using the procedure described by Weibel and Gomez [20] with modifications [21].

Statistical analysis

The Kaplan–Meier method to estimate survival probability, and the log rank test to compare the different survival curves between SHRsp treatment groups, were used [22]. One-way analysis of variance (ANOVA) for repeated measures was used to compare data on body weight, diuresis, proteinuria and food and fluid intake. After significant ANOVA, a paired t-test was performed to compare each time interval with the previous one within a treatment group. Newman–Student–Keuls’ test for multiple comparison was used a posteriori to compare the different treatment groups. All data were studied with the SPSS package for computer analysis (SPSS Inc., Chicago, IL, U.S.A.). Results are reported as means ± SEM; P<0.05 was chosen as level of significant statistical difference.

RESULTS

As expected, salt-loading and the Japanese-style diet produced an acceleration of vascular lesions in SHRsp. In fact, lesion signs and deaths were detected from the fifth week of salt administration onwards. In control animals, 63% of SHRsp showed functional and/or behavioural lesion signs during the days preceding death. They were principally suggestive of cerebral (hemiparesis, 60%), renal (hematuria, 20%) or pulmonary (dyspnoea, 20%) localization of lesions. The mean time interval between the appearance of lesion signs and death was 5.8±2.4 days. The 50% of control SHRsp died between the fifth and the seventh week of treatment (Fig. 1). On the other hand, SHRsp receiving a drug treatment showed a significantly higher survival rate (χ² = 29.41; P<0.001). Although in indapamide-treated animals one death occurred much earlier than in the other groups, this group exhibited the longest survival time, not reaching 50% mortality.

Fig. 1. Line graph illustrating the cumulative survival rate of SHRsp receiving a drinking solution (1% NaCl, saline-treated SHRsp) or a drug (delapril 12 mg day⁻¹ kg⁻¹, indapamide 1 mg day⁻¹ kg⁻¹, or their combination) dissolved in the drinking solution. At the beginning of fifteenth week of life the saline-treated group reached 50% survival rate. The log rank test showed a highly significant difference between the saline-treated SHRsp and the other treatment groups, which showed much longer survival times (χ² = 29.41; P<0.001). In fact, rats treated with the combination of delapril and indapamide, or with delapril alone, showed a half-life of 30 and 33 weeks respectively. Rats treated with indapamide showed a 25% mortality at the end of the period of observation. No significant differences were found between the groups administered different drugs.
during the entire period of observation (170 days). The delapril and combination treatment groups, instead, reached a 50% mortality after 168 and 147 days of treatment respectively. No statistically significant difference was found between drug treatment groups \( (\chi^2 = 1.76; P = 0.19) \). Time elapsed between the first death and the time of 50% mortality was 11 days for control rats, 69 days for the delapril-treated group and 84 days for the combination-treated group. Unlike saline-treated animals, in drug-treated SHRsp death was never preceded by signs of lesion. On the basis of post mortem examination, they died mainly due to acute pulmonary oedema. Only in one indapamide-treated rat was a thoracic haemorrhage found.

As illustrated in Fig. 2(A), control rats presented an increase in body weight of about 10–20 g/week during the first 4 weeks of treatment. Afterwards, when lesion signs appeared and death occurred, a highly significant \( (P < 0.01) \) decrease in body weight gain was observed. This phenomenon was accompanied by a progressive increase in fluid intake, diuresis and proteinuria (Fig. 2B), and by a decrease in food intake. These changes were particularly evident in the last 2 weeks of observation.

In drug-treated animals, none of the changes described in control rats took place, even in the case of spontaneous death (Fig. 3). Body weight increased constantly until the end of the experiment (Fig. 3A). Fluid intake (Fig. 3B) and diuresis (Fig. 3C), with some occasional oscillations, were substantially maintained at a fairly constant level in each treatment group, but showed marked differences between groups. In the combination-treated group we observed a significant \( (P < 0.01) \) increase in diuresis from the third week of treatment onwards (Fig. 3C). This effect was maintained for the entire period of observation and could justify the slower increase in body weight observed from the fourth week of treatment onwards in this group. It should be noted that the decrease in body weight gain in the combination-treated group was neither related to animal death, which took place later (Fig. 1), nor to loss of renal function. In fact, with the exception of small occasional differences, the same level of proteinuria was found in all treatment groups for the entire period of observation (Fig. 3D).

The appearance of signs of lesions in control animals followed the acceleration of blood pressure increase caused by salt-loading (Fig. 4). In fact, after 3 weeks of treatment, blood pressure levels were above the 99% confidence interval calculated in the first week of treatment (201–234 mm Hg), reaching the peak value 3 weeks later. Instead, in drug-treated rats the increase in blood pressure levels over the range observed in the first week was markedly delayed. In delapril-treated rats blood pressure levels increased above the 99% confidence interval of control animals after 8 weeks of treatment, reaching a peak value higher than the peak of control rats 6 weeks later. The same phenomenon was observed in indapamide-treated rats, but with a further delay of 4 weeks. Nevertheless, in this group the peak value was reached only 2 weeks after the first significant increase in blood pressure. In contrast, the combination of delapril and indapamide efficiently maintained blood pressure within the initial range of controls. In the control group, spontaneous deaths were recorded after only 2 weeks of increased blood pressure levels, and the highest death rate (55% total deaths) coincided with the peak value of blood pressure. On the other hand, in delapril-treated rats the first spontaneous death was observed after 6 weeks of increased blood pressure levels. In indapamide-treated rats (excluding the
Fig. 3. Time course of body weight (A), fluid intake (B), diuresis (C) and proteinuria (D) in SHRs treated with delapril (12 mg day⁻¹ kg⁻¹), indapamide (1 mg day⁻¹ kg⁻¹) or their combination. In the combination-treated group, significant increases in diuresis and fluid intake with respect to the other groups of animals were detected from the third week of treatment onwards. This effect is probably related to the lower body weight value observed in this group from the fifth week of treatment until the end of the experiment. In drug-treated animals, only random oscillations in proteinuria levels were observed, never showing the sharp increase found in control rats. * = P < 0.05, ** = P < 0.01 with respect to the other treatment groups; # = P < 0.05, ## = P < 0.01 combination compared with delapril (A and D) or indapamide (C) treatment groups, ANOVA followed by Newman–Student–Keuls’ test for multiple comparison.
dead animal when blood pressure levels were still low), spontaneous deaths were recorded starting from the ninth week of increased blood pressure levels. In the case of delapril or indapamide treatment groups, the peak value of blood pressure was not associated with a higher number of spontaneous deaths. In the combination-treated group spontaneous deaths occurred independently of marked changes in initial blood pressure levels.

The study of cerebral lesions and heart weight in surviving animals showed significant differences between control and drug-treated SHRsp, and also between the groups administered different drugs (Table 1). Haemorrhage and infarcts in the cerebral cortex were observed in 38% of control animals. In delapril- or combination-treated animals, no lesions were present at all. In the indapamide-treated group only one animal, which manifested hyperreactivity 4 weeks before death, presented a focal haemorrhage in the cerebral cortex. Heart weight was significantly lower in control animals with respect to drug-treated SHRsp ($P<0.01$), but the heart weight to body weight ratio was similar to that of delapril- and indapamide-treated animals. Interestingly, the combination-treated animals presented a lower heart weight with respect to delapril- and indapamide-treated rats ($P<0.01$), and the heart weight to body weight ratio was significantly lower than in control rats also ($P<0.05$). The indapamide-treated group presented a lower heart weight than that of delapril-treated animals ($P<0.05$), but the heart weight to body weight ratio was similar in both groups.

In the second experiment, we compared lesions in kidney and brain in drug-treated animals and control rats after 8 weeks of salt-loading. Histological
evaluation of the kidneys demonstrated the presence of glomerular and vascular lesions resembling those described for human accelerated hypertension. In particular, glomeruli presented focal mesangial proliferation with matrix expansion, segmental sclerosis, scattered areas of fibrinoid necrosis as well as ischaemic wrinkling of the floculi (Fig. 5A). All these conditions were found also in drug-treated SHRsp, but to a lesser extent when compared with control rats (Figs. 5C, D and E). In fact, as shown by computer-assisted morphometry, glomerular and flocular areas were significantly ($P < 0.01$) decreased in control rats with respect to the other treatment groups (Table 2). Accordingly, a higher number of nuclei per unit of flocular area was found in control rats ($P < 0.01$ compared with all drug treatment groups). On the other hand, the ratio between glomerular and flocular areas, which indirectly measures flocular wrinkling, was not significantly different between the various treatment groups, suggesting that sclerosis rather than ischaemia is responsible for decreased glomerular size in control rats. Differences between treatment groups were found also in the type of blood vessel lesions. In control animals, interlobular and afferent arterioles showed narrowing or obliteration of the lumen, caused by concentric proliferation of myointimal cells. Arteriolar walls frequently presented acute lesions (around 68% of vessels examined) in control but not in drug-treated rats (Fig. 5B). In contrast, chronic vascular lesions were more frequently observed in drug-treated (48%, 46% and 45% of vessels examined respectively in delapril, indapamide and combination treatment groups) than in control rats (22%).

The histological and immunocytochemical studies performed on brains of SHRsp showed no patent lesions in delapril- or indapamide-treated animals, and only minor signs of lesion were scored in the cortex of one animal of the combination-treated group (Fig. 6). On the other hand, infarcts and haemorrhages were both observed in three out of the
was observed in striatum, thalamus or in the hippocampus characterized by loss of neurons and by flogistic glial reaction studied by GFAP immunocytochemistry.

The lesions were widely distributed in the neocortex and occasionally were located also in the lateral hypothalamus. No lesion was observed in striatum, thalamus or in the hippocampus. At the microscopic level, lesions were present around the necrotic areas (results not shown).

DISCUSSION

The present study shows that delapril, indapamide and their combination significantly delay spontaneous death in salt-loaded SHRsp with respect to control animals. This result was probably achieved by preventing development of acute vascular lesions in kidneys and brain. In fact, drug-treated rats never developed a massive proteinuria and fatal stroke, but died as a consequence of acute dyspnoea caused by pulmonary oedema. Notably, the mean survival time of salt-loaded SHRsp receiving a drug treatment was similar to that of normally fed SHRsp of the Kyoto colony, that is around 33 weeks [4]. Thus, the reduction in life-span usually seen in SHRsp when fed a high-sodium diet was completely prevented by treatments with delapril or indapamide or their combination.

The average time of death was slightly different for the various treatments, but clearly demonstrates that the diuretic indapamide is as effective as the ACE inhibitor delapril in delaying spontaneous death in SHRsp. Whereas many studies on diet-induced stroke in SHRsp have reported protection by administration of ACE inhibitors, such as enalapril [7], captopril [8], trandolapril [23], quinapril [24], imidapril [25] and delapril itself [26], indapamide has never been shown to have similar effects. A previous study in salt-loaded SHRsp, designed to test the effects of treatments with low, non-hypotensive doses of several diuretics, namely chlorothiazide, amiloride, acetazolamide and furosemide, failed to show any positive result in relation to survival [9]. Moreover, furosemide was found to accelerate the occurrence of death after the onset of stroke. At variance with this, we have shown that indapamide is a very effective protective agent. It is possible that this difference to the other diuretics tested in SHRsp depends on peculiar pharmacological effects of indapamide, such as its ability to counteract the vasoconstrictor activity of some endogenous substances such as thromboxanes or biogenic amines [12, 27]. Accordingly, other drugs known to modulate vascular contractility have been shown to protect salt-loaded SHRsp from death [28]. In addition, indapamide was shown to possess a pronounced antioxidant activity, which was related to nephroprotection in an animal model of salt-induced hypertension [29]. Free radicals seem to be involved also in SHRsp vascular lesions, since increased levels of blood lipid peroxides and decreased levels of glutathione peroxidase were demonstrated during stroke development [30].

It is well accepted that treatments leading to reduction of blood pressure levels counteract the
appearance of spontaneous death in SHRsp [31]. This effect was demonstrated for drugs belonging to several classes of antihypertensive agents [5]. In particular, it has been hypothesized, on the basis of drug treatments and epidemiological observations, that cerebrovascular lesions may appear when blood pressure levels increase above 220 mmHg, and never develop when blood pressure is kept lower than 200 mmHg [31]. However, several lines of evidence indicate that the antihypertensive property of ACE inhibitors may not be sufficient or even necessary to explain their protective activity [7, 8, 32]. In fact, Stier et al. [7, 8] showed that administration of enalapril (15 mg day⁻¹ kg⁻¹) or captopril (50 mg day⁻¹ kg⁻¹) can prevent spontaneous death of salt-loaded SHRsp, causing only a small delay in the increase of blood pressure. Thus, it is possible that ACE inhibitors produce vascular protective effects in SHRsp without lowering blood pressure levels below 200 mmHg. In accordance with this hypothesis, our results show that SHRsp treated with delapril survived several months longer than control animals with blood pressure levels equal to or higher than those observed in this latter group. Interestingly, we have found the same effect in SHRsp treated with the thiazide-like diuretic indapamide. Indeed, the survival time of delapril- or indapamide-treated animals was comparable with that of the animals treated with the combination, which, on the other hand, did not show the progressive increase in blood pressure levels observed in the other groups. It should, however, be considered that the small delay in blood pressure increase that we observed in delapril- or indapamide-treated animals may be sufficient to prevent the development of the malignant consequences of accelerated hypertension or to activate protective mechanisms. In fact, it is known that repeated exposure to subthreshold ischaemic injury can induce, at least in the brain, adaptive changes that increase resistance to further insults [33]. Nevertheless, in the case of treatment with the angiotensin II (Ang II) antagonist losartan, complete protection of salt-loaded SHRsp at doses not capable of modifying the time course of the blood pressure increase [32].

Several lines of evidence suggest that Ang II is involved in stimulating smooth muscle cell hypertrophy and hyperplasia [34], increased endothelial permeability to plasma proteins [35] and neutrophil chemotraction [36]. On this basis, it has been hypothesized that the vascular protective activity of ACE inhibitors may depend on the antagonism of Ang II toxic effects on the arterial wall [32, 37]. In particular, Ang II was shown to increase the production of transforming growth factor β in kidney, leading to increased glomerular sclerosis [38]. As we have shown, glomerular sclerosis is a frequent finding in salt-loaded SHRsp and can be prevented by treatment with the ACE inhibitor delapril. Importantly, nephroprotection by the administration of an ACE inhibitor has previously been recognized as a phenomenon that is independent of the control of blood pressure levels [39], and can thus, itself, explain the prevention of stroke in SHRsp [19, 40]. In fact, in these animals, renal failure generally precedes the appearance of stroke [41]. Our results suggest that indapamide, similarly to delapril, can have nephroprotective effects, resulting in the prevention of acute vascular and glomerular lesions as well as in the maintenance of low levels of urinary protein excretion. In line with these results, indapamide was recently found to be as effective as captopril in the control of microalbuminuria associated with diabetes mellitus [42].

The combination of delapril with indapamide did not produce additional protective effects with regard to renal lesions and survival. On the other hand, a clear prevention of heart hypertrophy was found in animals treated with the combination of delapril and indapamide, but not with these drugs alone. This difference can be partly explained by considering that only the combination of delapril and indapamide was able to promote diuresis and to control the increase in blood pressure levels, thus limiting their impact on the heart. The absence of a diuretic effect in rats treated with indapamide alone was not surprising, since the dose administered was largely subthreshold for this effect [43]. However, it is known that diuretics and ACE inhibitors have synergistic effects when administered together, for instance in the treatment of hypertension [10]. Similarly, a synergism may exist also for other pharmacological effects, like the increase in natriuresis and diuresis that follows treatment with diuretics. Although diuresis is generally not potentiated by the combination of diuretics with ACE inhibitors [44, 45], indapamide was shown to be an exception [13]. In agreement, our results provide additional evidence for a long-term stimulation of diuresis by combining indapamide with delapril in salt-loaded SHRsp. There is no simple explanation for this effect. However, it has recently been shown that indapamide is able to enhance the bradykinin-dependent synthesis of cyclic guanosine monophosphate, which is an indirect index of nitric oxide (NO) synthase activity [46]. This effect could be of particular importance, since mice lacking the bradykinin B₂ receptor, which stimulates prostaglandin and NO synthesis, were shown to develop hypertension when fed a high-sodium diet [47]. In the presence of chronic ACE inhibition, the kinin breakdown is diminished, allowing accumulation of bradykinin in tissues [48]. In such a way, indapamide may synergize with delapril by potentiating bradykinin effects on NO, which is a modulator of pressure diuresis and natriuresis in kidney, as well as a vaso-dilator [49]. In line with this hypothesis, evidence of increased NO production during treatment with indapamide and the ACE inhibitor perindopril was recently obtained [50]. Notably, a similar mechanism is potentially also involved in preventing heart hypertrophy, since the B₂ receptor antagonist ica-
bant can block the cardioprotective effects of ACE inhibitors [48].

In conclusion, our study confirms that ACE inhibitors have a protective activity in salt-loaded SHRsp, independently of major effects on blood pressure. It demonstrates for the first time that also indapamide, a thiazide-like diuretic, possesses a similar activity at a dose that delays, but does not steadily counteract, the effect of high-sodium intake on blood pressure levels. No further advantages in survival were found by treatment with the combination of delapril and indapamide, although blood pressure was maintained within the lower range. These findings minimize the importance of high blood pressure as a causal factor of stroke in this SHR substrain. On the other hand, heart hypertrophy was significantly counteracted in animals treated with the combination of the two drugs. This result supports the notion that indapamide can synergize with ACE inhibitors in the control of hypertension-related cardiovascular diseases.

ACKNOWLEDGMENTS

We thank Dr R. Razzetti for useful suggestions and Dr B. Keeling for correction of the manuscript. Delapril and indapamide were kindly provided as gifts by Chiesi Farmaceutici S.p.A., Parma, Italy. The study was funded by the Italian National Research Council (CNR grant no. 95.02477.CT04).

REFERENCES

43. Landriani L, Barlocco D, Pinna GA, Demontis MP, Miele M, Enrico P, Anania V. Diuretic agents related to indapamide. III. Synthesis and pharmacological activity of N-(4-chloro-3-sulphamoylbenzamido)-1,2,3,4-tetrahydroquinolines and 1,2,3,4-tetrahydroisoquinolines. Farmaco 1989; 44: 1059–68.