ELSEVIER

European Journal of Pharmacology 333 (1997) 39–45

ejp

Angiotensin receptor antagonists delay nitric oxide-deficient stroke in stroke-prone rats

Saleem Ahmad *

Department of Pharmacology, Arnold and Marie Schwartz College of Pharmacy and, Health Sciences, Long Island University, Brooklyn, NY 11201, USA

Received 30 January 1997; revised 10 June 1997; accepted 13 June 1997

Abstract

We investigated whether chronic deficiency of nitric oxide (NO) in stroke-prone spontaneously hypertensive rats (SHRSP) precipitates stroke and whether exogenous nitrates and other pharmacological agents can prevent stroke. Groups of five-week-old male SHRSP rats chronically received saline, L-nitro-arginine methyl ester (L-NAME) in saline, L-NAME along with pharmacological agents (L-arginine, isosorbide dinitrate, enalapril maleate and L-158,809; angiotensin receptor antagonist; 5,7-dimethyl-2-ethyl-3(−)[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-imidazo[4,5-b]pyridine) in saline to drink. The development of visible neurological deficits following various treatments was considered as an occurrence of stroke. Within hours following onset of stroke, the rats were anesthetized, catheterized and attached to a Cardiomax blood pressure recorder. SHRSP treated with L-NAME (10 ± 2 mg/day) developed stroke in 11 ± 2 days while no neurological deficit was seen in animals receiving saline till the end of the study period (35 days). Blockade of the renin–angiotensin system with enalapril or L-158,809 significantly delayed the onset of stroke (19 ± 2 and 20 ± 2 days, respectively), but caused only slight reductions in mean arterial blood pressure. These results suggest that chronic inhibition of NO synthase in SHRSP is associated with the development of stroke and such stroke appears to be renin–angiotensin system-dependent. © 1997 Elsevier Science B.V.

Keywords: Angiotensin II; Enalapril; L-nitro-arginine methyl ester (L-NAME); Nitric oxide (NO); Stroke

1. Introduction

Endothelium derived relaxing factor (EDRF), now known as nitric oxide (NO) plays a major role in the regulation of peripheral and cerebral hemodynamics. Within the central nervous system, NO is released as a neurotransmitter where it has been shown to modulate the central regulation of blood pressure (Bredt et al., 1990; Togashi et al., 1992; Dawson and Snyder, 1994). Several studies have suggested that a continuous release of NO in blood vessels maintains vascular tone and tissue perfusion (Gardiner et al., 1990; Ribeiro et al., 1992; Buchanan and Phillis, 1993). Nitroxidergic nerves releasing NO in cerebral blood vessels have been identified (Toda and Okamura, 1990). NO is synthesized via the NO synthase-mediated conversion of the amino acid, L-arginine, to L-citrulline (Moncada et al., 1989). Whether impaired NO synthase activity is directly related with the occurrence of vascular diseases such as myocardial infarction and/or stroke remains to be determined. It has been suggested that predisposition to stroke in stroke-prone spontaneously hypertensive rats (SHRSP) may be due to reduced NO-mediated vasodilation as a consequence of impaired NO synthase activity (Yang et al., 1991).

The use of competitive NO synthase inhibitors to deplete NO has emerged as one of the primary tools to investigate the role of NO in the regulation of the cerebral circulation. *N*-nitro-L-arginine methyl ester (L-NAME) is one of the most potent NO synthase inhibitors available to date (Moore et al., 1990). Chronic administration of L-NAME has been shown to cause an elevation in mean arterial blood pressure (Arnal et al., 1992) and reduction in cerebral blood flow (Izuta et al., 1995). Whether the L-NAME-induced reduction in cerebral blood flow is renin–angiotensin system-dependent remains to be clarified. Using chronic administration of L-NAME in vivo, we designed the present study with two major objectives. The first objective was to examine whether chronic inhibition of NO can precipitate stroke in SHRSP. The second objective was to determine whether such stroke due to depletion of NO could be prevented by exogenous nitrates and/or angiotensin receptor antagonists.

* Tel.: (1-718) 488-1062; Fax: (1-718) 488-3437.

2. Materials and methods

2.1. Chemicals and drugs

Enalapril maleate, isosorbide nitrate, L-arginine, *N*-nitro-L-arginine methyl ester (L-NAME) and 2,3,5-triphenyl–tetrazolium chloride (TTC) were obtained from Sigma (St. Louis, MO, USA). The angiotensin AT_1 receptor antagonist, L-158,809 (5,7-dimethyl-2-ethyl-3(-)[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-imidazo[4,5-b]pyridine) was obtained from DuPont Merck (Wilmington, DE, USA).

2.2. Animals

Four-to-five-week-old male SHRSP (70–90 g) were obtained from a locally maintained colony at the Animal Care facility of Long Island University. Rats were housed in groups, allowed free access to water and standard rat chow which consisted of fat (4%), K^+ (1%), protein (23%) and Na^+ (0.30%) by weight (PMI feed, New York, NY, USA). Room temperature was maintained at 25°C and an alternate 12 h day and night cycle was maintained. At 5–6 weeks of age, the animals were transferred to metabolic cages and housed individually with free access to rat chow and drinking water. Prior to initiation of treatment, a 48–72 h period was allowed for adaptation of the rats. For each set of experiments, control and experimental groups of SHRSP were litter mates. The procedures used were in accordance with the US Health Service, National Institutes of Health guidelines for the care and use of laboratory animals.

2.3. Experimental protocol

After the adaptation period, four groups of SHRSP received the following treatments. Group I ($n = 11$) received saline (1%) and group II ($n = 14$) received L-NAME (0.5 g/l) in saline to drink. This dose was previously shown to produce maximal inhibition of vascular NO synthase and sustained elevations in blood pressure (Arnal et al., 1992). Group III ($n = 7$) of SHRSP received L-NAME (0.5 g/l) and arginine (5 g/l) in saline to drink. Group IV ($n = 8$) received L-NAME (0.5 g/l) and isosorbide dinitrate (20 mg/l) in saline to drink. Measurements of body weight and drinking volume were carried out every 48 h for five weeks. Physical activity of all the animals was monitored twice a day. Onset of stroke was detected with the sudden development of visible neurological deficits (e.g., monoplegia or paralysis of a limb). Within 12 h of onset of stroke, the rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and allowed to breathe spontaneously through a tracheostomy tube. Body temperature was maintained at 37°C using a heating lamp connected to a temperature regulator. A jugular vein was cannulated with polyethylene tubing (PE-50) for the supplemental doses of anesthetic agent. The right carotid artery was catheterized with PE-50 tubing and attached to a transducer, which was coupled to the Cardiomax-II, model 85 (CMX/IBM-PX computer; Columbus instruments, Columbus, OH, USA) for the determination of cardiovascular parameters. Following 20 min for stabilization after surgery, blood pressure and heart rate recordings were taken every minute for the period of 60–90 min. Immediately following cardiovascular recordings, the animals were decapitated and the brains of these SHRSP were rapidly removed, weighed and prepared for staining. Seven coronal sections (1.2–1.5 mm thick) were cut from the frontal pole of the brain of each rat, stained immediately by incubation for 30 min in 4% TTC at 37°C and fixed by immersion in 10% buffered formalin solution (Forsting et al., 1995). TTC stains normal brain tissue (intact cellular membranes) red, whereas ischemic tissue turns pink and necrotic tissue turns grayish.

In a second series of experiments, four groups of SHRSP were used. Group V ($n = 5$) and group VI ($n = 5$) received saline and L-NAME (0.5 g/l) in saline to drink, respectively. Group VII ($n = 8$) received L-NAME (0.5 g/l) and enalapril maleate (an angiotensin converting enzyme inhibitor; 20 mg/l) in saline. Group VIII ($n = 5$) received L-NAME (0.5 g/l) and L-158,809 (an angiotensin AT_1 receptor antagonist; 20 mg/l) in saline. All parameters were recorded as described above. Additional experiments were carried out to examine the pre-stroke status of cerebral tissues and cardiovascular parameters. SHRSP groups IX ($n = 9$), X ($n = 10$), XI ($n = 10$) and XII ($n = 9$) received saline, L-NAME in saline, L-NAME plus enalapril in saline and L-NAME plus L-158,809 in saline to drink. Half the SHRSP (4 or 5 rats) from each group were anesthetized on day 5 and the remaining half on day 10. Following cardiovascular measurements, histological parameters were recorded.

2.4. Statistical analysis

The data were expressed as means ± S.E.M. Data from different animal groups receiving the same treatment (repeat experiments) were not combined. A generalized Wilcoxon test was used to compare the time of onset of stroke between several SHRSP groups. One-way analysis of variance (ANOVA) was used to determine the treatment effect on cardiovascular and other parameters of different SHRSP groups. Bonferroni post hoc tests were performed to compare group means. $P < 0.05$ was chosen as the minimum criterion for statistical significance. GraphPAD InStat software was used for statistical analyses.

3. Results

Except for one animal, SHRSP drinking saline (group I) had not developed stroke at the end of the study period (5th week). However, mild infarctions in basal ganglia

were seen in all rats from this group when histologically examined. No cerebral infarctions were present in age-matched SHRSP that were drinking water (negative control).

In group II, chronic oral administration of L-NAME (0.5 g/l) precipitated stroke in most animals within 2 weeks (Fig. 1). At this concentration, the daily intake of L-NAME was 10 ± 2 mg/100 g rat. The first incidence of stroke occurred on day 7 (1 rat) whereas the majority developed stroke between days 10 and 12 while one rat did not develop stroke till day 14. The average stroke-free period observed in this group was 11 ± 2 days. The onset of stroke was identified with the development of left or right forelimb monoplegia and in one case with paralysis of both forelimbs. Gross examination of brains demonstrated massive hemorrhage in 2/14 SHRSP. Examination of brain slices after stroke also revealed the presence of cerebral infarctions. Infarcted areas were detected on the left or right side of 9 SHRSP while 5 animals presented with lesions on both sides of the cerebral hemispheres. However, one side was always more affected. This observation is consistent with the existence of contralateral monoplegia. The softening in the global regions of cerebral cortex was well recognized. Examination of coronal sections demonstrated infarction at the 3rd to 4th slice levels. The basal ganglion region was always infarcted (Fig. 2). In a separate set of experiments, age-matched normotensive Wistar Kyoto (WKY) rats also received chronic oral L-NAME treatment (0.5 g/l in saline) for 5 weeks. Although not histologically examined, none of the WKY rats developed monoplegia or any physical disability (data not shown).

Group III of SHRSP receiving L-NAME and arginine or group IV receiving L-NAME and isosorbide did not experience a delay in the onset of stroke (Fig. 1). Average post-treatment stroke-free periods in these groups were 11 ± 2 and 12 ± 3 days, respectively. Post-stroke examination of cerebral tissue revealed a picture similar to that of group II receiving L-NAME alone.

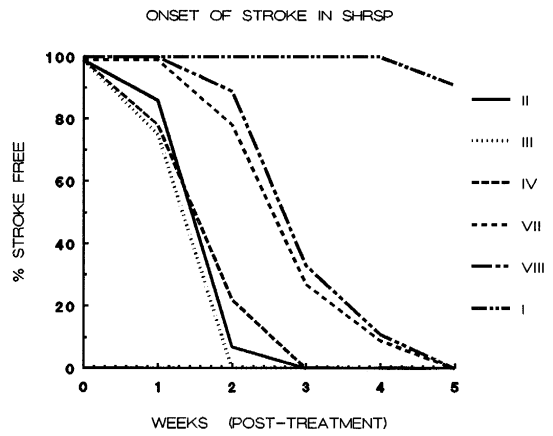

Fig. 1. Plot showing the onset of stroke in SHRSP following different treatments given in saline as drinking solution as described in Section 2. SHRSP received saline (group I; $n = 11$), L-NAME (group II; $n = 14$), L-NAME + L-arginine (group III; $n = 7$), L-NAME + isosorbide dinitrate (group IV; $n = 8$), L-NAME + enalapril maleate (group VII; $n = 8$) and L-NAME + L-158,809 (group VIII; $n = 5$). Statistical analysis with the Wilcoxon test showed that onset of stroke was significantly delayed in SHRSP in the enalapril ($P < 0.01$) and L-158,809 ($P < 0.01$) groups.

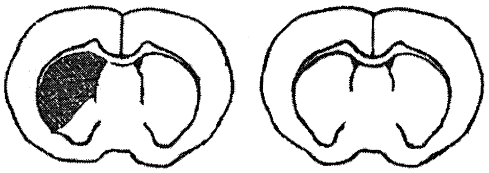

Fig. 2. Drawing of TTC-stained, 3rd coronal section from the frontal pole of SHRSP brains. L-NAME-induced stroke on the left (hatched lines; basal ganglion area) and control on the right (10th day following saline treatment).

SHRSP receiving L-NAME + enalapril (group VII) and L-NAME + L-158,809 (group VIII) had a delay in the onset of monoplegia with most strokes occurring between days 16 and 21. Average stroke-free periods in these groups were 19 ± 2 ($P < 0.01$ vs. group II) and 20 ± 2 days ($P < 0.01$ vs. group II), respectively (Fig. 1). In both groups, post-stroke examination of 3rd and 4th coronal sections of brain demonstrated the presence of infarctions in the basal ganglion area.

In subsequent experiments, pre-stroke gross examination of SHRSP brains (groups XI and XII) indicated no cerebral softening in the presence of angiotensin antagonists. This was further evidenced by the lower pre-stroke brain tissue weights (Table 1). However, examination of cerebral slices from SHRSP indicated the presence of mild infarctions on either side of the hemispheres while no physical disability was detected.

Consistent with the inhibition of NO synthase, chronic administration of L-NAME in saline elevated the blood pressure in SHRSP. Within five days, mean and systolic blood pressures rose to 200 ± 5 and 232 ± 5 mmHg, respectively (group X; Table 3). On day 10, the pre-stroke mean arterial pressure was even higher (209 ± 4 mmHg) and was consistent with the post-stroke mean arterial pressure of SHRSP in group II which developed stroke on

Table 1
Brain tissue weights in SHRSP

Treatment	Weight of SHRSP brain (g/100 g rat)	
	Post-stroke	Pre-stroke
Saline ($n = 5$)	—	1.20 ± 0.06
L-NAME ($n = 9, 7$)	1.28 ± 0.06	1.26 ± 0.04
L-NAME + L-arginine ($n = 7$)	1.32 ± 0.08	—
L-NAME + Isosorbide ($n = 8$)	1.33 ± 0.08	—
L-NAME + Enalapril ($n = 5, 4$)	1.30 ± 0.07	1.10 ± 0.04 [a]
L-NAME + L-158,809 ($n = 4, 5$)	1.32 ± 0.06	1.12 ± 0.08

Values are means ± S.E.M. Pre- and post-stroke comparisons of brain tissue weights in various treatment groups.
[a] $P < 0.05$.

Table 2
Post-stroke cardiovascular parameters in SHRSP

Treatment	Post-stroke blood pressure (mmHg)			Heart rate (beats/min)
	mean	systolic	pulse	
Group II [c]				
L-NAME ($n = 14$)	218 ± 4	241 ± 5	55 ± 2	375 ± 6
Group III				
L-NAME + Arginine (n = 7)	181 ± 5 [b]	215 ± 6 [a]	55 ± 4	385 ± 7
Group IV				
L-NAME + Isosorbide ($n = 8$)	172 ± 5 [b]	210 ± 6 [a]	64 ± 3 [a]	432 ± 8 [b]
Group VII				
L-NAME + Enalapril ($n = 8$)	189 ± 6 [a]	232 ± 5	53 ± 2	387 ± 7
Group VIII				
L-NAME + L-158,809 ($n = 5$)	205 ± 5	238 ± 6	54 ± 3	381 ± 8

Values are means ± S.E.M. Post-stroke recordings (average of 60–90 min) following chronic administration of various treatments.
[a] $P < 0.05$ vs. group II.
[b] $P < 0.01$ vs. group II.
[c] Groups I and V (receiving saline) did not develop stroke. Group VI was duplicate of group II.

day 11 following L-NAME treatment (Table 2). An increase in heart rate due to L-NAME was also seen ($P < 0.01$; Table 3). SHRSP from Group I (receiving saline) did not show the elevation in mean and systolic blood pressures (Table 3). On day 10, mean arterial and systolic blood pressures were 118 ± 4 and 139 ± 5 mmHg, respectively.

Co-administration of L-arginine in group III caused a ten percent (statistically significant) decrease in elevation of mean and systolic blood pressures (Table 2). Despite reducing the increases in blood pressure, L-arginine treatment had no effect on the L-NAME-induced onset of stroke. Administration of isosorbide dinitrate along with L-NAME caused a smaller increase in mean and systolic blood pressures as shown in Table 2 (group IV vs. group II; $P < 0.01$). We also observed significant increases in pulse rate and pulse pressure. However, isosorbide dinitrate did not delay the onset of L-NAME-induced stroke. Additionally, in a separate set of experiments, the pre-stroke blood pressure (on day 10) following arginine plus L-NAME and isosorbide dinitarte plus L-NAME was also measured (data not shown). These results were statistically similar to those for post-stroke blood pressure shown in Table 2.

Co-administration of angiotensin blockers (enalapril maleate and L-158,809) considerably lowered the L-

Table 3
Pre-stroke cardiovascular parameters in SHRSP

Treatment		Pre-stroke blood pressure (mmHg)			Heart rate (beats/min)
		mean	systolic	pulse	
Group IX					
Saline	A ($n = 4$)	110 ± 4	135 ± 4	37 ± 2	350 ± 6
	B ($n = 5$)	118 ± 4	139 ± 5	39 ± 2	341 ± 5
Group X					
L-NAME	A ($n = 5$)	200 ± 5	232 ± 5	55 ± 3	382 ± 7
	B ($n = 5$)	209 ± 4	238 ± 6	57 ± 4	378 ± 6
Group XI					
L-NAME + Enalapril	A ($n = 5$)	179 ± 5 [a]	211 ± 4 [a]	56 ± 3	390 ± 6
	B ($n = 5$)	186 ± 5 [a]	220 ± 5	55 ± 2	386 ± 7
Group XII					
L-NAME + L-158,809	A ($n = 4$)	180 ± 6 [a]	222 ± 5	55 ± 3	384 ± 6
	B ($n = 5$)	189 ± 5 [a]	226 ± 6	54 ± 4	382 ± 6

Values are means ± S.E.M. Pre-stroke recordings (average of 60–90 min) on days 5 (A) and 10 (B) following chronic administration of various treatments.
[a] $P < 0.05$ vs. group X; (A was compared with A and B was compared with B).

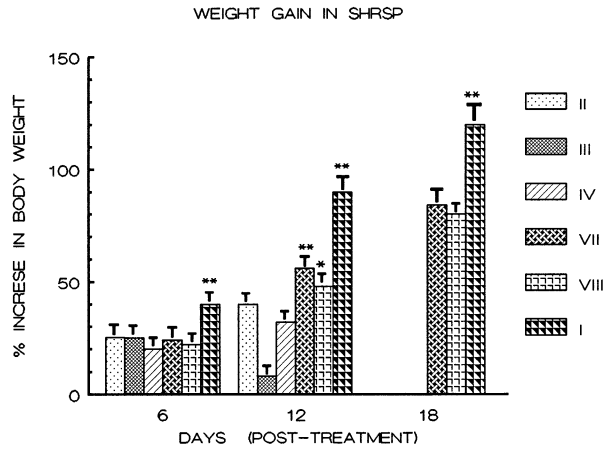

Fig. 3. Pre-stroke weight gain in SHRSP following different treatments given in saline as drinking solution as described in Section 2. SHRSP received saline (group I; $n = 11$), L-NAME (group II; $n = 14$), L-NAME + L-arginine (group III; $n = 7$), L-NAME + isosorbide dinitrate (group IV; $n = 8$), L-NAME + enalapril maleate (group VII; $n = 8$) and L-NAME + L-158,809 (group VIII; $n = 5$). Values are means ± S.E.M. * $P < 0.05$; ** $P < 0.01$ vs. group II (ANOVA).

NAME-induced elevations in mean and systolic blood pressures. The post-stroke mean pressure in the enalapril group was 189 ± 6 vs. 218 ± 4 mmHg for the L-NAME group ($P < 0.05$). The post-stroke mean pressure in SHRSP of the L-158,809 group was slightly lower than in the L-NAME group (205 ± 5 mmHg), and the difference did not reach statistical significance (Table 2). Pre-stroke blood pressures on day 10 from groups X, XI and XII (Table 3) were compared with post-stroke blood pressures of group II, VII and VIII (Table 2), respectively. No statistical difference was detected. These data indicated that the onset of monoplegia or stroke did not modify cardiovascular parameters.

SHRSP receiving various treatments were also monitored for weight gain up to the onset of stroke. The saline control group of SHRSP manifested the highest weight gain (10 g per day for 100 g of rat). Groups receiving enalapril plus L-NAME and L-158,809 plus L-NAME had a greater weight gain (6 g per day for 100 g of rat) than did the group receiving L-NAME alone (3 g per day) or other treatment groups (Fig. 3).

4. Discussion

In previous studies, SHRSP had a normal blood pressure up to the age of five weeks. After this there was a steady increase in systolic blood pressure, up to and over 200 mmHg by the age of 15 weeks, at which point SHRSP developed stroke (Okamoto et al., 1974). Both increase in blood pressure and stroke occurred one to two weeks earlier if SHRSP were fed Japanese chow and saline solution for drinking (Okamoto et al., 1974; Stier et al., 1989). The present study has shown that the increase in blood pressure and the onset of stroke occurred more rapidly following NO synthase inhibition. Most of the SHRSP developed forelimb monoplegia within 11 or 12 days following chronic oral intake of L-NAME. Histological examination showed cerebrovascular lesions in the basal ganglion areas of SHRSP receiving L-NAME treatment. These lesions included anemic infarcts, edema and rarefaction of the cerebral tissues. These observations are consistent with the report suggesting the presence of considerable NO activity (including vascular activity) in the basal ganglia of rat brain (Forstermann et al., 1990). Consistent with the present data, small infarcts (lacunar strokes) of basal ganglia are known to cause minor neurological deficits which may be associated with hemiplegia, hemiparesis and clumsiness of one limb (Fisher, 1969).

In the present study, isosorbide dinitrate did not offer any protection from stroke in SHRSP. The dose may not have been a factor, since over a similar dose range, isosorbide dinitrate has been shown to reverse NO synthase inhibitory effects of L-NAME in vivo (Mascolo et al., 1994). Several explanations can be given for the lack of beneficial effect of isosorbide dinitrate on L-NAME-induced stroke. Firstly, this may be due to the inability of exogenous NO to reach cerebral smooth muscle cells, presumably being engulfed by free radical scavenger proteins present on the blood–brain barrier (Reiter, 1995). Secondly, despite causing a lesser elevation in mean and systolic blood pressures when given along with L-NAME, isosorbide dinitrate raised the systemic pulse pressure. Increased pulse pressure, even in the absence of increases in systolic and mean pressures, can produce hypertrophy of cerebral arterioles (Baumbach, 1996). Such hypertrophy is likely to be associated with an increased media-to-lumen ratio (Christensen, 1991; Baumbach, 1996) and may precipitate stroke in SHRSP. Finally, beneficial effect of isosorbide dinitrate due to lowering of blood pressure may not be of significance if there is a simultaneous development of local cerebrovascular pathogenic events. As discussed below, inhibition of NO production enhances the activity of vascular angiotensin II. The administration of exogenous nitrate is also associated with an increase in circulating angiotensin II (Munzel et al., 1996). Angiotensin II up-regulates the activity of the vascular NADPH/NADH oxidase system (Fukui et al., 1997), which results in enhanced production of superoxide ($.O_2^-$) anion (Pagano et al., 1995). In vivo, superoxide binds to NO and generates a strong oxidant, peroxynitrite ($ONOO^-$) (Gryglewski et al., 1986). Peroxynitrite, a cytotoxic substance, can cause endothelial injury, and cerebral vessels are highly vulnerable to such injury (Medele et al., 1996).

The present in vivo data showed that the elevation in blood pressure was slightly less when L-NAME and a tenfold higher dose of L-arginine were given together than when L-NAME was given alone. However, L-arginine treatment did not offer any protection from L-NAME-induced stroke. Consistent with our observations, a recent

study showed that 20-fold higher concentrations of arginine only partially reversed the L-NAME-induced reductions in cerebral blood flow in normotensive Fisher rats (Horvath et al., 1994). Although additional studies may be needed to determine if the dose of L-arginine was a factor, the lack of beneficial effect of L-arginine may be independent of dose, since in other reports, a 10-fold higher concentration of L-arginine prevented the acute effects of L-NAME (Moncada et al., 1991). Additionally, the drinking volumes in two (L-NAME and L-NAME + L-arginine) groups were not significantly different (data not shown). Whether NO synthase isoforms present in the cerebral vasculature/endothelium may undergo a conformational change following chronic exposure to L-NAME that may not be reversible with L-arginine requires investigation. A recent study has suggested that there is heterogeneity in the mechanisms of L-arginine uptake in vascular endothelial cells (Durante et al., 1996). Due to the unique nature of the blood–brain barrier, it is possible that, in the cerebral vasculature and/or endothelium, the uptake of L-arginine is tightly regulated and the intracellular concentration needed to reverse the L-NAME-induced NO synthase inhibition may not be reached.

Enalapril has been reported to be relatively ineffective to lower blood pressure in spontaneously hypertensive rats receiving saline as a drinking solution (Sweet et al., 1981). It has been shown that giving SHRSP a 1% NaCl drinking solution results in an initial lowering of plasma renin activity at 1 week followed by recovery in the 2nd week with a marked increase in 3 to 4 weeks (Shibota et al., 1979). A recent study has also shown enhanced kidney renin activity in SHRSP (Volpe et al., 1990). Based on these findings, it is unlikely that plasma renin activity was suppressed in the SHRSP used in the present study. In vivo, NO acts as a physiological antagonist to angiotensin II, since most of the vascular effects of NO are found to be opposite to those of angiotensin II (Dubey et al., 1995; Fukuto and Chaudhuri, 1995). Thus, in the absence of vasodilator NO, the physiological activity of angiotensin II is expected to be considerably exaggerated. Both angiotensin blockers prolonged the survival of SHRSP, and considerably delayed the onset of stroke. Angiotensin blocker-induced prolongation of SHRSP survival may be a consequence of depleted angiotensin II or reduced mean and systolic pressures. It is likely that, in the absence of NO, increased activity of the systemic and local renin–angiotensin system caused rapid remodeling of cerebral vessels. Angiotensin II-induced remodeling has been shown to occur as a consequence of stimulation of migration (Bell and Madri, 1990), proliferation (Daemen et al., 1991) and hypertrophy (Itoh et al., 1993) of vascular smooth muscle cells along with increased production of extracellular matrix (Scott-Burden et al., 1990). These atherogenic effects of angiotensin II have been implicated in the development of stroke (Rossi et al., 1995), and angiotensin blockers such as enalapril prevent such effects (Powell et al., 1989; Stier et al., 1989). Additionally, enalapril has been shown to decrease collagen and elastin accumulation in the arteries of growing rats, independent of their effects on blood pressure (Keeley et al., 1990). On the other hand, minimizing the role of angiotensin II, results of some studies have suggested that the L-NAME-induced structural changes are seen predominantly in large arteries and are less important in microvessels (Schiffrin, 1995). However, in our experiments, treatment with angiotensin antagonists delayed the L-NAME-induced onset of stroke, even though systolic pressures had exceeded the stroke-precipitating critical value of 200 mmHg. This suggests that some beneficial effects of these antagonists were due to factors independent of blood pressure reductions.

A recent study has demonstrated that chronic inhibition of NO in vivo exacerbated hypertension and produced nephrosclerosis in spontaneously hypertensive rats (Ono et al., 1996). In these animals, chronic administration of an angiotensin converting enzyme inhibitor, quinapril, not only prevented but also reversed L-NAME-induced severe nephrosclerosis. Quinapril also decreased L-NAME-induced increases in peri-arteriolar fibronectin and smooth muscle actin deposits in renal afferent arterioles (Ono et al., 1996). This study had not ruled out the possibility that the beneficial effect of quinapril may have been the consequence of potentiated kinins. However, from the present data, it is clear that a lack of angiotensin II and not the increase in kinins minimized the deteriorating effects of NO synthase inhibition on cerebral vessels.

In summary, chronic systemic/cerebrovascular NO synthase inhibition precipitates stroke in SHRSP. The onset of such stroke is delayed with angiotensin converting enzyme inhibitor(s) or angiotensin AT_1 receptor antagonists. Depletion of angiotensin II counteracts cerebrovascular NO synthase inhibition-induced vascular damage either locally and/or by reducing mean and systolic blood pressures.

Acknowledgements

The author thanks Dr. Patrick E. Ward, Department of Physiology, Ohio State University, Columbus, OH, for his helpful suggestions during preparation of this manuscript.

References

Arnal, J.F., Warin, L., Michel, J.B., 1992. Determinants of aortic cyclic guanosine monophosphate in hypertension induced by chronic inhibition of nitric oxide synthase. J. Clin. Invest. 90, 647–652.

Baumbach, G.L., 1996. Effects of increased pulse pressure on cerebral arterioles. Hypertension 27, 159–167.

Bell, L., Madri, J.A., 1990. Influence of the angiotensin system on endothelial and smooth muscle cell migration. Am. J. Pathol. 137, 7–12.

Bredt, D.S., Hwang, P.M., Snyder, S.H., 1990. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 347, 768–770.

Buchanan, J.E., Phillis, J.W., 1993. The role of nitric oxide in the regulation of cerebral blood flow. Brain Res. 610, 248–255.

Christensen, K.L., 1991. Reducing pulse pressure in hypertension may normalize small artery structure. Hypertension 18, 722–727.

Daemen, M.J.A.P., Lombardi, D.M., Bosman, F.T., Schwartz, S.M., 1991. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. Circ. Res. 68, 450–456.

Dawson, T.M., Snyder, S.H., 1994. Gases as biological messengers: Nitric oxide and carbon monoxide in the brain. J. Neurosci. 14, 5147–5159.

Dubey, R., Jackson, E., Luscher, T., 1995. Nitric oxide inhibits angiotensin II-induced migration of rat aortic smooth muscle cell: Role of cyclic nucleotides and angiotensin receptors. J. Clin. Invest. 96, 141–149.

Durante, W., Liao, L., Iftikhar, I., O'Brien, W.E., Schafer, A.I., 1996. Differential regulation of L-arginine transport and nitric oxide production by vascular smooth muscle and endothelium. Circ. Res. 78, 1075–1082.

Fisher, C.M., 1969. The arterial lesions underlying lacunes. Acta Neuropathol. 12, 3–15.

Forstermann, U., Gorsky, L.D., Pollock, J.S., Schmidt, H.H., Heller, M., Murad, F., 1990. Regional distribution of EDRF/NO-synthesizing enzyme(s) in rat brain. Biochem. Biophys. Res. Commun. 168, 727–732.

Forsting, M., Reith, W., Schabitz, W., Heiland, S., Kummer, R., Hacke, W., Sartor, K., 1995. Decompressive craniectomy for cerebral infarction. Stroke 26, 259–264.

Fukui, T., Ishizaka, N., Rajagopalan, S., Laursen, J.B., Capers, Q. IV, Taylor, W.R., Harrison, D.G., Leon, H., Wilcox, J.N., Griendling, K.K., 1997. p22phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. Circ. Res. 80, 45–51.

Fukuto, J.M., Chaudhuri, G., 1995. Inhibition of constitutive and inducible nitric oxide synthase: Potential selective inhibition. Annu. Rev. Pharmacol. Toxicol. 35, 165–194.

Gardiner, S.M., Compton, A.M., Bennett, T., Palmer, R.M.J., Moncada, S., 1990. Persistent hemodynamic changes following prolonged infusions of N^G-monomethyl-L-arginine methyl ester in conscious rats, In: Moncada, S., Higgs, E.A. (Eds.), Nitric Oxide from L-Arginine: A Bioregulatory System. Elsevier, Amsterdam, pp. 489–491.

Gryglewski, R.J., Palmer, R.M.J., Moncada, S., 1986. O_2^- is involved in the breakdown of endothelium-derived relaxing factor. Nature 320, 454–456.

Horvath, I., Sandor, N.T., Ruttner, Z., Mclaughlin, A.C., 1994. Role of nitric oxide in regulating cerebrocortical oxygen consumption and blood flow during hypercapnia. J. Cereb. Blood Flow Metab. 14, 503–509.

Itoh, H., Mukoyama, M., Pratt, R.E., Gibbons, G.H., Dzau, V.J., 1993. Multiple autocrine growth factors modulate vascular smooth muscle cell growth response to angiotensin II. J. Clin. Invest. 91, 2268–2274.

Izuta, M., Clavier, N., Kirsch, J.R., Traystman, R.J., 1995. Cerebral blood flow during inhibition of brain nitric oxide synthase activity in normal, hypertensive, and stroke-prone rats. Stroke 26, 1079–1085.

Keeley, F.W., Johnson, D.J., Elmoslhi, A., Leenen, F.H.H., 1990. Enalapril suppresses normal accumulation of elastin and collagen in cardiovascular tissues of growing rats. In: Program and Abstracts of the 13th Sci. Meeting of the Int. Soc. of Hypertension; June 24-29, Montreal, Canada, Abstract IC. 24.

Mascolo, N., Izzo, A.A., Autore, G., Barbato, F., Capasso, F., 1994. Nitric oxide and castor oil-induced diarrhea. J. Pharmacol. Exp. Ther. 268, 291–295.

Medele, R.J., Stummer, W., Reulen, H.J., Steiger, H.J., 1996. Evidence for peroxidative damage by nitric oxide in experimental chronic cerebral vasospasm. Neurol. Res. 18, 277–280.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1989. Biosynthesis of nitric oxide from L-arginine: A pathway for the regulation of cell function and communication. Biochem. Pharmacol. 38, 1709–1715.

Moncada, S., Palmer, R.M., Higgs, E.A., 1991. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109–142.

Moore, P.K., Al-Swayeh, O.A., Chong, N.W.S., Evans, R.A., Gibson, A., 1990. L-N_G-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilation in vitro. Br. J. Pharmacol. 99, 408–412.

Munzel, T., Kurz, S., Heitzer, T., Harrison, D.G., 1996. New insights into mechanisms underlying nitrate tolerance. Am. J. Cardiol. 77, 24C–30C.

Okamoto, K., Yamori, Y., Nagaoka, A., 1974. Establishment of the stroke-prone spontaneously hypertensive rat (SHR). Circ. Res. 34–35 (Suppl. I), I143–I153.

Ono, H., Ono, Y., Frohlich, E.D., 1996. ACE inhibition prevents and reverses L-NAME-exacerbated nephrosclerosis in spontaneously hypertensive rats. Hypertension 27, 176–183.

Pagano, P.J., Ito, Y., Tornheim, K., Gallop, P.M., Tauber, A.I., Cohen, R.A., 1995. An NADPH oxidase superoxide-generating system in the rabbit aorta. Am. J. Physiol. 268, H2274–H2280.

Powell, J.S., Clozel, J.P., Muller, R.K., Kuhn, H., Hefti, F., Hosany, M., Baumgartner, H.R., 1989. Inhibitors of angiotensin-converting enzyme prevent myointimal proliferation after vascular injury. Science 245, 186–188.

Reiter, R.J., 1995. Oxidative processes and antioxidative defense mechanisms in the aging brain. FASEB J. 9, 526–533.

Ribeiro, M.O., Antunes, E., De Nucci, G., Lovisolo, S.M., Zatz, R., 1992. Chronic inhibition of nitric oxide synthesis: A new model of arterial hypertension. Hypertension 20, 298–303.

Rossi, G.P., Rossi, A., Sacchetto, A., Pavan, E., Pessina, A.C., 1995. Hypertensive cerebrovascular disease and the renin-angiotensin system. Stroke 26, 1700–1706.

Schiffrin, E.L., 1995. Vascular structure in N^G-nitro-L-arginine methyl ester-induced hypertension: Methodological considerations for studies of small arteries in hypertension. J. Hypertens. 13, 817–821.

Scott-Burden, T., Hahn, A.W., Resnik, T.J., Buhler, F.R., 1990. Modulation of extracellular matrix by angiotensin II: Stimulated glyco-conjugate synthesis and growth in vascular smooth muscle cells. J. Cardiovasc. Pharmacol. 16 (Suppl. 4), S36–S41.

Shibota, M., Nagaoka, A., Shino, A., Fujita, T., 1979. Renin–angiotensin system in stroke-prone spontaneously hypertensive rats. Am. J. Physiol. 236, H409–H416.

Stier, C.T. Jr., Benter, I.F., Ahmad, S., Zuo, H., Selig, N., Roethel, S., Levine, S., Itskovitz, H.D., 1989. Enalapril prevents stroke and kidney dysfunction in salt-loaded stroke-prone spontaneously hypertensive rats. Hypertension 13, 115–121.

Sweet, C.S., Gross, D.M., Arbegast, P.T., Gaul, S.L., Britt, P.M., Ludden, C.T., Weitz, D., Stone, C.A., 1981. Antihypertensive activity of *N*-[(*S*)-1-ethoxycarbonyl)-3-phenylpropyl]-L-ala-L-pro (MK-421), an orally active converting enzyme inhibitor. J. Pharmacol. Exp. Ther. 216, 558–566.

Toda, N., Okamura, T., 1990. Mechanism underlying the response to vasodilator nerve stimulation in isolated dog and monkey cerebral arteries. Am. J. Physiol. 259, H1511.

Togashi, H., Sakuma, I., Yoshioka, M., Kobayashi, T., Yasuda, H., Kitabatake, A., Saito, H., Gross, S.S., Levi, R., 1992. A central nervous system action of nitric oxide in blood pressure regulation. J. Pharmacol. Exp. Ther. 262, 343–347.

Volpe, M., Camargo, M.J.F., Mueller, F.B., Campbell, W.G. Jr., Sealy, J.F., Pecker, M.S., Sosa, R.E., Laragh, J.H., 1990. Relation of plasma renin to end organ damage and to protection of K^+ feeding in stroke-prone hypertensive rats. Hypertension 15, 318–326.

Yang, S.T., Mayhan, W.G., Faraci, F.M., Heistad, D.D., 1991. Endothelium dependent responses of cerebral blood vessels during chronic hypertension. Hypertension 17, 612–618.