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Commentary

Protection against ischemia: a physiological function of the renin-angiotensin system

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Abstract

The renin-angiotensin system (RAS) is involved in a complex mechanism that serves to preserve the blood supply to organs so that they can maintain cellular function. Angiotensin II exerts this effect, independently of the blood pressure generated, through two time-related events: a fast opening of the reserve collateral circulation and a much slower response of new vessel formation or angiogenesis. This effect is observed in rats with ligation of the abdominal aorta and in gerbils with abrupt or progressive unilateral carotid artery ligation. Inhibition of the angiotensin-converting enzyme (ACE) or the angiotensin II receptor represses this effect, and it appears that it is mediated through a non-AT_1 receptor site of angiotensin II. Many tumors, both benign and malignant, express renin and angiotensin. It seems that the stimulating action of angiotensin II on angiogenesis could also be involved in preserving the blood supply to tumor cells. Administration of converting enzyme inhibitors increases survival and decreases tumor size in tumor-bearing rats. These observations support the hypothesis that the RAS, directly or indirectly, is involved in situations in which the restoration of blood supply is critical for the viability of cells and that it is present not only in normal but also in pathological conditions such as tumors. In view of the ubiquitous presence of renins and angiotensins, it is also likely to be involved in other conditions, such as inflammation, arthritis, diabetic retinopathy, and retrolental fibroplasia, among others in which angiogenesis is prominent. In addition, angiotensin II could be involved, through the counterbalance of the AT_1 and AT_2 receptors, in the rarefaction of blood vessels as an etiologic component of essential hypertension.

Keywords: Renin; Angiotensin II; Angiotensin receptors; Converting enzyme inhibitors; Ischemia; Collateral circulation; Cerebral circulation; Stroke; Angiogenesis; Tumor

1. Introduction

Renin was discovered by Tigerstedt and Bergman in 1898 as a pressor substance present in the renal cortex [1]. Volhard in 1923 proposed that a vasospastic substance was a cause of hypertension, and Bohn, one of his associates, stated in 1932 that a vasoconstrictor substance was found in the blood of patients with hypertension [2,3]. Following the seminal experiments of Goldblatt *et al.* in 1934, in which the restriction of blood flow through the renal arteries was shown to produce a sustained increase of blood pressure, many investigators have used this methodology to produce experimental renal hypertension [4]. Two groups of investigators independently arrived at the conclusion that this ischemia-linked hypertension is produced by an enzymatic chain of events involving renin, in which a pressor substance is generated. This pressor substance was named "hipertensina" by a group headed by Braun-Menendez; Page and his collaborators called the compound "angiotonin" [5,6]. By a collective agreement among them, the hybrid word "angiotensin" was born. Since then, the tremendous interest generated has led to many advances in the knowledge of the RAS, phylogenetically one of the oldest hor-

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Abbreviations: RAS, renin-angiotensin system; ACE, angiotensin-converting enzyme; NO, nitric oxide; cGMP, cyclic GMP; GBM, glioblastoma multiforme; and SHR, spontaneously hypertensive rat.

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mone systems. Angiotensin II emerged as the most important effector peptide of the RAS, and its central physiological role in the regulation of salt homeostasis, kidney function, and blood pressure has been well established. The physiology of the RAS was thought to be comprehensively understood. The vasoconstrictive peptide angiotensin II released in the circulation whenever blood pressure falls was seen as being responsible for increased vascular resistance and stimulation of the renal reabsorption of sodium, thereby effecting restoration of blood pressure. As knowledge widened, it became increasingly clear that the original schema was over simplified. First, it was found that angiotensin II, unlike other classical circulating peptidic hormones, is not restricted to the blood and can be locally generated in almost all of the tissues of the body [7,8]. As elegantly discussed by Goldblatt, renin and angiotensins were probably never "intended" to circulate but rather to perform their actions locally [9]. At the same time, it was demonstrated that other peptides such as angiotensin III and angiotensin IV, initially regarded as inactive breakdown products of angiotensin I or II, were indeed able to produce physiological effects of their own [10]. Finally, a third level of complexity had to be integrated with the disclosure that the RAS acts through more than one receptor [11]. Specific receptors for angiotensin III and IV were discovered, and two different receptors for angiotensin II, AT_1 and AT_2, were characterized [12,13]. The functional dominance of the AT_1 receptor responsible for almost all of the hitherto known effects of angiotensin II thus raises the partially solved question of the physiological role of the AT_2 receptor. This new complexity of the RAS led to the idea that its physiological role may encompass broader functions than merely salt homeostasis and blood pressure control. Indeed, it was demonstrated that it participates in many additional regulatory processes, such as inflammation, athero-thrombosis, cardiac and brain function, and cell growth and apoptosis. Experimental evidence also indicates that angiotensin II tends to restore blood flow in the setting of acute ischemia and plays a role in angiogenesis, a process crucial in maintaining tissue perfusion during sustained ischemia [14,15]. This commentary is focused on these latter two aspects of the RAS function, which support the broad general concept that the ultimate physiological role of the RAS is to preserve blood supply to the tissues.

2. Protective effect of angiotensin II during acute ischemia

During the course of a study in which renal ischemia-dependent hypertension was induced by aortic ligation between the renal arteries of rats, the abrupt reduction of blood supply to the muscles distal to the ligature resulted in hind limb paralysis [14]. In animals in which the left ischemic kidney remained untouched, paralysis was transient and recovery was within 2 hr. On the contrary, animals in which

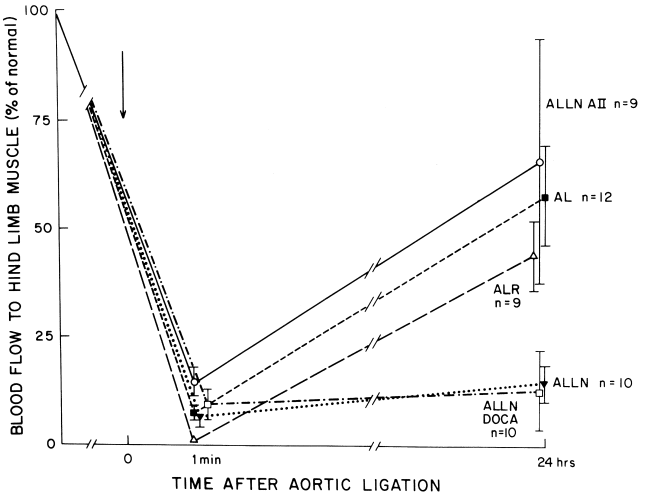

Fig. 1. Changes in blood flow to the gastrocnemius muscle prior to, at 1 min, and at 24 hr after aortic ligation in the same animal. AL: aortic ligation; ALLN: aortic ligation left nephrectomy; ALLN AII: aortic ligation left nephrectomy plus angiotensin II infusion; ALR: aortic ligation pretreated with reserpine; ALLN DOCA: aortic ligation left nephrectomy pretreated with desoxycorticosterone and saline solution to drink. The arrow indicates the time of aortic ligation; "n" indicates the number of animals in each group. Values are expressed as means ± SEM. Reproduced with permission from Am J Physiol 1982;243: H869–75. Copyright (1982) American Physiological Society. [Ref. 14].

left nephrectomy was performed remained paralyzed for up to 24 hr after surgery. This incidental observation suggested that the RAS could be responsible for a mechanism leading to rapid restoration of blood flow to the organs subjected to acute ischemia, presumably by enhancing the recruitment of the collateral circulation. This hypothesis was explored further in a series of experiments in rats with aortic ligation and in an experimental model of stroke using gerbils with unilateral carotid artery ligation [14,16].

Initially, it was considered that the recuperation from paralysis after aortic ligation was the result of a mechanical effect of the high blood pressure generated by the stimulated RAS. However, after pharmacological manipulation of the blood pressure, it was possible to establish that this outcome is related to a chemical effect of angiotensin II and not to a physical or mechanical influence of the elicited hypertension. Measurements of the blood flow to the hind limb muscle at different times after aortic ligation, using different labeled radioactive microspheres, showed that a significant decline immediately followed the ligation (Fig. 1). After 24 hr, the reduced blood flow recovered in the groups in which the left kidney remained intact and in nephrectomized rats infused with exogenous angiotensin II. The same trend was observed in aortic ligated-reserpinized animals, which have lower blood pressure. In contrast, blood flow remained low in animals with aortic ligation and left nephrectomy that had lower blood pressure, and in rats similarly prepared but made hypertensive by being given desoxycorticosterone (DOCA) and salt. A strong correlation was found between the absence of gait anomaly, restored blood flow, and ele-

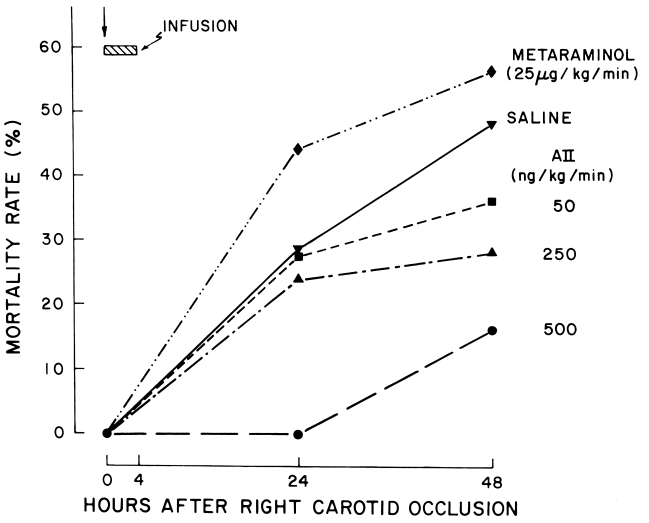

Fig. 2. Cumulative mortality rate in gerbils with unilateral carotid artery ligation after a 4-hr infusion with either saline, metaraminol, or angiotensin II at 50, 250, or 500 ng/kg/min. Twenty-five gerbils were used in each group. The chi-square test for trend yielded 5.26; df = 1; $P < 0.025$. Reproduced with permission from Stroke 1986;17:82–5. Copyright (1986) Lippincott Williams & Wilkins. [Ref. 16].

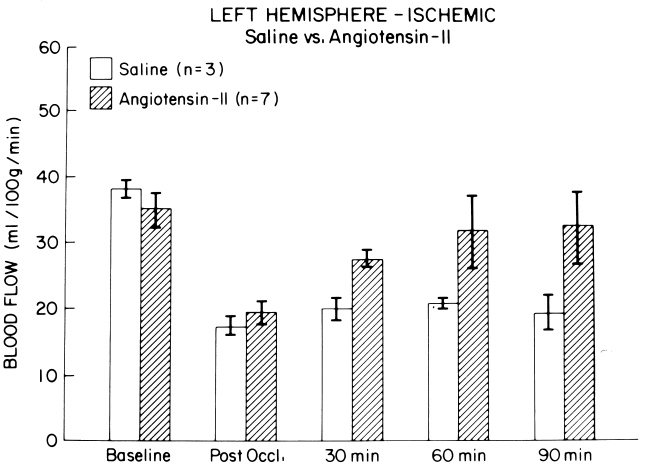

Fig. 3. Cerebral blood flow in the ipsilateral hemisphere in gerbils with unilateral carotid artery ligation infused with either saline or angiotensin II at 500 ng/kg/min. Using the technique of hydrogen clearance, measurements were taken before surgery, immediately after surgery, and 30, 60, and 90 min thereafter. Bars represent mean values ± SEM.

vated plasma renin activity. Animals with high plasma renin activity had increased blood flow and walked normally, whereas rats with unstimulated plasma renin activity had low blood flow and gait alteration. Exogenous administration of angiotensin II to nephrectomized animals restored blood flow to the muscles and prevented paralysis. These results indicated that the restoration of blood supply to the muscles of the posterior limb was dependent upon the high levels of angiotensin II but independent of the effect of elevated blood pressure. Aortographies showed that the restoration of blood flow distal to the ligature in animals with high angiotensin II levels was the consequence of a bypass of the ligation through collateral pathways, in this case mainly through the mesenteric circulation.

These observations raised the possibility that similar mechanisms could be operative in other situations of experimental ischemia such as those affecting the brain. The gerbil (*Meriones unguiculatus*) is characterized by a cerebral vascular tree lacking a complete anastomotic circle of Willis and is prone to development of focal brain ischemia following abrupt unilateral carotid artery ligation [17]. Fig. 2 shows that an abrupt ligation of a carotid artery produced a mortality rate of approximately 50% at 48 hr after surgery. Infusion of exogenous angiotensin II decreased the mortality rate to approximately 15%, while increasing blood pressure to the same extent with metaraminol had no protective effect from mortality [16]. Blood flow measurements in the ipsilateral hemisphere showed a trend of restoring blood supply in animals treated with angiotensin II (Fig. 3).

Gradual occlusion of a blood vessel produces a less detrimental effect on tissues than does sudden occlusion. Slow occlusion allows adaptive processes to engage more efficiently, and minimizes the decrease of blood supply to the ischemic area. If the RAS cascade plays a part in this process, then inhibition of the system under conditions of progressive carotid artery ligation should result in more severe neurological impairment. To test this assumption, progressive unilateral carotid artery ligation was done in gerbils infused for 4 hr with either enalaprilat, an ACE inhibitor, or saralasin, a competitive angiotensin II antagonist. The mortality in progressively ligated animals treated with enalaprilat or saralasin markedly increased and was no longer different from that of abruptly ligated animals [18] (Fig. 4). These results further support the role of angiotensin II in the protective adaptation to ischemia. In another study, the effects of pretreatment with enalaprilat and the specific AT_1 receptor antagonist losartan were compared in a model of an abrupt unilateral carotid artery ligation [19]. Pretreatment with losartan significantly increased the survival rate.

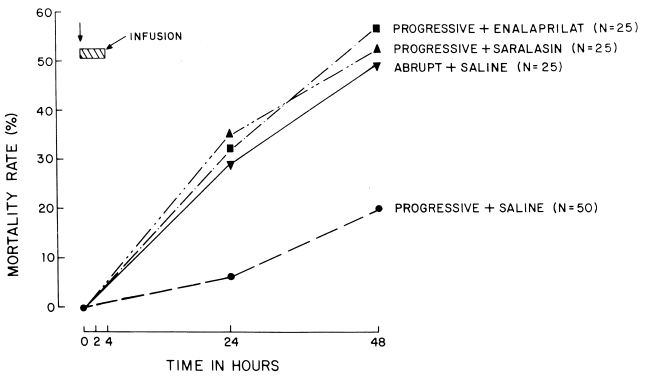

Fig. 4. Cumulative mortality rates in groups of gerbils with abrupt or progressive carotid artery ligation after a 4-hr infusion of saline, enalaprilat, or saralasin. The log-rank test indicated a significant difference across all four groups ($\chi^2 = 11.0$, df = 3, $P < 0.025$). Reproduced with permission from J Cereb Blood Flow Metab 1988;8:149–54. Copyright (1988) Lippincott Williams & Wilkins. [Ref. 18].

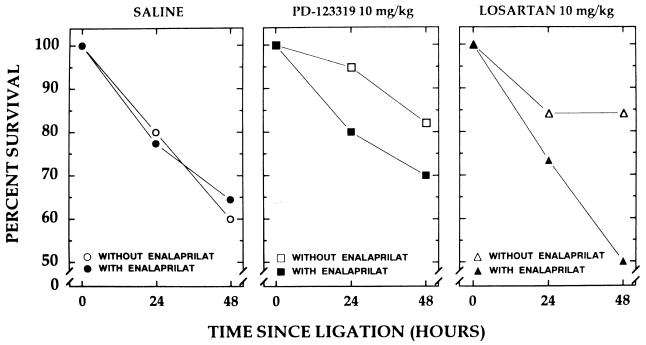

Fig. 5. Cumulative survival at 24 and 48 hr after unilateral carotid artery ligation with and without previous administration of enalaprilat after treatment with saline, PD-123319 (an AT_2 ligand), or losartan (an AT_1 blocker). Number of gerbils used in each group: saline, N = 60; saline plus enalaprilat, N = 31; PD-123319, N = 39; PD-123319 plus enalaprilat, N = 30; losartan, N = 38; and losartan plus enalaprilat, N = 30. Reproduced with permission from J Cardiovasc Pharmacol 1994;24:937–40. Copyright (1994) Lippincott Williams & Wilkins. [Ref. 19].

The beneficial effect of losartan was repressed completely when it was administered simultaneously with enalaprilat. Enalaprilat curtailed angiotensin II formation, whereas losartan, by blocking the AT_1 receptor-mediated negative feedback of angiotensin II on renin release [20] and increasing the circulating levels of angiotensin II, intensified the effect of the unopposed non-AT_1 receptors (Fig. 5). In another set of experiments, using gerbils pretreated with either saline, losartan, or enalaprilat, the left renal artery was ligated to stimulate endogenous renin-angiotensin II production. Again, only the addition of an ACE inhibitor markedly increased the mortality rate, while both enalaprilat and losartan curtailed the increase in blood pressure induced by the renal artery ligation. The deleterious effect on survival in gerbils after an abrupt carotid artery ligation was seen consistently with other ACE inhibitors such as captopril and lisinopril (Achard J-M, Mazouz H, and Fournier A, unpublished observations). Taken together, these studies provide strong experimental evidence that in two different ischemic territories, the brain and the area caudal to aortic ligation, high levels of angiotensin II have a protective effect against ischemia by limiting the decrease in blood flow through the rapid recruitment of pre-existing collateral circulation, which operates, most likely, via the non-AT_1 receptors.

Non-AT_1 receptors certainly represent appealing candidates to mediate the protective response of angiotensin II. In a series of studies aiming to compare the vasculoprotective effects of ACE inhibitors and AT_1 receptor antagonists, Unger and his group established that in spontaneously hypertensive rats aortic tissue cGMP content, an index of NO production, increased to a greater extent with losartan than with ramipril. The increase in cGMP with ramipril could be attributed to a bradykinin-induced stimulation of NO production [21–23], while the effect of losartan was reminiscent of a previous report that AT_2 stimulation increases NO production in the kidney [24]. Indeed, Siragy *et al.* [25] examined the role of the AT_2 receptor in vascular and renal responses to physiological increases in angiotensin II in mice with targeted deletion of the AT_2 receptor gene. Mice lacking the AT_2 receptor (AT_2-null mice) had slightly elevated systolic blood pressure compared with wild-type control mice. In AT_2-null mice, but not in wild-type mice, infusion of low doses of angiotensin II for 7 days produced a marked and sustained increase in systolic blood pressure and a reduction in urinary sodium excretion. AT_2-null mice had low basal levels of renal interstitial fluid bradykinin and cGMP, compared with wild-type mice. Finally, dietary sodium restriction or angiotensin II infusion increased renal interstitial fluid bradykinin and cGMP in wild-type mice by approximately 4-fold, whereas no changes were observed in AT_2-null mice. These results demonstrate that the AT_2 receptor is necessary for normal physiological responses of bradykinin and NO to angiotensin II and strongly suggest that the AT_2 receptor plays a regulatory protective role mediated via bradykinin and NO against the pressor actions of angiotensin II. A similar mechanism was observed in the aorta of stroke-prone spontaneously hypertensive rats. Increasing circulating angiotensin II, either exogenously or by losartan, raised aortic cGMP independently of the changes of blood pressure. This effect was sensitive to selective blockade of AT_2 receptors, bradykinin B_2 receptors and NO synthase, indicating that the activation of NO synthase and the stimulation of bradykinin B_2 receptors are both involved in the AT_2-dependent action of angiotensin II on vascular cGMP [26].

These converging results establish that, in various experimental models, AT_2 stimulation is linked to NO-dependent vasodilation, and support the hypothesis that such a mechanism could account for the protective effect of angiotensin II during acute brain ischemia. This hypothesis was examined in a model of acute carotid artery ligation in the gerbil. Pretreatment with PD-123319 (1-[[4-(dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*] pyridine-6-carboxylic acid, ditrifluoroacetate), an AT_2 receptor ligand, induced a significant decrease in mortality compared with saline-pretreated animals. Moreover, unlike the protective action of losartan, the beneficial effect of the AT_2 ligand was not inhibited significantly by pretreatment with enalaprilat (Fig. 5) [19]. This suggests that PD-123319 decreased mortality by, agonistically, stimulating AT_2 receptors. PD-123319 effectively displaces radiolabeled angiotensin II from AT_2 receptors, but seems to act as either antagonist or agonist, depending on the experimental conditions and the model used. These findings are consistent with the hypothesis that the protective effect of angiotensin II in acute stroke is mediated by AT_2 receptor stimulation. It has been demonstrated that both AT_1 and AT_2 receptors are expressed in the brain of rodents and humans, and the ligands for both receptors modulate cerebral blood flow autoregulation in the rat [27]. However, it is not known whether the AT_2 receptors involved in the adaptative response are cerebral, modulating a central con-

trol of blood flow redistribution, or vascular, responsible for a direct vasodilatory response. In the rat, AT_2 but not AT_1 is expressed in cerebral arteries. This may not be the case in the gerbil since no expression of AT_2 receptors was observed in the cerebral arteries of the gerbil [27]. However, a possibility that should be considered is that AT_2 receptors are poorly expressed under basal conditions and are efficiently and rapidly up-regulated in the cerebral arteries of the gerbil in response to ischemia. In this regard, it is interesting to note that AT_2 expression has been shown to be increased markedly by various pathological processes such as brain lesions [28], ischemia [29], nerve transection [30], myocardial infarction [31], or balloon-injured carotid artery of the rat [32]. Thus, the angiotensin II-dependent protective opening of the collateral circulation in the setting of acute ischemia may result from an AT_2 receptor-dependent pathway. Whether this effect is the consequence of a direct vasorelaxing effect of the vascular AT_2 receptor or a mechanism similar to the "reverse vascular steal" seen in the heart [33] or results from central cerebrovascular regulation mediated by AT_2 receptors in the brain, remains an unresolved question.

Experimental evidence has shown that both ACE inhibitors and AT_1 receptor antagonists are protective against myocardial ischemia. This finding, at first glance, supports the concept that high angiotensin II levels are deleterious rather than protective for the ischemic heart. However, the mechanism of cardiac protection with ACE inhibition has been shown to be mediated by the slowing down of bradykinin degradation and appears not to be related to RAS inhibition, at least in the chronic phase of heart failure after 2 months of coronary ligation [34]. Moreover, the protective effect of AT_1 blockade seems to be the consequence of the stimulation of AT_2 receptors rather than the inhibition of AT_1. Similarly, the use of the AT_1 receptor antagonist candesartan reduced infarct size after regional myocardial ischemia in pigs. Pretreatment with the AT_2-receptor ligand PD-123319, a bradykinin antagonist, or indomethacin did not affect infarct size but abolished the reduction of infarct size achieved by candesartan. Therefore, the reduction of infarct size by candesartan in pigs involves, again, AT_2 receptor activation, bradykinin, and prostaglandins, but is not due to any changes in blood flow in the ischemic area [35]. Similar results were seen with candesartan in dogs subjected to temporary occlusion and reperfusion of a coronary artery [36].

On the other hand, Leenen *et al.* evaluated the time course of changes in cardiac tissue and plasma angiotensins in rats subjected to an acute coronary artery ligation. Plasma angiotensins and ventricular angiotensin II that had become elevated after ligation returned to normal by 3 days in the infarct-free venticular wall, but only after 1–2 weeks in the infarcted ventricle. The rapid increase in cardiac tissue angiotensin II in both the infarct and infarct-free parts of the left ventricle was blocked by pretreatment with ACE inhibitors but not by nephrectomy [37]. These studies demonstrate the increase of local production of angiotensin II in response to ischemia, which probably stimulates the AT_2 receptor sites. This suggests that the protective effect of AT_2 stimulation during ischemia may not be restricted to a regulatory effect on local hemodynamics, but may also extend to a protective mechanism at the cellular level.

A non-hemodynamic protective mechanism that may also be present in the brain has been reported in rats with temporary middle cerebral artery occlusion. This ischemia induces an increase of transcription factors c-Fos and c-Jun in the cortex, which positively correlated with the degree of neurological deficits [38]. Intraventricular pretreatment with the selective AT_1 receptor antagonist irbesartan, which inhibits brain but not vascular AT_1 receptors, significantly improved the neurological outcome of focal cerebral ischemia and markedly reduced the expression of c-Fos and c-Jun proteins in the cortex on the ligated side of the brain. Also irbesartan pretreatment completely abolished the ischemia-induced c-Fos and c-Jun expression in the hippocampus, a brain area highly sensitive to hypoxia. Whether the beneficial effect of AT_1 blockade is due to the suppression of a deleterious effect of AT_1 receptors on neuronal outcome [27], or to a protective effect of AT_2 receptor stimulation as in the myocardial ischemic models is somewhat controversial. It is interesting to note that the AT_2 receptor mRNA level after brain ischemia or during glutamate neurotoxicity in cultured cortical cells from rats increased by 3-fold in both the cortex and the hippocampus [39]. Thus, ischemia induces an overexpression of AT_2 receptors in neurons, as would be physiologically expected if AT_2 stimulation were to induce cellular adaptation to hypoxia. However, the viability of cortical cells after glutamate stimulation *in vitro* was partially restored by the antisense oligonucleotide for the AT_2 receptor, leading the authors to conclude that the AT_2 receptor may be related to one of the processes in cell injury. It seems puzzling that, in the face of an ischemic injury, the physiological cellular response is the overexpression of the receptors, which increases the consequences of the insult, unless acceleration of cell death may be, in some way, beneficial. AT_2 receptors are involved in the processes of wound healing and tissue repair, and their role in the regeneration of neurons has been established both *in vitro* and *in vivo* [40]. Paradoxically, AT_2 receptor stimulation has also been shown to induce apoptosis in various cell types [40–43] including cultured neurons from newborn rat brain [44]. Several observations suggest that neuronal injury triggers early cellular molecular events that are identical for both apoptosis and regeneration, such as the activation of the mitogen-activated protein kinase phosphatase-1 [45,46]. The idea is thus compelling that activation of the early genes c-*fos* and c-*jun* in response to hypoxic insult could, in fact, bring the cell to a metabolic crossroads, at which point either cell repair or programmed cell death could still occur. Depending on whether or not energy supply has been restored at this point, the cell would be either engaged in a repair process or, on the contrary, would

complete apoptosis, a process regarded as a physiological mode of cellular waste disposal with minimal tissue reaction.

It is thus proposed that all of the physiological actions of the RAS can be viewed as a coordinated effort to preserve tissues against acute ischemia. As blood pressure falls, systemic activation of the RAS contributes to maintaining perfusion of the brain and other organs and functional glomerular filtration in the kidney. When blood supply is restricted in a focal area, local activation of the tissue RAS induces not only the rapid recruitment of the collateral circulation to limit the effect of decreased blood flow, but also contributes to cellular mechanisms for adaptation to the ischemic insult, allowing the cells to survive and recover. Ultimately, if the ischemic injury is severe enough, angiotensin II then promotes apoptosis of the most severely affected cells, thereby favoring the use of limited energy supplies to the still viable cells and reducing the costly inflammatory reaction that would have been induced by cellular necrosis.

Immediately after vascular occlusion, the rapid enhancement of hitherto present but functionally inactive vascular pathways represents the emergency adaptative response [47]. As time proceeds, a slower process takes place to restore blood flow to ischemic areas through angiogenesis, i.e. the formation of new vessels. The angiogenic properties of angiotensin II have now been firmly established, indicating that this compound participates in long-term homeostatic maintenance of tissue perfusion [15,48–51].

3. Angiotensin II as an angiogenic factor

Ilich *et al.* reported that after renal ischemia there is not only a development of collateral circulation but also an increase of endothelial cell turnover, as an indication of cellular mitosis, in the perirenal vessels. A humoral angiogenic factor produced by the ischemic kidney was implicated in this effect [52]. Moreover, Cuttino *et al.* [53] found that, besides the development of the collateral circulation, a factor extracted from ischemic kidneys induced the formation of new vessels when tested in the cheek pouch of the hamster. After the results of experiments in which collateral vessels were opened by angiotensin II, the effect of this octapeptide on vascular neoformation *in vivo* was demonstrated in 1985 [15]. Using the method described by Gimbrone *et al.* for the rabbit cornea [54], angiotensin II was incorporated in a slow release matrix and placed in a pocket surgically created in the cornea. Angiotensin II produced a progressive stimulation of new vessel formation, reaching a plateau in the rabbit in approximately 1 month. Vehicle alone or incorporated with cholecystokinin, an unrelated octapeptide, had no effect. The angiogenic effect of angiotensin II was further demonstrated in other models of angiogenesis, such as the chorioallantoic membrane of the chick embryo and sponge implantation in the rodent [48–50]. Angiotensin II indeed appears to be one of the most potent angiogenic factors known. Placement of angiotensin II, this time in the rat cornea and incorporated in a slow-releasing Hydron pellet, had an angiogenic effect in doses as low as 1.5 pg/pellet 6 days after surgery (Fig. 6). The mechanism by which angiotensin II stimulates vessel growth appears to be related to a chemotactic effect of this compound on endothelial cells [55] or, in addition, through the migration of pericytes [56]. Notwithstanding, the angiogenic effect of angiotensin II could be a direct action on endothelial cells or an indirect action through the expression of vascular endothelial growth or fibroblast growth factors [57,58]. Also, angiotensin II activates, among others, glycosaminoglycan, protein synthesis, G protein, phospholipase C, diacylglycerol, and the inositol trisphosphate pathway [50,59]. The stimulating effect of angiotensin II on cellular growth was reported in other cell lines such as cultured 3T3 and adrenocortical cells [60,61]. The AT_1 receptor appears to intervene in the growth-promoting effect of angiotensin II. Recent research has shown that cell growth and proliferation are mediated by AT_1 receptors, whereas stimulation of AT_2 receptors leads to an inhibition of cell proliferation and promotes cell differentiation [62–64]. Evidence for an antiproliferative effect of angiotensin II was reported in cultured coronary cells and seems to be mediated by AT_2 receptors [65]. On the other hand, Levy *et al.* reported that AT_2 receptor stimulation is the factor responsible for angiogenesis [66]. Under physiologic conditions, AT_1 and AT_2 receptors develop sequentially during microvascular maturation, and the role of the endogenous angiotensin system in angiogenesis depends on the balanced local expression of its various components [67]. Moreover, angiotensin-(1–7) could be another player involved in vascular growth [68].

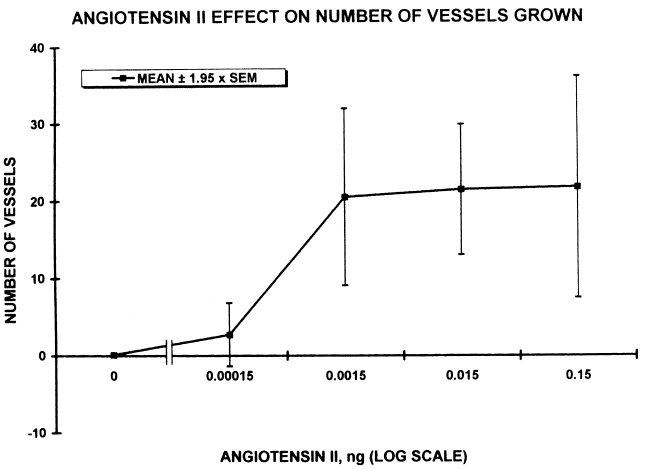

Fig. 6. Dose effect of angiotensin II, incorporated in a Hydron pellet and placed in a corneal pocket of the rat, on the number of vessels that penetrate through the corneal limbus. Number of eyes implanted with a different dose of angiotensin II: 0.0 ng, N = 9; 0.00015 ng, N = 10; 0.0015 ng, N = 15; 0.015 ng, N = 17; and 0.15 ng, N = 13.

An addition to these considerations is the provocative

concept that the etiology of essential hypertension could be related to the angiogenic effect of angiotensin II, not by excess but by defect. The presence of vascular rarefaction in spontaneous hypertensive rats and in cases of human disease [69–71] points to the probability that angiotensin II is involved in this action ([72,73]; for a review, see le Noble *et al.* [74]).

The role of angiotensin II on erythropoiesis is well known [75,76]. During fetal development, the main site of erythropoietin production is the liver. In rat fetuses and neonates, extrarenal and renal production of erythropoietin is regulated primarily by oxygen demand and is increased markedly by angiotensin II perfusion [76]. The role of angiotensin II in humans is largely supported by repeated observations that ACE inhibition worsens anemia in patients with chronic renal failure [77], and both ACE inhibition and AT_1 antagonists are effective in correcting post renal transplantation erythrocytosis [78,79]. The mechanism relies not only on the stimulation of erythropoietin production, but also on a direct stimulatory effect on proliferation of erythroid progenitors by angiotensin II [80,81]. The increase of red cells induced by angiotensin II increases oxygen transport and represents another mechanism by which this octapeptide contributes to the preservation of oxygen delivery to tissues [82].

4. The RAS and tumor angiogenesis

Several reports have described the production of renin by both benign and malignant tumors [83–88]. Some, but not all, of these neoplasms arise from the kidney. There appears to be an association between the presence of renin and the degree of vascularization in some tumors such as angiolymphoid hyperplasia with eosinophilia, GBM, alveolar sarcoma, small cell carcinoma, and adenocarcinoma of lung, pancreas, and ovary, suggesting that renin in tumors, most likely through the cascade to angiotensin II, could contribute to angiogenesis in tumors. The above-discussed stimulating effect of angiotensin II on endothelial growth, the fact that some tumors have been shown to produce renin, and the fact that tumors frequently express angiotensin II receptors [83,88] may yield the hypothesis that angiotensin II acts as a paracrine-autocrine factor promoting tumor growth. Experiments have shown that in rats implanted with Walker 256 carcinosarcoma a significantly increased survival rate was seen over a 25-day period when enalapril, an inhibitor of ACE, was administered orally (Fig. 7). A dose-dependent reduction in tumor size was seen with lisinopril, a different ACE inhibitor, when it was given to rats similarly prepared [89]. Moreover, another ACE inhibitor, captopril, curtails the growth of chemically induced and implanted tumors in rats and mice by reducing angiogenesis [55,90]. Most likely, the RAS in tumors stimulates neovascularization, a requirement for tumor growth and development, through the

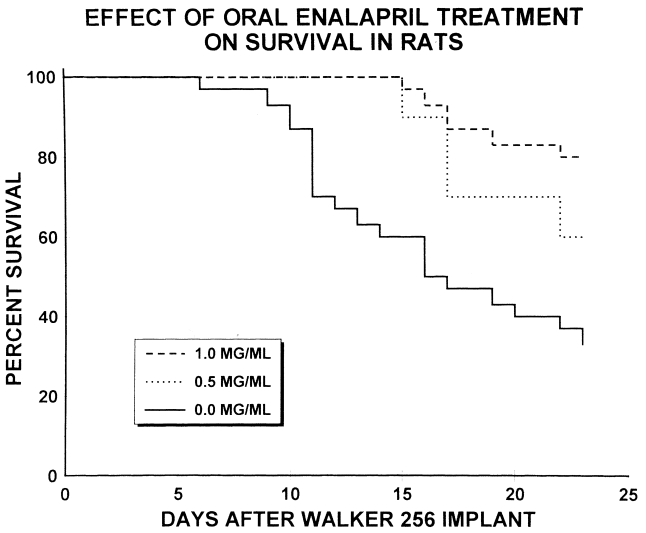

Fig. 7. Survival rate of rats with a subcutaneously implanted Walker 256 carcinosarcoma after *ad libitum* oral administration of enalapril in the drinking water at three different concentrations. Number of animals used in each group: 1.0 mg/mL, N = 30; 0.5 mg/mL, N = 10; 0.0 mg/mL, N = 30.

stimulating effect of angiotensin II on angiogenesis [15,48, 55,91–93]. If this is the case, a specific inhibitor of angiotensin II should block the angiogenic-stimulating effect of neoplasms.

GBM is the most prevalent tumor of the brain and is characterized not only by the expression of renin by the cells, but also by the histological feature of very prominent capillary formations. Interestingly, despite this there is evidence that GBM receives less blood than the surrounding host brain. Small arteriovenous communications are often identified in angiograms of GBM [94], but the observation of red veins over brain tumors suggests that a substantial portion of blood is traversing the lesion without releasing oxygen or reducing hemoglobin. Also, a quantitative confirmation of the red vein observation revealed an increase in the shunt flow but not in the nutritional flow in GBM [95]. It appears that these ischemic changes in GBM resemble those seen in kidney ischemia [96]. To test whether the presence of angiotensin II in GBM is involved in a mechanism of angiogenesis, extracts of this lesion were incorporated into Hydron pellets and placed in a surgically created pocket in the rat cornea. These extracts produced a marked stimulation of new vessel formation. Saralasin, a specific inhibitor of angiotensin II but not the unrelated octapeptide cholecystokinin, blocked the angiogenesis-induced by GBM extracts (Fig. 8).

5. Clinical and therapeutic implications

Although the RAS is increasingly being perceived to exert effects beyond blood pressure control on vascular structure and function, the beneficial effects of ACE inhib-

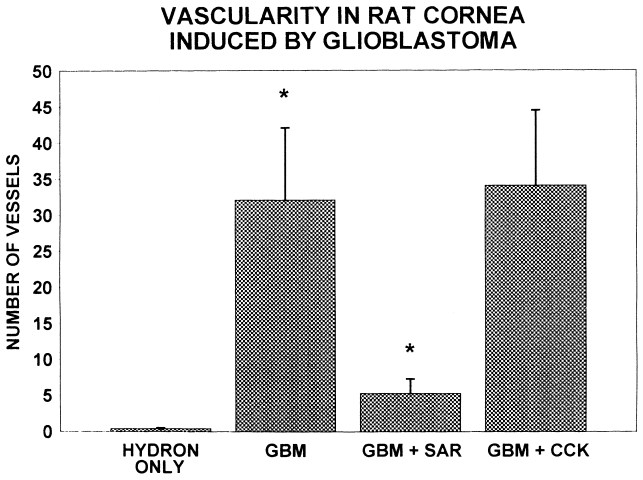

Fig. 8. Vascularity induced by glioblastoma multiforme (GBM) extracts in the rat cornea implanted alone or concomitantly with saralasin (SAR) or cholecystokinin (CCK) in a vehicle of Hydron. Number of eyes implanted in each group: Hydron only, N = 11; GBM, N = 12; GBM + SAR, N = 11; and GBM + CCK, N = 11. Bars represent mean values ± SEM. Asterisks (*) indicate a significant difference between those groups: $P < 0.02$.

itors in cardiovascular patients have created the general concept that activation of the RAS is, by nature, detrimental. This concept has influenced a pharmacological blockade of a natural system that appears to be an effective treatment in preserving cardiovascular function in at-risk patients. However, a critical review of large morbidity and mortality trials clearly points to the superiority of renin-stimulating diuretics over renin-inhibiting β-blockers in preventing strokes in hypertensive patients, not only at high doses but also at low doses [97]. This has been confirmed by the meta-analyses of Psaty *et al.* [98], which show that in elderly stroke patients mortality was not decreased by β-blockers. This discrepancy between the efficacy of diuretics and β-blockers in stroke protection even led Brown and Brown [99] to ask the provocative question: "Does angiotensin II protect against stroke?" soon after the publication of the MRC trial of 1985 [100]. The proposed explanation for this putative beneficial effect of angiotensin II was that its potent vasoconstricting effect reduces the risk of microaneurysm rupture. However, such a mechanism would account for the protection only against intracranial hemorrhages, although ischemic strokes are more frequent. The intrinsic protective effect of angiotensin II on tissue perfusion in the setting of both chronic and acute ischemia demonstrated in animal models could represent a more relevant explanation. Indeed, the first large trial comparing ACE inhibitors to conventional treatment in primary prevention of stroke in hypertensive patients (CAPPP) was published in 1999 [101]. It further confirms that in hypertensive patients ACE inhibition is less effective than angiotensin II-stimulating diuretics in preventing stroke. Captopril was associated with a greater risk of stroke for a similar blood pressure lowering effect, after adjustment for age, sex, presence of diabetes, initial systolic blood pressure, and previous treatment. It is necessary to point out that the age-adjusted stroke incidence has been evenly rising since 1994 [102,103]. This decreased efficiency in primary prevention of strokes could be due to the increasing use of antihypertensive treatments that decrease circulating levels of angiotensin II. Indeed, the trend of antihypertensive therapy has changed dramatically since 1986 with the increasing use of ACE inhibitors, their prescriptions rising from 10 to 68 million, whereas those for diuretics decreased from 105 to 85 million a year. Taken together, the analysis of the large clinical trials on cerebrovascular accidents supports the idea that complete inhibition of the RAS may not be the most effective preventive therapeutical approach for the cerebral complications of hypertension in humans. Even though the beneficial effects of ACE inhibition are not in dispute in cardiac and in severely atherosclerotic patients, as recently confirmed in the large clinical trial HOPE [104], AT_1 receptor antagonists could likely offer similar efficacy while preserving the potential beneficial effects of AT_2-dependent protective actions of angiotensin II against cerebral ischemia [105].

In relation to the angiogenic effect of RAS in tumors, Lever *et al.* [106] reported the first clinical evidence supporting the concept that long-term use of ACE inhibitors may protect against cancer. In a retrospective large cohort study based on the records of 5207 Scottish patients, they observed that the relative risks of incident and fatal cancer in the 1559 patients receiving ACE inhibitors were, respectively, 0.72 and 0.65 when compared with a control population in West Scotland. In patients receiving other antihypertensive drugs, the number of incidents and fatal cancer was not lower than expected. This study suggests the possibility of a relationship between RAS, likely intrinsic to tumors, and tumor development and provides the first available clinical data supporting such an hypothesis.

It is possible that, exactly as for the general growth factor properties of angiotensin II, its angiogenic action results from an equilibrium between an action of the AT_1 receptor, balanced by an effect of an AT_2 receptor. As a consequence, AT_1 antagonists would be expected to decrease angiogenesis more efficiently than ACE inhibitors. The specific property of AT_1 receptor antagonists to block its mediated hormonal action while, in contrast, increasing its AT_2 receptor-based effects may thus allow one to envisage a theoretical advantage of AT_1 antagonists over ACE inhibitors on tumor growth. The same is true for the angiogenic action of angiotensin II, the second putative mechanism by which angiotensin II in tumors might facilitate their growth and metastasis. If this hypothesis is correct, the differences in actions between ACE inhibitors and AT_1 antagonists would point to the latter being the more efficient. A comparative prospective trial on the long-term use of AT_1 antagonists rather than of ACE inhibitors versus other antihypertensive drugs appears justified.

6. Conclusions

A great deal remains to be learned about the complexity of the RAS system, but recent new insights into the opposing and balanced effects mediated through distinct receptors suggest a new view of the general physiological role of the RAS, which may now be viewed as a central player in the sequence of events leading to the protection of cells against ischemia. Metaphorically, angiotensin II not only opens detours to bypass roadblocks ahead and create new trails, but, additionally, stimulates the production of carriers necessary to deliver the commodities to the consumers. Although the development of potent inhibitors of angiotensin II converting enzyme has undoubtedly provided therapeutic progress in the management of patients with heart failure and atherosclerotic vascular disease, this new knowledge must be integrated to better define how the RAS should be pharmacologically manipulated in various types of patients. A large body of converging experimental evidence, together with preliminary clinical data, suggests that because of the duality of angiotensin II receptors, AT_1 receptor antagonists may have a greater protective effect than ACE inhibitors against stroke and at least similar potential protective effects against cancer. Because of the burden of these diseases on public health, the promotion of large multicenter randomized trials comparing ACE inhibitors and AT_1 receptor antagonists should be regarded as a priority by healthcare providers and the pharmaceutical industry.

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