Angiotensin II Decreases Mortality Rate in Gerbils with Unilateral Carotid Ligation

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SUMMARY Evidence indicates that after vascular occlusion, infusion of angiotensin II restores blood supply to ischemic tissues by stimulating the development of collateral circulation through a mechanism independent of the mechanical effects of increased blood pressure. To test this effect in focal cerebral ischemia, angiotensin II was intravenously administered for four hours immediately after unilateral carotid ligation. Three different pressor doses, 50, 250, and 500 ng/kg/min, were used, and mortality rate was evaluated at 1 and 2 days after vascular occlusion. Two additional groups similarly prepared were infused either with saline or with the pressor agent metaraminol. There was a significant inverse relationship between the infusion dose of angiotensin II and mortality: the greater the infusion dose of angiotensin II, the lower the mortality rate. Infusion of metaraminol, at the dose chosen to mimic the pressor effect of the highest angiotensin II dose, yielded a mortality rate which was statistically indistinguishable from that obtained with saline infusion. It is concluded that the mortality rate after unilateral carotid occlusion is significantly reduced by intravenous administration of angiotensin II through mechanisms unrelated to its hypertensive action. Evidence suggests that this may occur by the enhancement of the development of collateral circulation and therefore the reduction of the severity of brain ischemia.

CEREBROVASCULAR DISEASE caused by restriction of blood flow to the brain through normal vascular channels is a common clinical occurrence. Attempts have been made to protect the central nervous system from ischemia using pharmacological or surgical procedures or by elimination or inhibition of putative harmful products generated following the impairment of blood supply. The effectiveness of these therapeutic measures remains speculative. The removal of the causative factors of brain ischemia is still the treatment of choice. This is not often accomplished after a sudden ischemic event at which time increasing blood flow to the ischemic areas is critical. Therefore, the enhancement of the development of collateral circulation would be, under these circumstances, the optimum palliative mechanism to maintain the necessary blood supply to the brain. Stimulation of the development of collateral circulation to ischemic areas could represent a potential therapeutic tool in the treatment of the ischemia of the central nervous system.

Collateral circulation was defined by Liebow as a system of vessels which develops to substitute for major vascular pathways. There are at least two phases during collateral circulation development. Immediately after vascular occlusion, there is a rapid enhancement in function of previously present "in reserve" vascular pathways which is followed, as time proceeds, by the formation of new vessels. Several factors of neural, mechanical and chemical origin have been proposed as stimuli in the development of collaterals.

In the kidney, the renin-angiotensin system has been demonstrated to act as a stimulus for the development of collateral circulation. After induction of renal ischemia, the endogenous renin-angiotensin system or the administration of angiotensin II have been shown to rapidly restore blood flow to the affected area. Evidence indicates that this action is unrelated to the mechanical effect of the increased blood pressure produced by angiotensin II. Renin or the renin-angiotensin cascade is not exclusively present in the kidney but is found also in other organs, including the central nervous system. Its role to the ischemic kidney, serving as a protective mechanism against focal ischemia by promoting the development of collateral circulation, suggests that a similar function might be applied to the ischemic brain. If increased levels of circulating angiotensin II have an effect in the ischemic brain similar to that seen in the kidney, then infusion of this peptide should result in an increased survival rate after focal cerebral ischemia.

The present experiments were designed to study the effect of exogenous administration of angiotensin II to gerbils with focal brain ischemia. It is known that the
gerbil is prone, due to a particular anatomical configuration of the vascular supply to the brain, to develop focal cerebral ischemia leading to stroke and eventual death following acute unilateral carotid artery ligation. The results of these experiments show that the infusion of angiotensin II significantly reduces the mortality rate in gerbils with unilateral carotid ligation and that this effect is apparently not a consequence of the elevated blood pressure produced by angiotensin II.

Methods
Adult male gerbils (Meriones unguiculatus), purchased from Tumblebrook Farm, Inc., West Brookfield, Massachusetts, weighing between 59 and 92 grams were used. They were kept in controlled-temperature rooms at 24°C with periods of light and darkness of 12 h each. The animals were fed with rat chow (Ralston Purina, St Louis, Missouri), and tap water was given ad libitum. Surgical procedures were performed under sodium pentobarbital anesthesia intraperitoneally administered at a dose of 60 mg/kg of body weight using clean but not sterile technique. Animals were placed in the supine position, the neck region was cleaned with ethanol 95%, and a midline incision was made exposing the superficial left jugular vein and right carotid artery. A polyethylene catheter, primed with the solution to be infused was placed in the jugular vein. Unilateral carotid ligation was done with a 4-0 silk thread and infusion started immediately after connecting the venous cannula to a syringe driven by an infusion pump (Harvard Apparatus, Millis, Massachusetts). All infusions were maintained for four hours at a rate of 0.135 ml per hour, during which time the wound was covered with a gauze moistened with saline. A single supplementary dose of anesthesia (15 mg/kg, ip) was given to each animal as required or at three hours after carotid artery ligation. At the end of the four-hour infusion period, the venous cannula was removed, and the jugular vein was tied. The skin was sutured, and the animals were returned to their cages and allowed to recuperate from the anesthesia. At 1 and 2 days after carotid ligation mortality was evaluated. Differences in mortality rates between groups were analyzed using the logrank test for survival.

The following groups composed of 25 animals each were studied: a) Saline infused; b, c, d) Angiotensin II (1-L-asparaginyl-5-L-valyl angiotensin octapeptide, Ciba-Geigy, Summit, New Jersey) infused at a dose of 50, 250 and 500 ng/kg/min; e) Metaraminol bitartrate (Merck, Sharp & Dohme, West Point, Pennsylvania) infused at a dose of 25 μg/kg/min. All the drugs were dissolved in NaCl solution (0.9% w/v). Group e was administered at a dose of 25 μg/kg/min. The pressor agents infusion at the dose of 500 ng/kg/min, mortality rates were recorded at the dose of 500 ng/kg/min. All the drugs were dissolved in NaCl solution (0.9% w/v). Group e was administered at a dose of 25 μg/kg/min. The pressor agents infusion at the dose of 500 ng/kg/min, mortality rates were 50%. Mortality rates in group infused with saline were 28% and 48% at one and two days after surgery, respectively. In gerbils in which carotid ligation was followed by angiotensin II infusion at the dose of 500 ng/kg/min, mortality rates were reduced to 0% and 16% at one and two days after carotid ligation, respectively. Lower doses of angiotensin II produced a less marked effect on mortality rate. At 250 ng/kg/min, mortality rates were 24% at day one and 28% at day two. At 50 ng/kg/min, mortality rates were 28% at day one and 36% at day two. Chi-square test for trend yielded 5.26: d.f. = 1; p < 0.025. After the infusion of metaraminol, mortality rates were 44% and 56% at one and two days after surgery, respectively, in spite of the increase in mean blood pressure. No significant differences were present between these values when compared with those from the saline infused group. When in an additional group of gerbils the dose of metaraminol was doubled to 50 μg/kg/min with the purpose of increasing the mean blood pressure further, 100% of the animals died during or shortly after infusion.

Results
Results from experiments in which mortality rates were evaluated are summarized in figure 1. Death was preceded by neurological deficit of varying degrees. Mortality rates after unilateral carotid ligation in the group infused with saline were 28% and 48% at one and two days after surgery, respectively. In gerbils in which carotid ligation was followed by angiotensin II infusion at the dose of 500 ng/kg/min, mortality rates were reduced to 0% and 16% at one and two days after carotid ligation, respectively. Lower doses of angiotensin II produced a less marked effect on mortality rate. At 250 ng/kg/min, mortality rates were 24% at day one and 28% at day two. At 50 ng/kg/min, mortality rates were 28% at day one and 36% at day two. Chi-square test for trend yielded 5.26: d.f. = 1; p < 0.025. After the infusion of metaraminol, mortality rates were 44% and 56% at one and two days after surgery, respectively, in spite of the increase in mean blood pressure. No significant differences were present between these values when compared with those from the saline infused group. When in an additional group of gerbils the dose of metaraminol was doubled to 50 μg/kg/min with the purpose of increasing the mean blood pressure even further, 100% of the animals died during or shortly after infusion.
The results of mean blood pressure recording are shown in figure 2. In the group infused with angiotensin II at the dose of 500 ng/kg/min, mean blood pressure markedly increased to a peak in approximately 4 minutes and slowly decreased to a plateau stabilizing at a similar level for the remaining period of infusion. When angiotensin II administration was discontinued, blood pressure fell rapidly. In the group infused with 50 ng/kg/min, blood pressure rose to a maximum value lower than in the latter group at approximately 2 min after the infusion was commenced. Discontinuation of the infusion also produced a rapid decrease of mean blood pressure. Infusion of metaraminol produced an increase in mean blood pressure in approximately 2 minutes. Again, when the drug infusion was discontinued, mean blood pressure rapidly decreased. No significant differences of blood pressure are present between the angiotensin 500 ng/kg/min group and the metaraminol infused groups. In the saline-infused gerbils, the blood pressure remained stable throughout the recording period. Neither the carotid ligation nor the saline infusion produced any significant changes in mean arterial pressure.

Discussion

Previous reports have shown that unilateral carotid ligation in the gerbil is followed by neurological signs and morphologic changes consistent with focal brain ischemia. The results of the present experiments show that infusion of angiotensin II significantly reduces the mortality rate in gerbils after unilateral carotid ligation. These results indicate that the decrease in mortality rate is due, most likely, to a specific action of angiotensin II unrelated to the mechanical effect of the high blood pressure produced by this octapeptide. This conclusion is consistent with the finding that administration of a different pressor agent tended to increase rather than decrease mortality rate.

The mechanism of this effect remains speculative. If it is valid to extrapolate results from experiments in which ischemia was induced to the kidney after ligation of the abdominal aorta, it is possible to postulate that the effect of angiotensin II is due to the improvement of blood supply to the ischemic areas of the brain through the development of collateral pathways to bypass the surgically-created carotid occlusion. In support of this hypothesis, results from preliminary experiments measuring cerebral blood flow in identical animal preparation using hydrogen clearance, have shown a progressive restoration of cerebral blood flow during infusion of angiotensin II (Unpublished results). Cerebral blood flow was measured before and at several intervals after unilateral carotid occlusion and during angiotensin II administration. Immediately after carotid occlusion, blood flow decreased to 55% of preligated values (100%). Infusion of angiotensin II at 500 ng/kg/min brought about a gradual restoration in blood flow to the ischemic hemisphere over the next 90 minutes. In these animals, the increase in blood pressure produced by angiotensin II was controlled by blood withdrawal sufficient to maintain pressure at approximately preinfusion levels. In saline-infused animals, blood flow remained at reduced levels over the entire 90 minute period. These preliminary results indicate that an increase in blood flow to ischemic areas of the brain under angiotensin II treatment may be responsible for the decrease in mortality rate. This finding would be consistent with the notion of angiotensin II acting as a chemical stimulus for collateral circulation development to the ischemic stimulus for collateral circulation development to the ischemic brain in a fashion similar to that seen to the ischemic kidney.

The stimulus for the development of collateral circulation is not completely understood, and several theories have been postulated. It is widely accepted that the mechanical effect of the pressure gradient across the vascular obstruction plays a role in the mechanism of opening collateral pathways. Although it has been suggested that this mechanical action of blood pressure could be a responsible mechanism, the involvement of chemical mediators cannot be ruled out in any of the reported experiments, and no attempt has been made to determine which factor is more important. Previous work from this laboratory has demonstrated that the development of collateral pathways to ischemic kidney occurs regardless of changes in mean blood pressure. In that report, the high circulating levels of renin or angiotensin II, due to the stimulation of the intrinsic renin-angiotensin cascade or to the infusion of angiotensin II, were shown to play a role in the development of collateral circulation from above to below the aortic constriction. By independently manipulating the blood pressure and the plasma renin activity, it was possible to demonstrate that the opening of collateral pathways can be achieved by increasing plasma renin activity and not blood pressure. In the present experiments, the use of a different pharmacological agent with vasopressor action, metaraminol, showed that augment-
ed blood pressure does not necessarily decrease the mortality rate under the present experimental conditions. Metaraminol is an alpha-receptor stimulator which increases blood pressure by increasing vascular peripheral resistance through vasoconstriction. Angiotensin II also increases peripheral resistance through vasoconstriction. By infusing metaraminol, elevated blood pressure was obtained; however, it was accompanied by a mortality rate not different from that obtained with saline infusion. The present results indicate that other actions of angiotensin II may be more important than a mere increase of blood pressure and give credence to the proposal of a chemical rather than mechanical factor involved in the development of collateral circulation.

As Liebow pointed out, there are several ways that a chemical mediator can facilitate the development of collateral circulation: a) by vasodilatation; b) by controlling the proliferation of new vessels; c) by guiding vessels to specific destinations. As previously discussed, it appears that there is an incongruity between the concept of a vasodilator mechanism and the effect of angiotensin II. Angiotensin II is one of the most potent vasoconstrictors known and also exerts its vasoconstrictor properties on the cerebral vessels. If vasoconstriction plays a role in the development of collateral circulation, the interesting concept of "reverse vascular steal" described by Chiarello and his associates for the cerebral circulation could be proposed also for the cerebral circulation. It is necessary to point out that the other vasconstrictor used in the present experiments, metaraminol, had no significant effect on mortality rate, but it is also possible that metaraminol may act on a different anatomical location of the vasculature than angiotensin II. A possibility to be considered, moreover, is that the effect of angiotensin II could be mediated through vasodilators such as prostaglandins. In any event, the mechanism by which collaterals are developed may involve a more dynamic and complex process than a mere widening of the caliber of the vessels.

The cellular elements of the brain are extremely sensitive to a lack of blood supply. Therefore, the action of angiotensin II must be rapid enough to produce its effect before irreversible changes take place. It seems likely that in the relatively short time involved, collateral circulation must be formed by activating pre-existing but hitherto "in reserve" vascular channels, possibly in a fashion similar to the one described by Longland. As time proceeds, the continuous action of angiotensin II would possibly be involved also in the slow phase, producing the stimulation of new vessel formation.

The concept of a chemical mediator involved in the development of collateral circulation is not new. In 1940, Lewis proposed the idea of a chemical stimulant, produced as a result of tissue-need, which controls the development of collateral circulation. The renin-angiotensin system may be such a chemical stimulant.

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