Angiotensin Delays Platelet Aggregation After Injury of Cerebral Arterioles

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SUMMARY Endothelium of cerebral surface vessels (pial arterioles and venules) was injured with a light/dye technique in anesthetized mice. This induced platelet aggregation at the site of injury. The onset of aggregation was monitored through a microscope in mice given angiotensin II acetate, 4 μg i.v. 30 minutes earlier. Aggregation latency was compared with that in vehicle treated (saline) mice. Angiotensin II caused a highly significant delay in aggregation within the arterioles which was not related to a change in shear rate of blood. Angiotensin II added to platelet rich plasma, failed to influence the aggregation produced by subsequent addition of 0.5 μM ADP or 0.5 mM sodium arachidonate. Angiotensin is a well known stimulator of prostacyclin synthesis or release, and angiotensin has been reported to inhibit platelet aggregation in vivo by increasing prostacyclin in the effluent superfusing the mass of aggregating platelets. Our data represent the first report of an antiaggregating effect of angiotensin II in vivo in an intact microvascular bed. The data is consonant with the literature describing increased prostacyclin levels following angiotensin II infusion. The antiaggregating effect of angiotensin in cerebral microvessels may help explain a recent observation describing increased survival of gerbils treated with angiotensin following carotid ligation.

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calculated shear rate from RBC velocities measured in arterioles of all mice and we measured microvascular pressures in a parallel study of pial arterioles in 10 additional mice. RBC velocity was measured in the preselected arteriole using a 2-slit velocimeter (IPM, San Diego, CA) and cross correlator. Microvascular pressures were measured using a servo-null pressure measuring device (Eutectic Electronics, Raleigh, NC). The micropipettes used with this device had a tip 2–3 μm in diameter and were filled with 1.5 molar NaCl.

At the end of each experiment blood pH, CO₂, and O₂ were measured in blood from the carotid artery using an ultramicro blood gas analyzer (Radiometer, Cleveland, OH). Values in angiotensin and vehicle treated mice were identical and will not be mentioned again (CO₂ = 32 ± 5 vs 33 ± 5 mm Hg; O₂ = 93 ± 7 vs 92 ± 8 mm Hg; pH 7.39 ± 0.07 vs 7.37 ± 0.06).

Finally to test the capacity of angiotensin to directly influence platelet aggregation we prepared platelet rich plasma (PRP) from mouse blood. First we stimulated this PRP with angiotensin II acetate (4 μg/ml) in an aggregometer (Chronolog, Havertown, Pennsylvania). Aggregation was expressed as a change in optical density of the PRP. Since no aggregation occurred even after four minutes at 37°C we were also able to test the effect of angiotensin II acetate (4 μg/ml) on subsequent aggregation produced by adding either 0.5 mM sodium arachidonate or 0.5 μM ADP. The maximal aggregation (M ± SD) produced by each agent was 73 ± 3 (N = 4) and 64 ± 7 (N = 5) in the control PRP and 74 ± 4 (N = 4) and 63 ± 3 (N = 5) in the PRP to which angiotensin had been added.

**Discussion**

The data clearly show that an intravenous bolus of 4 μg angiotensin II acetate significantly delays the onset of platelet aggregation in injured pial arterioles. The endothelial injury inducing this aggregation was produced by our light/dye technique 30 minutes after the injection of angiotensin. The data also show that both shear rate and microvascular pressure were normal when platelet aggregation was elicited thirty minutes after injection of angiotensin. Therefore the delayed onset of aggregation was not caused by increased blood pressure or shear rate. This is important because we have previously shown that if shear rate is elevated platelet aggregation may be delayed.

Since the effect of angiotensin cannot be ascribed to a change in shear rate it is appropriate to consider a direct effect of angiotensin on platelets as a cause of delayed aggregation. Angiotensin receptors exist on platelets. However we were unable to demonstrate an effect of angiotensin on the platelet itself. When platelets were studied in platelet rich plasma in an aggregometer, angiotensin did not aggregate platelets nor did it alter their response to the aggregating agents ADP or sodium arachidonate. In particular, angiotensin failed to inhibit subsequent aggregation by these agents.

If angiotensin did not exert a direct inhibitory effect on aggregation we must consider an indirect effect, in vivo, via release of a second messenger such as prostacyclin. Many studies have shown that angiotensin increased prostacyclin levels in a wide variety of tissues and bodily fluids. Of these many studies we cite three which demonstrated that prostacyclin is released from vascular beds during perfusion by angiotensin. Many studies have shown that angiotensin increased prostacyclin synthesis (or release) by vessel
wall has been demonstrated by showing reversal of platelet aggregation \textit{ex vivo}. The platelet aggregates had already been permitted to form on a suffused tendon. Our study appears to be the first to show an effect of angiotension on platelet aggregation totally \textit{in vivo}.

In the present study, angiotension II delayed aggregation in arterioles but not in venules. Previously we had demonstrated that cyclooxygenase inhibitors delayed onset of aggregation only in pial arterioles and not pial venules. Moreover, we reported that tranylcypromine, a drug which inhibits prostacyclin synthesis, accelerated aggregation in arterioles. Venules were not mentioned in the latter report, but in fact we found no effect of tranylcypromine on aggregation in the pial venules. We can only speculate about the reasons why manipulation of cyclooxygenase or the endogenous products of cyclooxygenase activity only alter aggregation in the pial arterioles and not the venules. One explanation may be the more rapid onset and progression of endothelial lesions in the venules following damage by light/dye. Perhaps this makes it more difficult for inhibitors to act effectively in the venules, and perhaps the already brief aggregation latency in venules represents a response that would be difficult to shorten further.

Others have reported that angiogenins II protects gerbils from stroke. This effect could not be related to the pressor effects of angiotension. The basis for the protective effects of angiotension II was not clear. A beneficial effect on platelet aggregation was not considered. Nevertheless platelets do accumulate in ischemic regions of brain. It is probable that many of these platelets aggregate and release constricting substances such as thromboxane A, and serotonin. Both the physical obstruction of microvessels by aggregates, and the vasoconstriction may exacerbate the original ischemia. Consequently the beneficial effect of angiotension II on the progression of cerebral ischemia in gerbils may possibly be explained by the inhibition of platelet aggregation as demonstrated by our data. Such a suggestion could be supported by a study in which angiotension II is given to gerbils with carotid ligation and platelet accumulation in the ischemic tissue is compared with that of gerbils who do not receive angiotension II. Meanwhile, our data serve as evidence that angiotension II can impair platelet aggregation in the cerebral microcirculation, and also as the first \textit{in vivo} evidence of such an effect in any microvascular bed.

References

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Stroke. 1986;17:1203-1205
doi: 10.1161/01.STR.17.6.1203

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