Angiotensin Delays Platelet Aggregation After Injury of Cerebral Arterioles

WILLIAM I. ROSENBLUM, M.D., FAROUK EL-SABBAN, PH.D., AND PAUL D. HIRSH, M.D.

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 ex 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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IT WAS RECENTLY REPORTED that angiotensin II can protect gerbils against cerebral infarction.1 The protective effect was independent of the hypertensive effects of angiotensin and a clear explanation of its beneficial effect was not available. However, it is well known that angiotensin elevates prostacyclin levels in blood and that prostacyclin is a powerful inhibitor of platelet aggregation.2,3 The antiaggregating effects of angiotensin have always been reported in terms of the effect in an in vitro or ex vivo test system suffused by blood and that prostacyclin is a powerful inhibitor of platelet aggregation.2,3 The antiaggregating effects of angiotensin have always been reported in terms of the effect in an in vitro or ex vivo test system suffused by prostacyclin rich effluent from a donor infused with angiotensin.2 We now report the antiaggregating effect of angiotensin II as manifest by delayed onset of aggregation in injured cerebral surface arterioles (pial arterioles). This is, to our knowledge, the first wholly in vivo demonstration of the antiaggregating effect of angiotensin II. This effect of angiotensin may represent at least one factor explaining the beneficial effects of angiotensin in gerbils with carotid ligation.1 We offer this suggestion because it is well known that platelets rapidly accumulate in zones of cerebral ischemia,6,7 where their presence could exacerbate the effects of ischemia in several ways.

Methods

Male mice, ICR strain (Flow Laboratories, Dublin, Virginia) weighing 27–33 grams were used. The pial preparation has been exhaustively described.8,9 Briefly, the mice were anesthetized with urethane, a tracheostomy and craniotomy performed, the dura stripped, and the pial surface kept moist with artificial cerebrospinal fluid.8,10 The mouse was maintained at 37°C and the pial surface was observed with incident light via a Leitz Ultropak microscope.

The technique for injuring the microvascular endothelium, with resultant platelet aggregation has also been extensively and repeatedly described.9,11,12,13 The technique is notable for showing that in the microcirculation endothelial injury will trigger platelet aggregation long before any exposure of subendothelium occurs.11,12 The endothelium is injured by illuminating the microscopic field via the Ultropak illuminator, using a mercury arc lamp in the presence of sodium fluorescein. Neither light nor dye are injurious by themselves. The combination, with the system of objectives and illumination used here9,11,13 results in rapid endothelial injury and platelet aggregation. The adhering, growing aggregate fluoresces brightly and is readily recognized through the microscope. The dye (0.8 ml/100 g mouse, 2% solution in saline) is injected in 1–2 seconds via tail vein, and the onset of the first recognizable aggregate is timed with a stop watch in a preselected arteriole and venule. The arteriole is arbitrarily chosen for study based on its size (30–50 µ) and the presence of a nearby venule. The pairing of arteriole and venule makes possible the monitoring of both sides of the capillary bed in the same experiment.

A single injection of 4 µg angiotensin II acetate (Sigma, St. Louis, MO) was given via tail vein. Measurements of red cell velocity, calculation of shear rate, and induction of platelet aggregation were initiated 30 minutes after angiotensin injection in order to permit pressure to return to pre injection levels. On each day control mice were alternated with angiotensin injected mice; an equal number of each were studied on any given day. Controls were injected with saline (the vehicle for angiotensin).

Angiotensin is a pressor, and aggregation may be delayed due to the increased shear rate that accompanies elevated blood pressure.14 To rule out elevated pressure and shear rate as a cause of the results we

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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. It is reasonable to assume that a similar phenomenon occurred in our study and offers the most likely explanation for the delayed onset of aggregation. Herefore, the effect on platelet aggregation of such increased prostacyclin synthesis (or release) by vessel

<table>
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<th>Table 1 Angiotensin Pretreatment Delays Onset of Platelet Aggregation in Injured Pial Arterioles</th>
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<tr>
<td><strong>Angiotensin</strong> Group</td>
</tr>
<tr>
<td>(N = 10)</td>
</tr>
<tr>
<td>Weight (gm)*</td>
</tr>
<tr>
<td>Diameter arteriole (µ)</td>
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<tr>
<td>Latency aggregation arteriole (sec)</td>
</tr>
<tr>
<td>Shear rate arteriole (sec⁻¹)</td>
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<tr>
<td>Diameter venule</td>
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<td>Latency aggregation venule (sec)</td>
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*All values in this table are expressed as M ± SD.
†Significantly longer than control (p < .001, Students "t" with Welch-Aspin modification for significantly different variances (22)). An analysis of covariance with shear rate as the covariant also showed highly significant delay of aggregation after angiotensin (p < .001).
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 angiotensin on platelet aggregation totally in vivo.

In the present study, angiotensin II delayed aggregation in arterioles but not in venules. Previously we have 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 angiotensin II protects gerbils from stroke. This effect could not be related to the pressor effects of angiotensin. The basis for the protective effects of angiotensin 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 angiotensin 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 angiotensin 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 angiotensin. Meanwhile, our data serve as evidence that angiotensin II can impair platelet aggregation in the cerebral microcirculation, and also as the first in vivo evidence of such an effect in any microvascular bed.

References
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