Local application of prostacyclin (PGI₂) to cerebral (pial) microvessels, inhibited the aggregation of platelets induced in the vessels by exposing them to a filtered mercury light source following intravenous sodium fluorescein. The inhibition was consistently observed in venules rather than arterioles and was manifest by a lengthening of the time required for the noxious stimulus to produce an initial aggregate, and/or by a lengthening of the time required for enlarging aggregates to totally block the venule. The consistency of the inhibition diminished at doses below 100 μg/ml. Inhibition was observed whether or not alcohol was used as the vehicle for PGI₂ and whether or not the body temperature of the anesthetized mouse was permitted to fall. 

Support of this suggestion, we have demonstrated that application of PGI₂ to microvessels in the hamster cheek pouch inhibited platelet aggregation within cerebral microvessels. Others have reported that application of PGI₂ to microvessels in the hamster cheek pouch inhibited platelet aggregation within them. There are no reports of a similar effect, produced by exogenous PGI₂ applied to cerebral vessels, though a similar effect would be expected from our in vivo study of the action of tranylcypromine. A direct demonstration of PGI₂ action on cerebral microvessels is important to support our interpretation of the tranylcypromine studies and to extend observations beyond a single vascular bed.

Methods

The method used has been reported previously. Male mice of the ICR strain (Dublin Farms) were anesthetized with urethane. Tracheotomy was performed, and about 35 minutes after the anesthetic was given a craniotomy was performed, the dura was anesthetized with urethane. Tracheotomy was performed, and 25 min after craniotomy. After finding a representative field with tungsten illumination, the mouse was injected via a tail vein with 0.2 ml 2% Na fluorescein and the pial vessels illuminated with appropriately filtered light from a mercury source. The combination of light plus dye produces fluorescing venules rather than arterioles and was manifest by a lengthening of the time required for the noxious stimulus to produce an initial aggregate, and/or by a lengthening of the time required for enlarging aggregates to totally block the venule. The consistency of the inhibition diminished at doses below 100 μg/ml. Inhibition was observed whether or not alcohol was used as the vehicle for PGI₂ and whether or not the body temperature of the anesthetized mouse was permitted to fall. 

Summary

IT HAS BEEN SUGGESTED on the basis of in vitro studies, that a prostaglandin, PGI₂, may be an important endogenous inhibitor of platelet aggregation. In support of this suggestion, we have demonstrated that mice treated with tranylcypromine, an inhibitor of PGI₂ synthesis, display enhanced platelet aggregation within cerebral microvessels. Others have reported that application of PGI₂ to microvessels in the hamster cheek pouch inhibited platelet aggregation within them. There are no reports of a similar effect, produced by exogenous PGI₂ applied to cerebral vessels, though a similar effect would be expected from our in vivo study of the action of tranylcypromine. A direct demonstration of PGI₂ action on cerebral microvessels is important to support our interpretation of the tranylcypromine studies and to extend observations beyond a single vascular bed.

William I. Rosenblum, M.D. and Farouk El-Sabbab, Ph.D.
prohibited the potentially misleading comparison of control animals examined at one period of time, with experimental animals examined at another as such a comparison might give spurious results because of changes in basal or control values from one period to the next. Thus, each study consists of an experimental group and its control, 2 groups of mice equal in number, and studied together over exactly the same period of time.

Results

PGI₂ inhibited aggregation in cerebral microvessels if sufficiently high concentrations were used. The effect was consistently demonstrable only in venules, at the highest dose tested (100 μg/ml) and using time required for the noxious stimulus to produce an initial aggregate as the critical parameter (table). As the table shows, body temperature and the presence or absence of ethanol failed to influence the inhibiting effect of PGI₂, which was manifest as a prolongation of the time required to initiate aggregation. With respect to body temperature, mice in the second study were examined on the same days as those in the first study, but in study 2, body temperatures were permitted to fall to 28°C. The temperatures of the mice in study 3 were also permitted to fall. To determine the effects of ethanol we compared mice exposed to one of 2 vehicles, one with ethanol, the other without. Platelet aggregation was the same in the 2 groups.

When we used as our monitored parameter the time between onset of noxious stimulus and blockage of flow by accumulating aggregates, results paralleled those reported above, but, as noted in prior publications, this parameter was not as sensitive to drug effects as was the time required to initiate aggregation. Thus, a significant prolongation of time to block flow was found in studies 1 and 2, but not study 3.

<table>
<thead>
<tr>
<th>Study #</th>
<th>Treatment (Dose of PGI₂ in μg/ml or Control)</th>
<th>Time (sec) to first aggregate in venules</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100** (N = 10)</td>
<td>39 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Control** (N = 10)</td>
<td>25 ± 3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100 (N = 10)</td>
<td>38 ± 5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Control (N = 10)</td>
<td>24 ± 4</td>
<td></td>
</tr>
<tr>
<td>3*</td>
<td>100 (N = 10)</td>
<td>38 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Control (N = 10)</td>
<td>22 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

*No ethanol used in either experimental or control vehicles. **Mice kept at 37°C.

The results of 3 studies are shown. Data are expressed as mean ± standard deviation, and show consistent prolongation of time to first aggregate at 100 μg/ml PGI₂. Body temperature and the presence (studies 1 & 2) or absence (study 3) of ethanol had no effect on the results. Student's t-test was used to evaluate the results.

When the dose of PGI₂ was reduced from 100 μg/ml to 50 or 10 μg/ml the effect of the compound was greatly diminished. Thus at 50 μg/ml only 2 of 3 studies showed a significant lengthening of either time to initiate aggregation or to block flow, and a single study at 10 μg/ml showed no significant effect on either parameter.

Discussion

These experiments were based upon our prior studies in which systemic administration of an inhibitor of PGI₂ synthesis, tranylcypromine, potentiated aggregation of platelets in cerebral (pial) arterioles, but had no effect on venules. The present results, showing a demonstrable effect of exogenous PGI₂ on aggregation in venules, is consistent with the original tranylcypromine data. For example, if endogenous PGI₂ synthesis is high in arterioles, addition of exogenous PGI₂ might have no discernible effect on the platelets in arterioles, while inhibition of PGI₂ synthesis by tranylcypromine would greatly affect aggregation in arterioles. Venules might lack sufficient endogenous PGI₂ synthesis to influence aggregation, therefore exogenous PGI₂ would observably inhibit aggregation as found in the present experiments and tranylcypromine would have no effect. Differential PGI₂ synthesis in arterioles and venules has been reported for the rat, indicating greater synthesis of PGI₂ by arteries than by veins. Greater synthesis of endogenous PGI₂ in arterioles in our model could also explain the slower formation of platelet aggregates there as compared with the time required for aggregation in the venules.

Reports of greater PGI₂ synthesis by arteries as compared with veins, do not establish similar differential synthesis between the smaller arterioles and venules. An alternative explanation for an effect of PGI₂ on venules could be that PGI₂ may more easily penetrate the thin walls of venules than the thicker walls of arteries or it may be differentially inactivated enzymatically or nonenzymatically in the wall. In addition, flowing blood may act as an effective sink, causing a regional dilution of PGI₂. This action could reduce its concentration to very low levels compared with the concentration in the drop covering the vessel at the beginning of the experiment. As the velocity of flow is so much greater in arterioles than venules, it may be that local dilution of PGI₂ is much more marked in arterioles, and that this may account for our ability to demonstrate an effect only in venules.

Aggregation was only affected by extravascular doses far in excess of physiological concentrations. A number of mechanisms like those mentioned above, may serve to greatly reduce the dose before it reaches the platelet. Thus the high concentration of externally applied PGI₂ required in these experiments does not mitigate against a physiologic role for endogenous PGI₂ in regulating platelet aggregation in cerebral microvessels.

There have been few earlier demonstrations of PGI₂ effect in vivo. These are our previous, indirect,
demonstration using tranylcypromine,4 a similar demonstration by others,5,6 and a direct demonstration in which PGI2 was locally applied to injured vessels in hamster cheek pouch.6 Our data thus offer a different type of supporting evidence on the capacity of PGI2 to influence platelet aggregation in vivo. We have, in addition, extended previous data to another organ and another species, suggesting that PGI2 modulation of platelet function is a widespread phenomenon.

Acknowledgment

Ms. Anne Litten provided essential technical assistance. PGI2 supplied by Dr. John E. Pike, The Upjohn Co., Kalamazoo, MI.

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Effect of Phenoxybenzamine on Cerebral Blood Flow and Metabolism in the Baboon During Hemorrhagic Shock

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SUMMARY Experiments were performed on 2 groups of baboons anesthetized with Sernylan. One group served as control and the other was premedicated with 5 mg/kg phenoxybenzamine (PBZ). A 2-step hypovolemic shock model was used followed by retransfusion of the shed blood. Cerebral blood flow was measured by the 133Xe clearance method. Arterial and cerebral venous samples were taken and analyzed for blood gases as well as glucose and lactate content. The cerebral metabolic rates of oxygen, glucose, and lactate were calculated. In addition, the effect of CO2 inhalation was studied before shock was induced. PBZ produced no effect on either CBF or the flow response to CO2 prior to bleeding. PBZ pretreatment prevented the fall in cerebral blood flow and CMRO2 produced by systemic hypotension due to bleeding. Lactic acid showed no evidence of change either in production or uptake by the brain during the experimental procedure. The cerebral metabolic pathway of glucose, however, seemed to be affected by PBZ both before and during shock.

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PHENOXYBENZAMINE (PBZ) has a beneficial effect on various forms of shock.1-4 It has been demonstrated that the tissue blood flow to different organs was improved and metabolic changes induced by shock were reduced or abolished by pretreatment with phenoxybenzamine.

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It is generally assumed that owing to its well-developed autoregulation of blood flow, the brain's vital functions are protected during hypotension and shock. However, it has been demonstrated that the EEG and evoked potentials disappear during hypovolemia and do not return after reinfusion.2,7,8 In addition, metabolic9,10 and functional12 alterations occur which lend support to the concept that the central nervous system (CNS) is seriously affected in shock. Earlier studies in dogs showed that blood flow in different regions of the brain did not decrease to the same extent after PBZ premedication and the electrical activity of the cortex seemed to be preserved.9 Because earlier studies with PBZ in shock were per-
Topical prostacyclin (PGI2) inhibits platelet aggregation in pial venules of the mouse.
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