Incomplete global cerebral ischemia alters platelet biology in neonatal and adult sheep

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LITTLETON-KEARNEY, Marguerite T., Patricia D. Hurn, Thomas S. Kickler, and Richard J. Traystman. Incomplete global cerebral ischemia alters platelet biology in neonatal and adult sheep. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1293–H1300, 1998.—Platelets are implicated as etiologic agents in cerebral ischemia and as modulators of neural injury following an ischemic insult. We examined the effects of severe, transient global ischemia on platelet aggregation during 45-min ischemia and 30-, 60-, and 120-min reperfusion in adult and neonatal lambs. We also examined postischememic platelet deposition in brain and other tissues (120-min reperfusion) using indium-111-labeled platelets. Ischemic cerebral blood flow fell to 5 ± 1 and 5 ± 2 ml·min⁻¹·100 g⁻¹ in lambs and sheep, respectively. During ischemia, platelet counts fell to 47.5 ± 5.1% of control (P < 0.05) in lambs and 59 ± 4.9% of control in sheep (P < 0.05). Ischemia depressed platelet aggregation response (P < 0.01) to 4 μg collagen in lambs and sheep (20.4 ± 29.2 and 26 ± 44.7% of control, respectively). Marked platelet deposition occurred in brain and spleen in sheep, whereas significant platelet entrapment occurred only in brain in lambs. Our findings suggest that ischemia causes platelet activation and deposition in brain and noncerebral tissues.

PLATELETS HAVE BEEN IMPLICATED etiologically as agents in cerebral ischemia associated with thromboembolic vessel occlusion and as mediators of neuronal injury following an ischemic insult (14, 24, 38). Considerable evidence indicates that platelets aggregate within the brain vasculature after focal (23, 34) or global (8, 20) cerebral ischemia or ischemic stroke (35). Platelet aggregation contributes to hypoperfusion and microcirculatory dysfunction during recirculation after prolonged global cerebral ischemia (20) and as part of the pathophysiology of stroke (16, 26, 31). Furthermore, platelet activation triggers release of dense granule constituents including adenine nucleotides, thromboxane A₂, calcium, and serotonin, leading to functional alterations within the vascular endothelium (41) and significant vasoconstrictive consequences. Clinical studies demonstrate increased dense granule secretion (24), platelet release of microparticles (32), platelet activation in subtypes of ischemic stroke (22), increase in mean platelet volume (36), and platelet cytosolic Ca²⁺ efflux (11, 25, 28) after stroke, suggesting altered platelet biology. Little information exists regarding the effects of transient ischemia on platelet function in the newborn. Platelets of newborns are known to be less responsive to aggregants such as collagen, thrombin, and ADP compared with those of adults (3, 33, 37). Therefore, it is possible that differences in platelet function associated with immaturity may confer some degree of protection during transient cerebral ischemia in the young. The purpose of the present study was to determine whether incomplete global cerebral ischemia-reperfusion directly and rapidly alters platelet biology and whether this effect is age dependent in newborn versus adult animals. We also sought to determine whether platelets remain sequestered in the brain during early reperfusion.

METHODS

Animals. Nineteen neonatal lambs ranging in age from 2 to 5 days (mean age 3 days) and 20 adult sheep from 1 to 5 yr of age (mean age 1.5 yr) were used in these studies. Groups I (n = 8 lambs) and II (n = 9 sheep) were used for the ischemia and platelet aggregation studies; groups III (n = 4 lambs) and IV (n = 5 sheep) served as their respective nonischemic controls. For the platelet labeling studies, four lambs and four sheep comprised the ischemic groups (groups V and VI, respectively), whereas three lambs and two sheep (groups VII and VIII) formed the respective nonischemic groups.

Animal preparation. All the protocols for this study were approved by the Johns Hopkins Animal Care and Use Committee. Anesthesia was induced by external jugular vein injection of pentobarbital sodium (25–30 mg/kg). As previously described (30), either a tracheostomy (sheep) or endotracheal intubation (lambs) was performed, and all animals were mechanically ventilated to maintain normal blood gases. Both axillary arteries were cannulated with polypropylene catheters (PE-120). The left axillary catheter was advanced into the left ventricle for microsphere injection, and the right catheter (advanced to the aorta) was used for reference sample withdrawal and arterial blood gas sampling. Additional catheters were inserted into a femoral artery and both femoral veins for hemodynamic monitoring and drug and fluid administration.

The animals were repositioned prone to permit cannulation of the superior sagittal sinus. Temporalis skin and muscle were retracted, and a burr hole was drilled midline between the lambdoidal and coronal sutures. A polypropylene catheter (PE-90) for cerebral venous blood samples was threaded into the sagittal sinus. A Silastic multiple-port ventricular catheter (model 901302, Cordis, Miami, FL) was inserted into the lateral ventricle through another burr hole in the skull for monitoring of intracranial pressure (ICP) and infusion of artificial cerebrospinal fluid (CSF). A thermistor was inserted between the bone and dura to monitor epidural temperature, and wound edges were approximated to minimize temperature losses from the open cranial areas. After surgery, pancuronium (0.1 mg/kg) for muscle paralysis was administered with continuous intravenous pentobarbital infusion (3 mg/kg). Each animal was warmed with heating lamps and water blankets to maintain an epidural temperature of 38–39°C.

Measurements. Arterial blood pressure and ICP were continuously recorded via a Gould-Brush polygraph. Regional

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cerebral blood flow (CBF) was measured at baseline, 45 min of ischemia, and 30, 60, and 120 min of reperfusion, using the radiolabeled microsphere technique (15 ± 0.5-μm diameter spheres) (18). Briefly, a dose of ~1.5 × 10^5 microspheres labeled with 111In, 113Sn, 58Nb, or 65Sc (DuPont NEN, Boston, MA) was injected into the left ventricle as previously described (1, 30), followed by a 3-ml saline flush. A reference sample was withdrawn from the right aortic catheter at a rate of 2.5 ml/min. After harvesting and fixation, we dissected the brain into cerebellum, medulla,pons, midbrain, hippocampus, caudate nucleus, and cerebrum. Radioactivity of the brain samples and the blood reference samples was counted on an autogamma scintillation spectrometer (model 5530, Packard Instruments, Downers Grove, IL). Blood flows were determined using the reference organ technique as previously described (18, 30).

Arterial and sagittal sinus blood gas samples were analyzed for pH, PO_2, and PCO_2 with a Radiometer ABL30 electrode system (Radiometer, Copenhagen, Denmark). Oxygen content, saturation, and hemoglobin were measured by a CO-oximeter. Glucose and lactate were measured with a glucose analyzer. Cerebral oxygen consumption (CMRO2) was calculated as the product of CBF and the arterial-sagittal sinus O_2 content difference. Fractional O_2 extraction was calculated as the ratio of cerebral arteriovenous O_2 content difference to arterial O_2 content.

Platelet studies. Platelet aggregation and ATP secretion were measured on a whole blood, dual-channel lumiaggregometer (Chrono-Log, model 500VS; Havertown, PA) using established methods (2). Briefly, 2.7 ml of blood were collected from the sagittal sinus and arterial catheters at baseline, 45 min of ischemia, and 30, 60, and 120 min of reperfusion, and gently mixed with 0.3 ml of 3.8% citrate. Platelet counts were performed by phase-contrast microscopy using a Neubauer hemocytometer before 1:1 sample dilution with isotonic saline. Aliquots of the diluted samples (950 μl) were pipetted into cuvettes, and 50 μl luciferase-luciferin (Chrono-lume; Chrono-Log no. 395) were added. The samples were warmed to 37°C and stirred with a Teflon-coated stir bar for 2 min before the addition of collagen. Collagen is classified as a strong aggregant because of its interaction with the membrane receptor, which triggers platelet adhesion, shape change, activation, aggregation, arachidonate release, and secretion (19). Therefore, all samples were individually tested with 1, 2, and 4 μg of collagen (final concn). Maximum aggregation response was determined as the maximum amplitude of the impedance curve at 6 min after collagen addition. Platelet secretion of ATP was evaluated by peak amplitude of collagen-induced ATP release compared with a preset 20 nM ATP standard. Platelet aggregation response and ATP secretion were quantified by computer-assisted data reduction system (BIO/DNR Aggrolink, Chrono-Log). To test for possible effects of platelet number on aggregation, whole blood (60 ml) was collected in 3.8% citrate via jugular venous puncture in adult sheep and prepared as above, then diluted to 55% of baseline values with autologous sheep platelet-rich plasma (PRP). Aggregation was quantified by paired samples from the same animal (n = 7).

For the isotopic platelet labeling studies, 60 ml (sheep) or 30 ml (lambs) of blood were gently aspirated from a femoral catheter into an acid-citrate-dextrose (ACD) solution (6:1 ratio), mixed thoroughly, and centrifuged [1,800 revolutions/min (rpm), 25°C] for 5 min. The PRP supernatant was separated and centrifuged again at 2,300 rpm (25°C) for 10 min to form a platelet pellet. The platelet was isolated and gently washed with ACD-saline (2 ml) and centrifuged (2,300 rpm, 25°C) for 10 min. The platelet pellet was then resuspended in ACD-saline (2 ml) containing 850 μCi of indium-111-tropolone solution prepared as described (5). This mixture was incubated for 30 min (25°C) and then centrifuged (3,200 rpm, 10 min). The supernatant was discarded, and the labeled platelets were resuspended in ACD-saline (2 ml) with subsequent determination of radioactivity. Thirty minutes before initiation of experimental protocols, animals were injected with 200–300 μCi of autologous labeled platelets. Forty-five minutes of incomplete global cerebral ischemia was induced, followed by 120 min of reperfusion. At 120 min of reperfusion, venous and sagittal sinus blood samples were obtained. The animal was killed, and samples of brain, skin, muscle, lung, heart, liver, spleen, and kidney were harvested. The radioactivity of blood and tissue was measured by autogamma spectrometer as for microsphere studies, but with a preset window for photo peaks of 171, 245, and 426. All tissue samples were normalized to radioactive counts per gram of weight, and the ratio of tissue to blood (per ml) was calculated.

Experimental protocol. Baseline measurements were obtained for arterial pH, PCO_2, and P_0_2 and CBF. Sagittal sinus (3 ml) and arterial (3 ml) blood samples were collected at baseline, 45 min of ischemia, and 30, 60, and 120 min of reperfusion for analysis of pH, PCO_2, hematocrit, O_2 saturation, O_2 content, and platelet aggregation. Incomplete global ischemia was produced by infusion of warmed artificial CSF (in mM: 151 Na^+ 3 K^+ 2.5 Ca^2+ 1.2 Mg^2+ 134 Cl^− and 25 HCO_3^− and 6 meq/urea) for 45 min through the Silastic multiple-port ventricular catheter, which was inserted into the lateral ventricle through a burr hole. ICP was controlled by CSF infusion, resulting in a cerebral perfusion pressure (CPP) of 5–5 mmHg (21). The arterial pressure was permitted to vary spontaneously, and ICP was adjusted accordingly. To initiate the 120-min reperfusion, the infusion was halted and the ICP was allowed to fall to normal values spontaneously. At end reperfusion, the animals received an overdose of pentobarbital and were killed by KCl injection.

Statistical analysis. Data are expressed as means ± SE. All repeated measurements were evaluated by two-way analysis of variance (ANOVA). If the F statistic for group treatment or the interaction between group and time was significant (P < 0.05), means among groups at individual time points were compared by a Newman-Keuls multiple-comparison test. If the F statistic for time effects or interactions between group and time were significant (P < 0.05), a one-way ANOVA with repeated measures was performed individually on each group to determine in which group the time effect was significant. Dunnett's test was then used to determine which time periods differed from baseline values. For the platelet labeling studies, a t-test for independent samples was used to test differences between counts per gram and counts per milliliter of blood in the various organs compared with control values. A P value of <0.05 was considered significant.

RESULTS

Arterial blood gases and hematocrit are summarized in Table 1. Ischemic arterial oxygen tension (P_AO_2) was higher in sheep compared with lambs, reflecting additional inspired oxygen administered to support cardiac function. Baseline arterial pH of the lambs was lower than that of the adult sheep but fell by the same amount during ischemia. By 120 min of reperfusion, arterial pH recovered in sheep but not lambs (7.4 ± 0.0 vs. 7.25 ± 0.03).

No differences in CPP were observed between lambs and sheep during ischemia or reperfusion (Fig. 1). ICP
Table 1. Physiological data during incomplete global ischemia and reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
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<td>Hct, %</td>
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Values are means ± SE. Hct, hematocrit; CMRO₂, cerebral oxygen consumption; O₂ Ext, oxygen extraction. Significant difference between sheep and lambs: *P < 0.05, †P < 0.01; significant difference from baseline control; ‡P < 0.05.

elevation reduced CBF during the ischemic period from 40 ± 6 to 5 ± 1 ml·min⁻¹·100 g⁻¹ in lambs and from 43 ± 3 to 5 ± 2 ml·min⁻¹·100 g⁻¹ in sheep (Fig. 2). During reperfusion, there were no age-related differences in CBF recovery. However, hyperemia occurred in sheep at 30 (90 ± 9 ml·min⁻¹·100 g⁻¹; P < 0.001) and 60 (70 ± 12 ml·min⁻¹·100 g⁻¹; P < 0.05) min of reperfusion, whereas lambs evidenced hyperemia only at 60 min (120 ± 32 ml·min⁻¹·100 g⁻¹; P < 0.05). In both groups, CBF returned to baseline values by 120 min of reperfusion. Ischemia reduced lambs CMRO₂ to 1.0 ± 0.5 ml·min⁻¹·100 g⁻¹ and sheep CMRO₂ to 1.6 ± 0.7 ml·min⁻¹·100 g⁻¹, with return to baseline values at 30 min of reperfusion (Table 1). At 45 min of ischemia, circulating platelet counts fall in both lambs (271,000 ± 50,489/µl; P < 0.05) and sheep (368,000 ± 29,510/µl; P < 0.05) compared with nonischemic controls (lambs, 525,500 ± 66,400/µl; sheep, 436,000 ± 39,900/µl). Figure 3 shows that this fall in platelet numbers was 47.5 ± 5.1% of baseline values in lambs and 59 ± 4.9% of baseline values in sheep. Furthermore, platelet counts remained similarly depressed throughout reperfusion (P < 0.05; Fig. 3).

To evaluate the effects of severe global ischemia on platelet function, we simultaneously tested platelet aggregation and platelet dense granule secretory response (ATP release) to three concentrations of collagen using whole blood lumiaggregometry. After ischemia, both lambs and sheep demonstrated a depressed dose-response curve for all collagen doses, as measured by platelet impedance aggregometry (Figs. 4 and 5). Both the magnitude (Fig. 6) and the slope (Fig. 7) of platelet aggregation were depressed during ischemia and remained depressed throughout reperfusion (P < 0.01) in

Fig. 1. Time course of changes in cerebral perfusion pressure during 45 min of ischemia and 120 min of reperfusion. Ischemic sheep (IS, n = 9) and lambs (IL, n = 8) are compared with saline-infused, nonischemic sheep (NIS, n = 5) and lambs (NIL, n = 4). All data are reported as means ± SE.

Fig. 2. Time course of changes in cerebral blood flow during 45 min of ischemia and 120 min of reperfusion. IS (n = 9) and IL (n = 8) are compared with NIS (n = 5) and NIL (n = 4). All data are reported as means ± SE. *P < 0.05, ***P < 0.001 from control.
both lambs and sheep. Stimulation with 4 µg of collagen after 45 min of incomplete global ischemia resulted in reduced platelet aggregation in sheep and lambs (20.4 ± 29.2 and 26.8 ± 44.7% of baseline, respectively; Fig. 6). Similar reductions were observed in response to stimulation with 2 and 1 µg of collagen. Depressed platelet responsiveness was not observed in nonischemic animals. After adjustment for the depression of circulating platelet numbers, ATP secretion was not different from baseline values in either lambs or sheep. However, at 45 min of ischemia, six of nine sheep and three of six lambs demonstrated a marked increase in platelet secretion of ATP (>100% of control) in response to 4 µg of collagen. This heightened ATP release was observed in only one of five nonischemic sheep and was not detected in any nonischemic lambs.

To determine whether quantitative changes in aggregation accompanied the fall in platelet numbers, we evaluated the effects on aggregation by dilution of whole blood with autologous sheep PRP. A reduction of platelet counts to 55 and 40% of baseline attenuated aggregation to collagen (P < 0.01) in a dose-dependent manner.

We examined the effects of ischemia-reperfusion on indium-111-labeled platelet deposition in brain, muscle, skin, heart, lung, liver, spleen, and kidney. Figure 8 demonstrates that sheep (P = 0.045) and lamb (P = 0.013) brain sequestered significant platelet numbers compared with nonischemic brain. The greatest platelet-associated radioactivity was detected in the spleen of sheep (P = 0.03; Fig. 9). No difference was found between nonischemic and ischemic lamb (P = 0.71) spleen platelet entrapment. However, the spleen of nonischemic lambs trapped large numbers of platelets, probably subsequent to platelet injury during labeling. Therefore, the difference between platelet deposition in

**Fig. 3.** Fall in platelet counts expressed as percentage of control during 45 min of ischemia and 120 min of reperfusion. IS (n = 9) and IL (n = 8) are compared with NIS (n = 5) and NIL (n = 4). All data are reported as means ± SE. At 45 min of ischemia and throughout reperfusion all platelet counts are depressed (*P < 0.01).

**Fig. 4.** Mean dose-response curve in sheep for platelet response to stimulation with 1, 2, and 4 µg of collagen as measured by amplitude (magnitude in ohms) of aggregation. Values are shown at baseline (C), 45 min of ischemia (ISC), and 120 min of reperfusion (RP).

**Fig. 5.** Mean dose-response curve in lambs for platelet response to stimulation with 1, 2, and 4 µg of collagen as measured by amplitude (magnitude) of aggregation. Values are shown at baseline (C), 45 min of ischemia (ISC), and 120 min of reperfusion (RP).

**Fig. 6.** Platelet response to stimulation with 4 µg of collagen expressed as percentage of baseline values. IS (n = 9) and IL (n = 8) are compared with NIS (n = 5) and NIL (n = 4). All data are reported as means ± SE. At 45 min of ischemia and throughout reperfusion, all platelet aggregations for IS and IL are different from baseline (#P < 0.01). Both ischemic groups are different from time controls (*P < 0.05).
nonischemic versus ischemic lambs was reduced. None of the other tissues, in either sheep or lambs, evidenced significant platelet entrapment during ischemia.

**DISCUSSION**

This study demonstrates four major findings. First, severe, transient global cerebral ischemia produces marked reduction of circulating platelets in both adult sheep and neonatal lambs. The decline in platelet count persists throughout reperfusion, suggesting ongoing platelet activation. Second, platelets are sequestered during early reperfusion in brain, independent of age and despite full restoration of CBF. The preponderance of isotopic activity was found in the spleen, an organ that is a common site for platelet sequestration and platelet clearance. Third, platelets are clearly hyporeactive to a well-known and physiologically strong aggregator (collagen) throughout early reperfusion. Platelet dense granule ATP secretion during ischemia is quantitatively inconsistent and independent of age. Finally, postischemic CBF abnormalities such as hyperemia are present in both newborn and adult sheep; however, hyperemia occurs earlier and persists longer in the mature brain. Nevertheless, prolonged derangement of CBF does not correspond with greater platelet physiology, at least not in the short time window of our observations. In light of these findings, we conclude that severe, incomplete global ischemia alters platelet biology equivalently in both newborn and adult brain. We hypothesize that platelet aggregates are actively formed in both newborn and mature brain vessels as a consequence of exposure to an ischemic microvascular bed subsequent to an early and evolving endothelial injury.

Platelets have been implicated both as etiologic agents in cerebral ischemia associated with thromboembolic vessel occlusion and as mediators of neuronal injury following an ischemic insult. Furthermore, platelet activation triggers release of dense granule constituents and mediators with significant vasoconstrictive consequences. However, it is not known whether cerebral ischemia specifically induces abnormalities in platelet function, thereby creating a vehicle for secondary brain injury during reperfusion or revascularization. Our experimental model allowed observation of “isolated” cerebral ischemia by selectively reducing perfusion pressure only within the cerebral circulation. We examined not only systemic aortic platelet samples but platelets obtained from the sagittal sinus, which reflects venous composition and drainage directly from brain. The data suggest that severely reduced flow to the brain induces significant thrombocytopenia that persists during early reperfusion. Accounts of thrombocytopenia following clinical ischemic stroke are variable; however, our findings are generally consistent with previous reports in experimental global, but not focal, ischemia in animals (13, 17, 20). Several clinical studies report moderate depression of circulating platelet numbers after stroke, ranging from 15 to 26% compared with age- and gender-matched controls (10, 36, 40), whereas others report no differences (16, 24, 31). Because initial postischemia platelet counts have not been reported in acute human stroke, it is difficult to determine whether our animal data are consistent with findings after clinical cerebral ischemia. However, we detected a drastic fall in circulating platelets to as low as 50% of control values in animals in which ischemic CBF was most severely reduced (e.g., to 5
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The reduction could not be attributed to hemodilution, because hematocrit was unchanged in lambs and slightly more concentrated in sheep over the study time course. Furthermore, newborn animals were not spared from ischemia-induced thrombocytopenia; it appears to be an age-independent response to ischemic brain insult.

Consequently, we examined both cerebral and noncerebral tissues to determine whether the location and magnitude of platelet deposition was also age independent. We found platelets widely dispersed throughout the reperfused brain within 2 h of recirculation in both the neonatal and mature brain. This finding is similar in time frame to that observed after prolonged global ischemia in adult cat (20) and photochemical carotid injury in rat (6), effectively within hours of the acute injury. Similar to the reports by others (6, 20), platelet deposits formed in visceral tissues such as spleen during reperfusion, to a lesser extent in the newborn in which splenic entrapment was not elevated relative to nonischemic lambs. It is possible that, in the adult, platelets become temporarily adherent and are subsequently released to be removed later by the spleen. In contrast to others (6, 20), we found platelet deposition only in the spleen, but not liver, lung, or kidney. Most likely these differences may be attributed to either species or technique differences rather than perfusion changes in these tissues. In the current study it is unclear whether cerebral ischemia resulted in blood flow reduction in peripheral organs. However, earlier studies from our laboratory and from others indicate that cerebral ischemia does not result in diminished blood flow to skin, intestine, kidney, muscle, lung, or liver (20, 30). On the basis of these data, it is unlikely that reduction in splenic blood flow occurs, either. However, both liver and spleen are known sites for removal of damaged platelets, and our findings most likely represent enhanced normal splenic function rather than ischemic endothelial injury. Eligio and Hovig (9) demonstrated extensive platelet sequestration in spleen sinusoids as well as phagocytic removal of damaged platelets. Others show that normal platelet loads can be cleared by the liver, but increased platelet load results in heightened platelet localization in spleen and lung (29). These data are consistent with reports of platelet entrapment in liver, lung, and spleen in patients with inflammatory disorders such as sepsis. Therefore, it is not surprising that we observed increased platelet entrapment in spleen in the adult sheep. The lack of significant sequestration in the newborn spleen may be caused by its relative immaturity in the 2- to 5-day-old lamb (12). Therefore, it is likely that our data reflect augmented splenic removal of platelets damaged as a consequence of cerebral ischemia.

The effect of global cerebral ischemia on platelet biology has not been extensively investigated. Therefore, we sought to determine whether exposure to a large segment of the cerebral vascular bed made acutely ischemic triggers platelet hyperreactivity. Platelet aggregates increase in jugular venous blood after unilateral carotid artery occlusion (7). However, clinical studies examining platelet responsiveness to collagen after ischemic stroke demonstrate either no change in aggregation (24) or decreased magnitude of the aggregation response (14, 40). Our data indicate that circulating platelets in whole blood are hypoaggregable to stimulation after global cerebral ischemia, and the hyporeactivity persists throughout early reperfusion. These data are novel in that we examined platelet responsiveness to increasing concentrations of collagen in whole blood sampled directly from the sagittal sinus draining blood from brain regions where CBF was homogeneously and reversibly reduced. It seems unlikely that the reduced number of platelets over the experimental course resulted in a lowered threshold for aggregation; others have demonstrated no correlation between similar platelet numbers and aggregation (40). Our dilutional studies did reveal attenuated platelet responsiveness when samples were diluted by 50%; however, dilution with PRP diminished sample hematocrit, increasing the acellular volume fraction. Therefore, the final collagen concentration presented to the platelet as a stimulus for aggregation was reduced.

At all doses of collagen, there was a significant reduction in both the magnitude of platelet aggregation and the rapidity of the response. These data do not directly suggest a mechanism for the loss of reactivity, but postischemic hyporeactivity is clearly independent of animal age. This finding is surprising given previous reports that platelets in the normal human newborn show less sensitivity to aggregants such as ADP, collagen, thrombin, and epinephrine relative to adult platelets (3, 4, 33, 37). We did not observe baseline differences in platelet aggregation between the newborn lambs and sheep. This may be related to species differences or may indicate that an age of 3–5 days in lambs is not equivalent to the same age in neonatal humans. However, ischemia-sensitized platelets harvested from both lambs and sheep responded in a similar, depressed manner to collagen stimulation.

Although we detected higher ischemic PaO2 values in sheep, it is unlikely that these differences had an effect on platelet function, particularly because we observed depressed platelet function in both lambs and sheep, regardless of differences in PaO2. Ischemia in both lambs and sheep elicited a profound Cushing response. Sheep were unable to tolerate the stress on the myocardium incurred by the cardiovascular response, which often raised mean arterial blood pressure to >225 mmHg, whereas lambs tolerated ischemia better, demonstrating a lower ischemic blood pressure. Therefore, the differences in PaO2 reflect administration of higher inspired oxygen concentrations required in sheep to support cardiac function. Additionally, we observed lower pH in lambs than in sheep throughout the study. Normally, neonatal lambs have a lower pH than adults because of an inability to regurgitate the cud. Furthermore, neonates possess less functional ability to adjust serum bicarbonate during acidosis as a result of immature renal distal tubule function. However, ischemia resulted in lowered pH in lambs proportionally as in
sheep, making it unlikely to significantly depress platelet function.

Ischemic exposure could alter platelet biology by potentiating either primary (reversible) or secondary (irreversible) aggregation via alteration of membrane-bound surface receptors, depletion of granular constituents, or increasing the fraction of exhausted, disaggregated platelets in the reperfused brain. Platelet aggregation requires receptor binding of fibrinogen (15), and in vitro studies suggest a correlation between time-dependent loss of platelet aggregation and internalization of surface-bound fibrinogen (42). Postischemic redistribution of bound fibrinogen to inaccessible intracellular storage sites could explain the platelet hyporeactivity we noted. Furthermore, clinical ischemic stroke (26) induces α-granule depletion, indicating increased platelet secretion after cerebrovascular insult. A reduction in granular constituents may denote primary aggregation and cause attenuation of the aggregation response. Loss of α-granule constituents could also account for the marked hyporesponsiveness in both adult and newborn sheep.

Enhanced ATP secretion from dense granules (14, 40), increased platelet calcium release (24, 27), and liberation of α-granule and β-thromboglobulin (11, 39) and thromboxane from activated platelets (31) have been noted in patients after stroke. Because ATP production is platelet dependent, whole blood platelet numbers affect the measurement accuracy. Therefore, we normalized data for ATP secretion per 100,000 platelets/µl to determine whether ischemia alters ATP release. Consequently, we did not detect a statistically significant change in ATP secretion. We found extreme variability in platelet ATP release postischemia likely caused, in part, by combinations of partially activated and circulating platelet aggregates within our samples. On the basis of their findings of poststroke enhanced ATP release and depressed aggregation, Joseph and colleagues (24) suggest that dense granular activation may be independent of aggregation. Our observations would support this hypothesis.

In conclusion, global cerebral ischemia triggers a drastic fall in circulating platelet numbers in both the newborn and adult. Whole blood platelet aggregation studies with collagen indicate that postischemic platelets are refractory to strong platelet agonists, possibly as a consequence of preformed platelet aggregates, which are no longer responsive to physiological levels of agonists, sequestration of platelet-bound fibrinogen, or diminished granular constituents. During reperfusion, platelet aggregates are sequestered in the brain equivalently in the young and the adult animal. It seems likely that platelet aggregates are actively formed in both newborn and mature brain vessels as a direct consequence of exposure to an ischemic microvascular bed with progressive endothelial injury. It is unclear what impact this has on the outcome of cerebrovascular injury. In addition to endothelial denudation, the mass of accumulated platelets may contribute to microvascular obstruction and the release of platelet secretory products, potentiating cerebrovascular vasospasm. The accompanying ischemia could intensify neuronal injury and negatively affect neurological outcome.

This work was supported by grants from the National Institutes of Health (NR-03816, NR-03521, and NS-20020) and the Maryland Affiliate of the American Heart Association (MDG-20595).

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Received 12 September 1997; accepted in final form 19 December 1997.

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