Prevention of Persistent Cerebral Smooth Muscle Contraction in Response to Whole Blood

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SUMMARY Using an in vitro system designed to measure arterial constriction, we have demonstrated the importance of platelet function in maintaining cerebral smooth muscle contraction after whole blood injection. We tested two agents, acetyl salicylic acid (ASA) and phthalazinol, both known to interfere with platelet function. In control tests normal rabbit and monkey blood produced a reliable and persistent arterial constriction. In experimental tests blood drawn from animals premedicated with ASA and phthalazinol failed to produce a persistent contraction. These results support the hypothesis that chemicals released during platelet aggregation may be important in persistent vasospasm.

THE MANAGEMENT of ruptured intracranial aneurysms is frequently complicated by persistent spasm of the large cerebral vessels. Not only has this complication hampered the early treatment of these aneurysms, but it has also been extremely resistant to various modes of therapy.

Clinically, cerebral vasospasm has also been noted following trauma, cerebral embolectomy, and surgical manipulation of aneurysm-bearing vessels. Experimentally it has been shown to be reproducible in vivo by the subarachnoid injection of blood, arterial injury, or the application of vasoactive chemicals. The resultant spasm is characterized by a transient phase of vasoconstriction which is followed by a prolonged period of vasospasm. Vasospasm can be demonstrated by cerebral angiography and it is associated with arterial intimal proliferation and myonecrosis.

The exact cause of persistent cerebral vasospasm is not known. The agent or agents responsible have been shown to be released from clotting blood and to be associated with platelets. Spasmogenic activity has been shown to persist in the CSF of patients who have suffered cerebral vasospasm. Serotonin (5-HT) and prostaglandin F2a (PGF2a) have been implicated by authors as being important in this process.

Recently, in a study by Ellis et al., a prostaglandin metabolite, thromboxane A2 (TXA2), which is released during platelet aggregation, was implicated in coronary artery spasm. Its spasmodic activity was tested in vitro with middle cerebral, carotid, renal, and coronary arteries and cerebral arteries were shown to be 4 times more sensitive than carotid or renal arteries. In addition, cerebral vessels are at least 2 times more sensitive to TXA2 than to either 5-HT or PGF2. Our studies were aimed at evaluating the importance of platelets in cerebral artery spasm. We have used acetyl salicylic acid (ASA), which is known to block the synthesis of prostaglandins in platelets, and phthalazinol, a potent phosphodiesterase inhibitor, which has been shown to elevate platelet cyclic AMP and inhibit platelet aggregation, to test our hypothesis that functioning platelets contribute to the prolonged cerebral arterial contraction after subarachnoid hemorrhage.

In order to insure maximum activation of all functioning platelets present, thrombin was added to the system in some of the animal groups.

Materials and Methods

Adult dogs of both sexes were anesthetized with sodium pentobarbital and one common carotid artery was catheterized. Animals were sacrificed by rapid exsanguination and the brain was perfused with warm (37°C), aerated (95% O2-5% CO2), modified Krebs solution. The brain was removed and the basilar artery was excised and placed in warm, aerated Krebs buffer. The interval between sacrifice and removal of the artery varied between 10 and 30 minutes. The artery was cut into 5 mm tubular segments. The segment to be studied was mounted longitudinally on 2 stainless steel pins which were suspended between a tension transducer and tension micrometer (figs. 1 and 2) to create an in vitro system to measure cerebral artery contraction similar to that originally described by Nielson. The arterial preparation was housed in a 10 ml plexiglass chamber filled with Krebs buffer at 37°C + 0.1°C and allowed to equilibrate at 400 to 500 mg resting tension for 1 hour. After the 1 hour period of stabilization the resting tension was increased to 3 grams. Prior to proceeding with experiments the reactivity of each artery was tested by replacing the Krebs buffer with a depolarizing solution (DPS) high in potassium. Only arterial segments which contracted briskly to depolarization were used for the study. The arterial chamber was flushed 3 times with Krebs buffer and the artery was allowed to resume its resting tension before test injections were performed. For test injections whole blood drawn in a tuberculin syringe from rabbits or macaca monkeys was introduced into the bath immediately after removal from the animal. The blood was therefore diluted by the 10 cc of Krebs buffer already in the chamber. All contractions induced by blood were expressed in terms of %DPS con-
traction. In one-half of the experiments thrombin was also added to the in vitro bath.

I. Control Groups

Control Group I. Seventeen canine basilar arteries were prepared as described and then induced to contract with 0.4 cc of normal rabbit blood. The response was followed for at least 30 minutes.

Control Group II. Eight canine basilar arteries were tested as in Group I except that after 30 minutes 100 units of thrombin were delivered to the bath slowly in 5 cc of Krebs buffer. The ensuing response was followed for 30 additional minutes.

Control Group III. Eight preparations were stimulated to contract with 0.4 cc monkey blood. These trials were followed for 30 minutes.

Control Group IV. Eight trials were tested as in Group III, except that after 30 minutes 100 units thrombin was delivered to the bath. The response was followed for an additional 30 minutes.

II. ASA Experiments

Test Group I. Eight canine basilar arteries were prepared as described except that ASA was added to the Krebs bath to a concentration of 3 mg per ml. ASA at this concentration has been shown to inhibit platelet aggregation in vitro. These specimens were tested with 0.4 cc rabbit blood drawn from rabbits premedicated with ASA.

Rabbits were injected intravenously with 100 mg ASA per kg 30 minutes prior to blood withdrawal. The progress of this contraction was followed for 30 minutes.

Test Group II. Eight specimens were prepared exactly as in Test Group I, and after a 30 minutes test period 100 units thrombin were delivered to the bath. The response was followed for an additional 30 minutes.

III. Phthalazinol Experiment

Test Group III. Eight specimens were prepared as described, and tested with 0.4 cc monkey blood drawn from monkeys premedicated with phthalazinol a dose of 50 mg per kg for 10 days prior to blood withdrawal. Phthalazinol (EG626) was administered to monkeys orally as a suspension in orange flavored "Tang" at a dose of 50 mg per kg per day. The progress of the response was followed for 30 minutes.

Test Group IV. Eight specimens were prepared exactly as in Test Group III, and after their response was followed for 30 minutes 100 units of thrombin were added to the bath. The response was then followed for an additional 30 minutes.

Prior to termination of any experiment in which the arterial segment had relaxed, DPS was re-administered to determine the viability of the vessel.

Results

I. In Vitro Response of Canine Basilar Artery to Monkey and Rabbit Blood

Control Group I. The addition of 0.4 cc whole rabbit blood to the arterial bath resulted in an almost immediate contraction reaching a maximum in about 8 minutes.
II. Response of Basilar Arteries to Blood Drawn From Rabbits Pretreated with ASA

Test Group I. Rabbits treated with ASA at a dose of 100 mg per kg had serum levels of 370 micrograms ASA per ml 30 minutes later. This level has been associated with prolongation of the bleeding time and inhibition of platelet aggregation. Blood from an ASA treated animal injected around an arterial segment bathed in ASA induced an initially smaller contraction than that produced by non-ASA treated blood and was followed by an early relaxation (fig. 4, closed circles).

Control Group I. The response of in vitro canine basilar arteries to monkey blood was even more variable than their response to rabbit blood. After an initial contraction similar to those induced by rabbit blood, several (3/8) specimens relaxed to baseline by 30 minutes.

Control Group II. The addition of thrombin to the bath after the administration of blood resulted in a more consistent constriction. (fig. 6, open circles).

Test Group II. The addition of thrombin to an arterial preparation which had been incubated 30 minutes with ASA treated blood (fig. 5, closed circles) did not produce the prolonged vasoconstriction seen in the controls. DPS was added before termination of these specimens to insure their viability, and they were found to be responsive.
III. Response of Basilar Arteries to Blood Drawn From Monkeys Pretreated with Phthalazinol

Test Group III. The response of the in vitro arterial segments to blood from animals pretreated with phthalazinol suggested inhibition of vasoconstriction. A mean value of 10.2% DPS contraction was noted for 8 such preparations at 30 minutes. This result, however, is difficult to interpret in light of the variability noted in Control Group III.

Test Group IV. The addition of thrombin to the arterial chamber containing phthalazinol-treated blood did not produce the persisting contraction reliably produced in the controls. (fig. 6, closed circles).

Discussion

Our studies indicate that persistent cerebral arterial constriction in response to blood is dependent on intact platelet function. Numerous reports over the past several years have suggested that platelet aggregation may be the source of the substance responsible for cerebral vasospasm.11, 14, 24 When endothelium is lost from the surface of an artery, platelets adhere to the exposed collagen, the basement membrane, and the microfibrils around the elastin23-27 (fig. 7). Platelet adherence has been observed even after minimum vessel injury without endothelial disruption.25 A principal effect of the interaction of platelets with collagen is stimulation of the discharge of the platelet granule contents.21, 25 These include ATP, ADP, 5-HT, epinephrine, histamine, calcium, magnesium, platelet factor 4, and mucopolysaccharide. ADP release in addition is known to stimulate platelet aggregation.26 Compounds which increase cyclic 3'-5' adenosine monophosphate (cAMP) concentration33, 34 in platelets inhibit ADP release and prevent platelet aggregation, whereas substances which induce platelet aggregation, including ADP, epinephrine, collagen, and thrombin, reduce cAMP concentration.34, 35 cAMP and ADP, therefore, appear to act antagonistically on the platelet contractile protein effecting the release of granules and subsequent platelet aggregation.24, 26, 29, 34

An aggregate induced by ADP provides a site for the activation of platelet and clotting factors which then result in the generation of thrombin. Thrombin has at least 3 effects: it causes the polymerization of fibrin, it frees platelet arachadonate to form prostaglandin endoperoxides and TXA₂,40 and it induces the platelets to release ADP which causes further platelet accumulation on the initial platelet mass. As fibrin polymerizes the platelets adhere to it and the platelet aggregate becomes more stable.40 Recent work has demonstrated that unstable intermediates of prostaglandin metabolism are formed during platelet aggregation, especially prostaglandin endoperoxides (PGH₂ and PGG₂) and TXA₂.40-42 These have been shown to be the major arachidonic acid products in aggregating platelets (fig. 8). TXA₂ causes platelet aggregation by inhibiting adeny
prostaglandins are essential for the maintenance of prolonged arterial constriction and perhaps for the genesis of cerebral vasospasm. If these in vitro observations can be confirmed in vivo it is possible that a rational approach to the prevention of spasm can be developed.

References

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Effect of Aminophylline on Cerebral Infarction in the Mongolian Gerbil

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SUMMARY The effects of aminophylline in Mongolian gerbils subjected to unilateral carotid ligation were studied. The drug was given in varying intraperitoneal doses at varying postoperative intervals and the animals observed for 5 days for clinical signs of stroke. Doses of 100 mg per kg caused early death and were discontinued. Doses of 50 mg per kg had no significant effect on morbidity, mortality, time until death, stroke incidence or lesion size, as compared to saline given as a control. Doses of 80 mg per kg caused a higher mortality, higher morbidity, and a shorter interval to death, but a smaller infarct. Thus, aminophylline did not have a protective effect against stroke in gerbils and was actually detrimental during the first 16 hours following the carotid ligation.

INTRACEREBRAL microcirculation is depressed following cerebral infarction; therefore, one treatment of stroke is the augmentation of blood flow to the ischemic cerebral tissue. Vasodilatory drugs have not been successful in this regard, possibly because the blood-brain barrier has a higher drug threshold than peripheral vessels, and the more widely dilated peripheral vessels receive the greater proportion of available oxygenated blood. There has been conflicting evidence as to the effects of aminophylline on cerebral blood flow. Aminophylline was originally thought to be a cerebral vasodilator, and it has been shown to reverse acute cerebral vasospasm following subarachnoid hemorrhage in monkeys and cats. However, more recently, cerebral vasconstriction has been demonstrated following aminophylline therapy in human subjects. Some authors have observed such vasoconstriction only in healthy cortical tissue; thus, by an “inverse steal,” it is possible that blood flow could be increased in the diseased cortical areas. Aminophylline also increases ventilation and cardiac output, which may also be beneficial to ischemic cerebral areas.

These findings led us to study the effectiveness of aminophylline in the treatment of experimental stroke in the gerbil. The Mongolian gerbil (Meriones unguiculatus) was used as the experimental model because a high incidence of ipsilateral cerebral infarction is seen following ligation of one common carotid artery, probably due to the presence, in a large number of these animals, of an anomalous circle of Willis.

Methods

Two hundred young adult gerbils weighing from 30 to 40 gm were used, 20 serving as nonoperated controls, 180 being anesthetized with an intraperitoneal injection of ketamine (44 mg per kg) and undergoing a midline ventral cervical incision with exposure of the

45. Tullis J: Clot. Springfield IL, Charles C Thomas, 1976
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