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 also has 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 F2α (PGF2α) 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 spasmogenic 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 PGF2α. 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° 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-

*Transducers were made by Statham (60 g range) and Grass (50 g range) and connected to a H-P7754A chart recorder with a 8805C pressure amplifier.

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MUSCLE CONTRACTION AND WHOLE BLOOD/Linder & Alksne

1. STAINLESS STEEL PIN
2. 5mm SECTION OF BASILAR ARTERY

TO TRANSDUCER

1. RINGSTAND
2. CLAMP
3. TENSION TRANSDUCER
4. ARTERIAL PREP
5. TEN ML BATH
6. 95-5% O2-CO2 MIXTURE
7. WATER BATH
8. TENSION MICROMETER
9. LEAD TO RECORDER

TO TENSION APPARATUS

FIGURE 1. Apparatus used to monitor the in vitro activity of basilar artery. Note arterial preparation is suspended between the transducer (3) and the tension micrometer (8) around a fixed pivot rod mounted in the bath.

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.19 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 at 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

†Purchased from Cal Biochem
‡Purchased from Matheson, Coleman, and Bell.
§Obtained from Takio Shimamoto, M.D., Director, Japan Atherosclerosis Research Institute, Tokyo, Japan.
minutes. In figure 3 the progress of this contraction was followed for 3 hours (N = 3). The same response was followed for 30 minutes in figure 4 (open circles). Although in all rabbit control samples constriction persisted at least 30 minutes, many were found to relax to baseline between 45 and 60 minutes. Other authors have described similar variability in the reactivity of cerebral vessels in response to blood.8,20

Control Group II. The addition of thrombin to the chamber appears to overcome the problem of variability in the duration of arterial contraction. In this group of specimens persistent vasoconstriction occurred. The response is shown in figure 5 (open circles).

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.19 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).

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.
Non-treated Monkey, N = 8
Phthalazinol-treated Monkey, N = 8
p < 0.005 @ 20° + 30°

FIGURE 6. Effect of thrombin on preps incubated with whole blood drawn from normal and phthalazinol treated
monkeys. As in Fig. 5 thrombin was added 30 minutes after the blood and the new contraction results. The % DPS
contraction was again measured relative to tension present when thrombin was added. Note standard error and significance
levels at 20 and 30 minutes.

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 in-
tact 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 elastin25-28 (fig. 7). Platelet
adherence has been observed even after minimum
vessel injury without endothelial disruption.26 A prin-
cipal effect of the interaction of platelets with collagen
is stimulation of the discharge of the platelet granule
contents.21, 22 These include ATP, ADP, 5-HT,
epinephrine, histamine, calcium, magnesium, platelet
factor 4, and mucopolysaccharide. ADP release in ad-
dition is known to stimulate platelet aggregation.32
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.36-37

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 in-
duces 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 aggregation becomes more stable.46 Re-
cent work has demonstrated that unstable in-
termediates of prostaglandin metabolism are formed
during platelet aggregation, especially prostaglandin
endoperoxides (PGH2 and PGG2) and TXA2.40-47 These
have been shown to be the major arachidonic acid
products in aggregating platelets14 (fig. 8). TXA2
causes platelet aggregation by inhibiting adenyl
DIN TXA 2, and phthalazinol, which prevents the decrease in cAMP produced by TXA 2, both prevent blood. This suggests that normally functioning blood conclusions: 1) The production of prostaglandins is indicated.

cyclase, and thereby depleting platelets of their cAMP (fig. 7). Several authors have proposed that TXA 2 is important in the genesis of arterial vasospasm, specifically coronary artery spasm and cerebral artery spasm.

In our experiment we noted a statistically significant inhibition of vasocostriction when aspirinated blood was compared with control blood. The addition of thrombin confirmed this inhibition. The inhibitory effects of aspirin on platelet aggregation appears to be related to inhibition of the synthesis of prostaglandin endoperoxides, precursors of TXA 2.

In our study we also see that phthalazinol is capable of inhibiting the persistent vasospasm induced by monkey blood treated with thrombin (fig. 6). Phthalazinol blocks the action of TXA 2 and subsequent platelet aggregation by increasing cAMP levels secondary to inhibition of platelet phosphodiesterase which is responsible for degrading cAMP (fig. 7). It is, of course, impossible to apply these conclusions unequivocally to the clinical condition of chronic vasospasm seen after subarachnoid hemorrhage but there may be a direct relationship. To the degree that vasospasm is platelet mediated these experiments suggest that it could be prevented or modified by the interruptions of prostaglandin synthesis or the elevations of cAMP. Further work in this area is indicated.

In vitro systems are definitely limited in their ability to model chronic disease process but they do simplify drug screening. If these compounds do not prove to be beneficial after in vivo testing additional pharmacologic agents can be evaluated.

From these results we have drawn the following conclusions: 1) The production of prostaglandins is necessary for in vitro persistent cerebral arterial smooth muscle contraction in response to blood. 2) The elevation of platelet cAMP levels caused by a phosphodiesterase inhibitor prevents the persistent arterial contraction produced by normal blood.

ASA, which inhibits the synthesis of the prostaglandin TXA 2, and phthalazinol, which prevents the decrease in cAMP produced by TXA 2, both prevent the prolonged in vitro vasoconstriction produced by blood. This suggests that normally functioning blood platelets which can aggregate and liberate vasoactive 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.

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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 in-
farct. 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 on the effects of aminophylline on cerebral blood flow. Aminophylline was originally thought to be a cerebral vasodilator but 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 ungu-
culatus) 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

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