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BRAIN MICROCIRCULATION OBSERVED 'IN VIVO' AFTER TRAUMA.(U)
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BRAIN MICROCIRCULATION OBSERVED IN VIVO AFTER TRAUMA

FINAL REPORT

William I. Rosenblum, M.D.

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Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia 23298

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William I. Rosenblum
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Brain Microcirculation Observed in Vivo After Trauma

Annual Report DAMD 17-76-C-6020

As noted in our application, we have confined our work this year, almost exclusively to the effects of penetrating brain injury on adjacent, undamaged (i.e. untouched) microvessels. The complexity of this problem demanded that our research associate devote himself almost entirely to its study.

One publication, largely of the previous years work, has appeared. In addition, we have had two publications accepted for publication, one in Stroke and one in Microvascular Research. The data in these publications, still in Press, may be summarized as follows.

(1) Effects of brain puncture on adjacent undamaged brain: When brain is punctured by a needle (see item 7 later in this report) two effects are produced on surface vessels adjacent to the puncture. These effects were platelet aggregation and vasoconstriction. The aggregation occurred only in venules, the vasoconstriction only in arterioles. These effects were manifest when the puncture was within 500μ of the vessel in question. By utilizing fluorescein, as described in an earlier publication and our prior annual report, we were able to be certain that vessels were narrowed at supposed sites of constriction, rather than partially occluded by platelet aggregates.

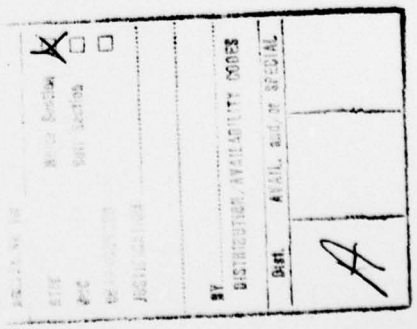
(2) The effect of distance on the vascular response: Aggregation of platelets occurred in venules even when the puncture center was as far as 600μ from the vessel wall, but not when longer distances were used.

(3) Effects of drugs on the response to brain puncture: Aspirin inhibited the effect of puncture on venules. That is, aggregation in untouched venules, occurred significantly less frequently when preceded by aspirin treatment.

	Frequency of Aggregation
Aspirin 25 mg/kg i.p. (N=10 mice)	0% of punctures
Control (N=10 mice)	33% of punctures*

*significantly less than control; $p<.05$

Indomethacin and aspirin each inhibited constriction of arterioles produced by puncture of adjacent brain.



	Frequency of Constriction	% Change in Diameter
Aspirin (25 mg/kg) N=10	20% of punctures	6±7*
Control N=10	43% of punctures	22±15

*Expressed as % of resting diameter. Significantly less than control; $p<.05$

Indomethacin (12.5 mg/kg i.p. N=10)	33% of punctures*	21±21*
Control (N=10)	59% of punctures	40±20

*$p<.05$ compared with control

One paper in press replicates the data tabulated above in 4 additional studies utilizing 80 additional animals. It also reports two studies (40 mice) employing another drug, dipyridamole. The latter failed to influence either platelet aggregation or arteriolar constriction.

(4) Effects of direct contact of needle with vessel: Great care was taken to avoid contact of needle with vessel. This was required since our experiments were testing the hypothesis that brain injury could alter microvascular phenomena in uninjured vessels. Accidental penetration of the vessel wall was ruled out by direct microscopic observation of the needle tip as it entered the brain. Since the tip was relatively "far" from the vessel this was not a problem. Since the needle shaft tapered to larger size away from the tip, there was a remote possibility that as the needle was inserted further into the brain, its wider shaft would brush or distort the nearby vessel. Although direct microscopic observation never revealed such a phenomenon, experiments were performed to test the effect of such accidental contact. We found that touching the vessels, and even displacing them or deforming them with blunt probes, failed to produce either platelet aggregation or arterial constriction. Consequently our results could not have been produced by accidental contact of the vessel by the side of the needle after penetration of adjacent brain.

(5) Effects of brain puncture on microvascular phenomena in adjacent vessels injured independently of the puncture: In these studies brain puncture preceded the injury of surface arterioles and venules by approximately 30 seconds. The injury of the vessels themselves was produced by exposing the vessels to a mercury lamp and injecting sodium fluorescein intravenously. The latter acts as a target for the radiant energy which presumably causes endothelial injury by generating heat. Injury of arterioles and venules is followed by platelet aggregation. Aggregation can be retarded by aspirin or indomethacin. Complete details are contained in last years annual report and are published (Circ Res 40:320-328, '77).

When brain puncture preceded the vascular injury just described, we predicted that the noxious effects of the preceding injury would be made worse because the effects of the brain injury would be superimposed on the effects of the vascular injury. Instead the effects of vascular injury were diminished.

	Vascular Injury	Vascular Injury preceded by Brain Puncture
Seconds to first aggregate	34±8	45±11*
Seconds to complete stoppage of flow	124±19	138±27**

*p<.01 **p<.05

The data in the above table summarize the experiments with statistically significant difference. The values are mean standard deviation, and were obtained in arterioles. In venules we found no effect of brain puncture on the effects of subsequent vascular injury. The table discloses, that rather than shorten the time required for a noxious stimulus to produce intravascular platelet aggregation, preceding brain puncture lengthens the time required for evidence of vascular injury to appear.

The inhibiting effects of brain puncture could be caused by release of materials that inhibit platelet aggregation, such as prostacyclin X (PGX). Why such release should occur, we cannot say. Since puncture alone causes aggregation of platelets in adjacent vessels, we obviously have a complex situation in which puncture is associated with the release of two kinds of forces, aggregate stimulating as well as aggregate inhibiting. It may be that production of the latter is responsible for the fact that after brain puncture alone, recognizable aggregates occur in only about 1/3 of venules and were never seen in arterioles (see items 1 and 3 of this report).

The data in the preceding table shows data for 50 mice (25 in each group). The distance of puncture center from the arteriolar wall was 171±30 . Experiments in 60 additional mice showed that significant inhibitory effects of brain puncture could be recognized after punctures as far as 610±59 from the wall, but not beyond.

(6) Miscellaneous data not included in manuscripts published or in press.

We have performed exhaustive studies on the effects on adjacent venules of distance and depth of puncture as well as on the effects of altering the material of which the needle is made. No differences were observed after using steel vs. glass microneedles. The tips of the needle were held constant in size for any series of experiments and were less than 100μ in width. Depth of puncture was varied systematically from 0.5 to 2 mm. Since the needle widens, as one

leaves the tip, the needle tract widened up to 300μ in width. Altering the depth of puncture over this range (0.5 - 2 mm) had no effect on aggregation frequency.

Significance of Data to Problem of Brain Trauma and Future Plans

We have shown that trauma to brain can induce adverse microvascular phenomena in adjacent vessels. These phenomena include platelet aggregation in venules, and vasoconstriction of arterioles. We have also shown that brain trauma is associated with anti-aggregating phenomena that require a subsequent aggregating stimulus in order to become manifest. The significance of these data to vascular consequences of penetrating brain injury are self-evident.

Because of the unexpected complexity of our results (i.e. evidence of both noxious and "helpful" effects after penetrating injury) we have had to consider the nature of possible chemical mediators being released as a result of brain injury. Because indomethacin and aspirin have affected our model we have had to consider prostaglandins and related endoperoxides. These considerations are relevant because both aspirin and indomethacin are well known inhibitors of prostaglandin synthesis. It should be noted that dipyridamole, a drug without such properties, failed to influence our system. During the past year investigators by others of prostaglandins and related compounds have mushroomed. We now have evidence that prostaglandins themselves may be only intermediates or byproducts of reactions producing much more potent, but extremely short-lived compounds. These include thromboxanes which may induce vasoconstriction and platelet aggregation, as well as prostacyclin X (PGX) which may inhibit platelet aggregation (Prostaglandins 12:715, '76). Inhibitors of each of these compounds have been reported (Prostaglandins 12:685 '76), but there are questions as to their specificity. For example (PNAS 74:1716 '77) imidazole is reported by one laboratory to inhibit thromboxane synthesis, but another lists it as a weak, but definite inhibitor of PGX synthesis (Prostaglandins 12:685 '76). It seems to us that pharmacologic developments are moving so rapidly in this area that we may shortly have available to us, potent and specific drugs to aid the analysis of our data. Because their appearance will influence the path of our research in a major way we thought it advisable to delay further studies of brain puncture until their appearance. For this reason we have not sought renewal of this contract, but rather, a no cost extension, which has been granted. This enables us, at no additional expense, to await the expected pharmacological developments and to take advantage of their appearance should they occur during the coming year. This, in turn, may permit more productive planning of future applications for contractual support, should this seem to be an appropriate course of future action.

We have also obtained, in part with funds from this contract, and with the permission of the contracting office, equipment for measuring platelet aggregability in vitro. In vitro studies, using an aggregometer, were not part of the original protocal, but were

suggested by several reviewers of our manuscripts. Now we will be able to see whether brain puncture is accompanied by alterations in aggregability of platelets throughout the body, or just within several hundred microns of the puncture. Obviously the present data suggests only the latter possibility, but could not rule out the former. In addition, as new drugs become available we will be able to test their effect on isolated platelets, in vitro, as well as their in vivo effect on the consequences of brain injury. We may then be able to detect drugs whose in vivo effect and in vitro effects may differ, thus pointing to important effects of drugs on brain tissue or vascular walls, as opposed to effects of drugs on the platelet itself.

Publications

1. Platelet Aggregation in the Cerebral Microcirculation. Effect of Aspirin and Other Agents. Circ Res 40: 320-328, 1977.

2. Effects of Combined Parenchymal and Vascular Injury on Platelet Aggregation in Pial Arterioles of Living Mice: Evidence for Release of Aggregate Inhibiting Materials. Stroke In Press

3. Platelet Aggregation and Vasoconstriction in Undamaged Microvessels on Cerebral Surface Adjacent to Brain Traumatized by a Penetrating Microneedle. Microvascular Research In Press