The blood-brain barrier following experimental subarachnoid hemorrhage

Part I: Response to insult caused by arterial hypertension

ERIC W. PETERSON, M.D., F.R.C.S.(C), AND ERICO R. CARDOSO, M.D., M.Sc., F.R.C.S.(C)

Division of Neurosurgery, University of Ottawa Medical School, Ottawa, Ontario, Canada

In three groups of cats, the authors studied the effect of subarachnoid hemorrhage (SAH) on the permeability of the blood-brain barrier (BBB) to the penetration of Evans blue-protein complex. One group received arterial hypertension alone, one group SAH alone, and one group SAH followed by arterial hypertension. Animals subjected to arterial hypertension alone showed areas of BBB breakdown. However, when cats were rendered hypertensive after SAH, there were no demonstrable BBB lesions. The SAH was produced by intracisternal injection of whole blood and hypertension by the intravenous injection of metaraminol. The preservation of the BBB after SAH is discussed. Vasospasm is considered as a possible hemodynamic variable responsible for the protection of the BBB from hypertensive damage. The need for a new model is proposed to further investigate the state of the BBB after SAH.

KEY WORDS: blood-brain barrier · Evans blue · hypertension · subarachnoid hemorrhage

The integrity of the blood-brain barrier (BBB) is essential for maintaining a constant environment for the nervous tissue. Numerous conditions have been found to alter the BBB in the course of disease of the nervous system, including hypertension, infarct, seizures, trauma, and sudden increase in intracranial pressure (ICP).

It is logical to inquire whether changes in the properties of the BBB may follow bleeding in the subarachnoid space. It is well recognized that elevation of blood pressure may follow subarachnoid hemorrhage (SAH) in clinical cases. It is of interest to evaluate possible changes that might occur in experimental models with SAH or with SAH combined with hypertension, and to contrast these findings with the state of the BBB in animals subjected to hypertension alone.

Materials and Methods

Animal Preparation

Cats of either sex, weighing 2.2 to 6.5 kg, received enough sodium pentobarbital (Nembutal, 30 mg/kg of body weight) to produce light anesthesia, supplemented with further small doses as required. Cannulation of the right femoral artery by a Portex cannula, with an inner diameter of 1.19 mm, permitted continuous blood pressure monitoring with a Statham pressure transducer.*

The animals were paralyzed with gallamine (Flaxedil, 10 mg/kg body weight/hr), and were given artificial ventilation with an A. C. Palmer animal ventilator† (respiration rate 26/min and stroke volume 14 to 25 ml, as determined by blood gas measurements). The inspired air was supplemented by O2, and the arterial pO2 determined the O2 flow. At least four times during each experiment, arterial blood gases and pH were measured and adjusted.‡

* Portex cannula manufactured by Portex, Ltd., Hythe, Kent, England; and Statham pressure transducer, Model P23 Db, manufactured by Statham Laboratories, Inc., Hato Rey, Puerto Rico.
‡ Blood gas analyzer manufactured by Instrumentation Laboratory, Inc., Lexington, Massachusetts.
Blood-brain barrier after SAH

The animals were divided into three groups: hypertensive (Group I), SAH (Group II), and combined hypertensive and SAH (Group III). Group I animals underwent only acute hypertension after intravenous injection of the tracer. Animals in Group II were submitted to cisternal injection of blood and intravenous injection of the tracer. Group III animals underwent SAH, injection of tracer, and acute hypertension in this order.

Production of SAH and Hypertension

In Group II and III animals, after blood gases and vital signs were stabilized, 4 ml of nonheparinized autogenous arterial blood was injected manually into the cisterna magna over a 4-minute period. The head was kept at 45° flexion, which facilitated an even distribution of the blood over the whole brain surface. The ICP was monitored during the injection, through the catheter that was used for injection by means of a four-way stopcock, and was not allowed to rise beyond 40 mm Hg below the arterial diastolic pressure. Thirty minutes after the subarachnoid blood injection, the animal was slowly injected with Evans blue dye (100 mg/kg body weight) diluted to 2% with Elliott's B solution. \( ^5 \) In Group III animals, this was followed immediately by an intravenous injection of 0.1 to 0.4 mg/kg body weight of metaraminol bitartrate (Aramine), diluted in saline, over a 3-minute period. The injection was controlled manually according to the blood pressure level. In most animals, further supplemental doses of metaraminol were required to maintain high blood pressure levels.

Tissue Fixation Technique

The animals were sacrificed by exsanguination through a right atrial incision, 15 minutes after hypertension was induced. \( ^5 \) In situ perfusion-fixation was immediately started by means of a No. 12 blunt needle placed in the ascending aorta through an incision in the left ventricle. An intravascular saline wash-out of blood for 2 minutes was followed by infusion of 1200 ml of 10% phosphate-buffered formalin. The perfusion pressure was kept constant at the level of the animal's initial systolic blood pressure.

Examination of the Specimens

The brains were immediately removed, examined, and photographed. The occurrence of lesions was checked first with the naked eye and subsequently under the operating microscope. The brains were kept immersed in the fixative that had been used for perfusion.

Sections were made after 24 hours of immersion. Coronal sections, 2 mm thick, starting at the sigmoid sulcus (approximate anterior coordinate 27), \( ^67 \) were continued posteriorly to include the forebrain, cerebellum, and brain stem. Blue-stained areas and homologous unstained areas were selected for microscopic fluorescence studies. Frozen sections (10 to 25 \( \mu \) thick) were obtained with a cryostat and/or a freezing microtome, and mounted in 50% glycerin-water. The sections were examined under a Zeiss photomicroscope.

Results

The mean values and standard errors (SE) for the blood samples of the 20 animals were as follows: pH 7.293 ± 0.004; PaCO\(_2\) 31.758 ± 0.400 mm Hg; and PaO\(_2\) 116.963 ± 1.469 mm Hg. These levels are within normal values of blood gases and pH for cats. \( ^{18,45,49} \) The results in the three experimental groups on the BBB are shown in Table 1.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>No. of Animals</th>
<th>Animals with BBB Breakdown</th>
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<tbody>
<tr>
<td>I: acute arterial hypertension</td>
<td>8</td>
<td>7 87.5</td>
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<tr>
<td>II: SAH</td>
<td>5</td>
<td>0 0</td>
</tr>
<tr>
<td>III: SAH &amp; arterial hypertension</td>
<td>7</td>
<td>0 0</td>
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* SAH = subarachnoid hemorrhage; BBB = blood-brain barrier.

Catheters were introduced into the right femoral artery and vein, and advanced as far as the aorta and inferior vena cava, respectively. The arterial line served for blood pressure monitoring and gas sampling and the venous line for the injection of drugs and Evans blue dye. Occasional flushing with heparinized saline (500 units of heparin/100 ml of physiological saline) kept the catheters patent. The rectal temperature was continuously monitored, \( ^8 \) and kept around 38°C using an electric heating pad as necessary. The electrocardiogram tracing was recorded.

Dissection of the posterior aspect of the neck exposed the atlanto-occipital ligament, and a \( \frac{1}{2} \) in. (38 mm) Cathlon intravenous catheter \( ^9 \) was used to puncture the cisterna magna. It was connected to a P23 Db Statham pressure transducer for recording the ICP. Arterial blood pressure and ICP were continuously monitored using an Electronics for Medicine polygraph, connected in series to the pressure transducers and to a Grass polygraph for intermittent recording. \( ^* \)

The animals were sacrificed by exsanguination through a right atrial incision, 15 minutes after hypertensive injection, through a stab incision in the left ventricle. An intravascular saline wash-out of blood for 2 minutes was followed by infusion of 1200 ml of 10% phosphate-buffered formalin. The perfusion pressure was kept constant at the level of the animal's initial systolic blood pressure.

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Group I

Following metaraminol injection, all animals developed an abrupt increase of systolic arterial blood...
pressure in excess of 95 mm Hg above the initial level (Fig. 1). It has been demonstrated that an acute hypertensive insult will not otherwise be effective in damaging the BBB. The mean (± SE) values of blood pressure rise induced by metaraminol were 74.4 ± 1.9 mm Hg for the mean arterial blood pressure and 101.5 ± 3.5 mm Hg for the systolic arterial blood pressure. The average (± SE) length of time required for the blood pressure to reach its maximum value was 8.33 ± 0.64 seconds. The length of time during which the blood pressure stayed elevated was variable. It has been demonstrated that the effectiveness of an acute hypertensive insult in inducing BBB breakdown is directly proportional to the magnitude of the blood pressure rise and inversely proportional to the length of time it takes to elevate it. The length of time during which the blood pressure remains elevated does not seem to influence the final results.

The brains were examined by gross inspection and with magnification under the operating microscope. They showed areas of discoloration, mostly localized in the gray matter of the cerebral hemispheres, but occasionally occurring in the thalamus or cerebellum. No areas of discoloration were seen in the brain stem. Their distribution was scattered, with some tendency to be symmetrical in the anterolateral and parasigmoid gyri (nomenclature after Snider and Niemer). The limits of the usual faint blue discoloration were well defined. The size varied from pinpoint to areas measuring up to 3 mm in diameter. Confluent pinpoint extravasations were frequent. In a few instances, the blue extravasation extended through the entire depth of the gray substance to the underlying white matter. Blue staining was also seen in those areas without a BBB, that is, the median eminence, pineal body, neurohypophysis, and area postrema.

The areas of extravasated dye-protein complex were identified under the fluorescence microscope by means of their red fluorescence; these were mainly localized in the neuropil and in the cell bodies (Fig. 2 left). In a few instances the fluorescent material was confined to the vessel wall. There was a striking contrast between the red fluorescence and the usual dark greenish autofluorescence displayed by nervous tissue. In areas without Evans blue exudation, the brain vessels were identified as “negative” round areas, distributed in a pattern resembling a honeycomb. Lipofuscin pigment, displaying a strong yellow fluorescence, was seen associated with the vessels. Evans blue tracer was not seen in the vessels.

**Fig. 1.** Typical tracing of the blood pressure (BP) response to the intravenous metaraminol.

**Fig. 2.** Fluorescence photomicrographs. × 232. **Left:** An area displaying blood-brain barrier (BBB) breakdown in Group I animals. The extravasated Evans blue-protein tracer, seen in orange-red, appears predominantly intracellular. **Right:** Group III animal. The normal greenish autofluorescence displayed by nervous tissue is seen. There is no evidence of BBB breakdown.
Blood-brain barrier after SAH

since it had been washed out by the perfusion-fixation solution.

**Group II**

Animals included in Group II underwent a subarachnoid injection of blood, followed by an intravenous injection of Evans blue dye. The subarachnoid injection of blood led invariably to slight increases of blood pressure and ICP. However, the ICP was prevented from reaching values more than 40 mm Hg below the diastolic arterial blood pressure, by injecting the blood slowly.

Upon examination, brains in Group II animals showed no blue discoloration except in the areas normally lacking a BBB. Therefore, no BBB breakdown to proteins had occurred. The amount of blood covering the hemispheres showed some individual variation, in spite of the same volume being injected in each instance. In all animals, the injection of 4 ml of blood was sufficient to cause spread of blood over the hemispheric convexities, cerebellar hemispheres, and in the median longitudinal fissure. However, it was more heavily localized in the basal cisterns.

The absence of BBB damage was confirmed by fluorescent microscopic examination. Sections were taken at random from the cerebrum and cerebellum, and in no instance was there any red fluorescence. The dark greenish autofluorescence normally displayed by nervous tissue was similar to that described for Group I animals, except for the outermost layer of the cortex and for the tissue surrounding the perivascular space of large penetrating vessels (Virchow-Robin spaces). These areas exhibited an intense yellow fluorescence which was similar to that displayed by the blood elements in the subarachnoid space. The areas of yellow fluorescence were confined to a narrow superficial layer.

**Group III**

Group III comprised animals that underwent SAH, followed in 30 minutes by an acute arterial hypertensive episode. The changes in the ICP and mean blood pressure caused by subarachnoid injection of blood were comparable to those seen in Group II animals. The mean (± SE) metaraminol-induced rise in blood pressure was 105.1 ± 5.0 mm Hg for the mean arterial blood pressure, and 124.7 ± 5.4 mm Hg for the systolic blood pressure. When compared with the corresponding values found for Group I animals, the mean increase in blood pressure in Group III animals was significantly larger (t = 3.05, df = 11, p < 0.01) (Fig. 3). We believe that these values exclude the possibility that the absence of lesions in Group III was due to ineffective blood pressure increase. The mean (± SE) time required for blood pressure rise was 7.45 ± 0.69 seconds, a time close to that found for Group I.

Examination of the brains of animals in Group III yielded findings exactly similar to those described for Group II. No evidence of BBB breakdown was seen. The distribution of blood in the subarachnoid space and the findings under fluorescent microscopy were no different from those described for Group II (Fig. 2 right).

**Discussion**

The results of this study may be summarized as follows: 1) acute arterial hypertension induces BBB breakdown to proteins; 2) SAH does not cause BBB breakdown to proteins during its acute stage; and 3) during the stage immediately following SAH, acute arterial hypertension fails to induce BBB breakdown.

**Experimental Considerations**

The experimental techniques most commonly used to mimic intracranial aneurysm bleeding are arterial puncture with a needle, 1,66,74 and injection of blood into the subarachnoid space. 14,46,56,57 The experimental reproduction of clinical SAH is troublesome. The standardization of variables such as speed and volume of bleeding is difficult due to the lack of exact clinical information. Clinical evidence obtained from ICP measurement suggests that SAH from an aneurysm occurs within a few seconds, raising the ICP to blood pressure levels.26,50 It has been demonstrated experimentally that a rapid rise in the ICP induces loss of cerebrovascular autoregulation and breakdown of the BBB. 24,39,54,78 Therefore, a sharp increase in the ICP is to be avoided if the effects of subarachnoid blood on the BBB are to be considered. In the present study, the duration of blood injection was determined by concomitant ICP measurement. It was found that the injection of 4 ml of blood over a 4-minute period would always prevent a significant ICP rise, as previously observed by other investigators. 26,46

Autopsies from clinical SAH material suggest the existence of a large variation in the amount of blood, from localized hemorrhage in the basal cisterns to
widespread distribution over the cerebral hemispheres. In adult cats, the injection of 4 ml of blood leads to an even spread in the subarachnoid space, associated with a denser concentration of clotted blood in the basal cisterns. This volume was comparable to that used by other investigators for animals of similar size.

Blood-Brain Barrier and SAH

An SAH is accompanied by aseptic meningitis, release of serotonin into the subarachnoid space by the blood elements, and altered cerebrovascular reactivity. Any pathophysiological changes may induce BBB breakdown. In spite of that, no BBB breakdown was seen during the acute stage following SAH in the present experiment. It might be that those pathophysiological changes were not intense enough to induce BBB damage. Acute arterial hypertension induces BBB breakdown. Furthermore, acute hypertension has been used as an experimental tool, in association with other BBB insults, to induce a more extensive breakdown of the BBB. It is said that a condition impairs BBB function if the amount of extravasated material after its association with hypertension is greater than that seen with hypertension alone. This supposition is corroborated by the findings of increased passage of plasma compounds through the cerebral endothelium that occurred during the acute stage following SAH in this study did not result in the BBB breakdown that is usually caused by arterial hypertension. These results may be due either to an atypical response by the BBB, or to a possible hemodynamic factor that could be interfering with the increase in intraluminal pressure, therefore rendering the hypertensive insult ineffective. The latter hypothesis deals with the possibility that vasospasm of major intracranial vessels, known to occur within minutes after an experimental SAH, could be preventing the transmission of the hypertensive insult into the distal vessels.

A BBB-damaging agent acting independently of systemic blood pressure and cerebral perfusion, could help to differentiate between the two possibilities. This is the object of our next report.

References
Blood-brain barrier after SAH


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Address reprint requests to: Eric W. Peterson, M.D., F.R.C.S.(C), 1081 Carling Avenue, Suite 703, Ottawa, Ontario K1Y 4G2, Canada.