Intracranial arterial spasm in the dog

A chronic experimental model

ROBERT H. WILKINS, M.D., AND PHILIP LEVITT, M.D.
Division of Neurosurgery, Durham Veterans Administration Hospital, and the Duke University Medical Center, Durham, North Carolina

A chronic canine model for the investigation of intracranial arterial spasm was designed and used to study spasm produced by rapid and slow cisternal injections of fresh or heparinized autogenous whole blood, or serum from incubated autogenous blood. Spasm so produced begins within 10 minutes after the injection and lasts from several hours to days. It affects primarily the major arteries of the circle of Willis, especially the proximal anterior cerebral arteries, and it does not seem to involve the extradural arterial tree.

The pathogenesis of intracranial arterial spasm in humans is not well understood, and no satisfactory treatment has yet been devised to combat its adverse effects. Although these unsolved clinical problems have prompted a number of investigators to study intracranial arterial spasm in animals, most of the experimental models that have been used have been acute preparations employing direct visualization of the cerebral arteries through an opening in the skull, usually for periods of less than 12 hours. Chronic experiments in animals, which more closely simulate the phenomenon in humans, were not begun until it was shown by Allcock in 1966 that arterial spasm in dogs could be demonstrated indirectly by cerebral arteriography. Since then, a few initial chronic studies have been made in monkeys and dogs.

The present investigation represents an attempt to establish a satisfactory chronic canine model for the study of intracranial arterial spasm.

Material and Methods

Anesthesia

Mongrel dogs of both sexes, weighing between 30 and 45 lbs, were anesthetized either with sodium pentobarbital (12 mg per lb body weight) or with a combination of sodium thiopental (between 250 and 500 mg per experiment) and chloralose (between 250 and 1500 mg per experiment), injected intravenously. Endotracheal intubation was performed, and the dogs either were allowed to breathe spontaneously or were paralyzed with intravenous succinylcholine chloride (10 to 20 mg periodically as needed) and placed on a respirator at a specified volume and rate of respiration.

Subarachnoid “Hemorrhage”

Several methods of inducing subarachnoid hemorrhage in the dog were tried. These included direct injection of fresh autogenous whole blood into the cisterna magna as well as several techniques for exposing and

J. Neurosurg. / Volume 33 / September, 1970
Experimental arterial spasm

Fig. 1. Arteries most frequently visualized in the mentovertex view of a left carotid arteriogram in the dog. The extracranial arteries, stippled in the drawing, are the common carotid (C.C.), occipital (Oc), external carotid (E.C.), internal maxillary (I.M.), and maxillo-carotid anastomotic (M.C.A.) arteries. The extradural portion of the internal carotid artery (I.C.) is also stippled, but its short terminal portion, which is intradural, is shown in solid black. The other intracranial arteries, drawn in solid black, are the middle cerebral (M.C.), internal ethmoidal (I.E.), anterior communicating (A.Co.), anterior cerebral (A.C.), posterior communicating (P.Co.), posterior cerebral (P.C.), superior cerebellar (S.Ce.), and basilar (B.) arteries. (This drawing is modified from de la Torre, E. Netsky, M. G., and Meschan, I. Intracranial and extracranial circulation in the dog: anatomic and angiographic studies. Am. J. Anat., 1959, 105:343-381).

piercing the internal carotid artery intracranially. The former, although performed in only three dogs, did not produce recognizable arterial spasm, and the latter resulted in very limited subarachnoid hemorrhage with focal spasm only at the point of injury.

Of the methods tested, the most successful for producing intracranial arterial spasm was found to be the injection of fresh autogenous whole blood into the chiasmatic cistern by the technique of Lougheed and Tom or of McQueen and Jeannes. Blood injected by either technique remained fairly well localized in the subarachnoid spaces about the circle of Willis.

Carotid Arteriography

Several techniques for carotid arteriography were also tested before we decided upon the one most satisfactory for our purposes. Our preferred method is described as follows. The bifurcations of both common carotid arteries are exposed surgically, using sterile technique. A flexible catheter* is inserted through a puncture in the ipsilateral common carotid artery, and is threaded up into the internal carotid. The contralateral common, external, and internal carotid arteries are then temporarily occluded.

* 18G-2 ½ Longdwell Catheter, Becton, Dickinson and Company, Rutherford, New Jersey.
with bulldog clamps. Single or serial mentovertex x-ray films are made as 8 ml of 75% Hypaque-M are injected through the catheter (Fig. 1). As the catheter is withdrawn at the end of the procedure, the puncture wound in the common carotid artery is closed with a 5-0 silk purse-string suture. In this way the arteries remain patent, and arteriography may be repeated several times on later occasions.

At the start of each experiment, an initial carotid arteriogram was performed as a baseline for comparison with subsequent arteriograms on the same dog.

**Blood Gas Measurements**

In the various experiments, determination of pH, pO_2, %O_2 saturation, and pCO_2 were made on heparinized blood samples drawn from the carotid catheter, using the IL Micro pH and Blood Gas Analyzing System.*

In three additional dogs, given only local anesthesia and intravenous succinylcholine chloride, the rates of controlled respirations were varied to artificially alter the values of blood pH, pO_2, and pCO_2. In each dog the respirator was consecutively set for periods of 10 to 30 min to produce "normal" ventilation, hyperventilation, and hypoventilation. This was done both before and after the induction of intracranial arterial spasm by the injection of 10 ml of fresh autogenous whole blood into the chiasmatic cistern. Carotid arteriograms and measurements of pH, pO_2, %O_2 saturation, and pCO_2 were made after each 10- to 30-min period. Despite marked variations in the pH and blood gas measurements, there was relatively little change in the caliber of the cerebral arteries. Hyperventilation was associated with a slight diminution in arterial caliber, but not as marked as that occurring as a result of the injection of blood into the chiasmatic cistern. With hyperventilation there was a slight increase in arterial diameter, but not enough to overcome previously-induced cerebral arterial spasm.

**Pressure Measurements**

Systemic arterial pressure was measured via a catheter in one of the femoral arteries, using a transducer and preamplifier.† Cerebrospinal fluid pressure in the cisterna magna was measured with a similar system via an 18-gauge lumbar puncture needle inserted percutaneously at the cranio-spinal junction.

Supratentorial subdural pressure measurements were performed with a subminiature pressure transducer,‡ attached to a Sanborn preamplifier. The transducer was inserted into the subdural space through a small craniectomy opening in the left parietal area. To insure a closed pressure system, the bone defect was filled with bone wax and the temporalis muscle was sutured closed around the small cable leading from the transducer. All pressures were recorded on an amplifier-recorder.§

**Electroencephalography and Electrocardiography**

Four or eight needle electrodes were placed into the scalp symmetrically, and EEG recordings were made in one of four montages. Electrocardiograms (Leads I, II, and III) were obtained simultaneously by the same electroencephalograph machine, using needle electrodes in the left hind limb and both fore limbs.

**Experimental Groups**

After the optimum experimental methods were established in 55 dogs, 57 more were studied in six groups (Table 1). Injections of fluid into the chiasmatic cistern were either given manually at a rate of about 1 ml/3 sec, or by a constant infusion pump at a rate of about 1 ml/9 min.

The dogs in Group 1 were injected either with sterile isotonic saline or with sterile dextran.** The blood used in the other groups was drawn into a sterile syringe from a femoral artery or vein of the dog to be injected, using antiseptic technique. The syringe was wet with sterile heparin if heparinized blood

---

* Model 113-S2, Instrumentation Laboratory, Inc., Watertown, Massachusetts.


‡ Model SA-SA-M7-BW, Sensotec Division, Comtel Corporation, Columbus, Ohio.

§ Model 322, Sanborn Company, Waltham, Massachusetts.

**6% Dextran w-v in Dextrose 5%, Abbott Laboratories, North Chicago, Illinois.
Experimental arterial spasm was to be used. Serum was removed, using sterile technique, from some of the fresh blood samples, and from other blood samples that had been incubated at 37°C in sterile test tubes from 2 to 8 days (simultaneous bacteriological cultures were made of some of the incubated blood samples, and were negative). The serum removed from fresh blood was straw-colored, whereas the serum removed from incubated blood was brownish-purple as a result of hemolysis.

Pathological Examination

At the end of most of the experiments, the dog's brain was removed and fixed in a 10% formalin solution. A few days later the brain was cut into multiple coronal sections for gross examination.

Results

Groups 1-4

Rapid Injection (10 ml, Saline, Dextran, Blood, or Serum). The dogs that were allowed to breathe spontaneously usually had a brief tonic seizure near the end of the injection, followed by a respiratory arrest that lasted 2 to 3 min. Their EEG tracings were difficult to interpret at these times because of muscle artifact. In contrast, the dogs that were paralyzed with succinylcholine chloride and were placed on a respirator had no gross or electroencephalographic evidence of sei-

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of Dogs</th>
<th>Fluid Injected into Chiasmatic Cistern</th>
<th>Amount of Fluid (ml)</th>
<th>Rate of Injection</th>
<th>Anesthesia</th>
<th>Respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>saline or dextran solution</td>
<td>10</td>
<td>1 ml/3 sec</td>
<td>pentobarbital (3)</td>
<td>spontaneous (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thiopental and chloralose (3)</td>
<td>controlled (1)</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>fresh autogenous whole blood (16)</td>
<td>10</td>
<td>1 ml/3 sec</td>
<td>pentobarbital(12)</td>
<td>spontaneous (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thiopental and chloralose (4)</td>
<td>controlled (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>heparinized fresh autogenous whole blood (2)</td>
<td>10</td>
<td>1 ml/3 sec</td>
<td>pentobarbital (2)</td>
<td>spontaneous (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>controlled (1)</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>serum from fresh autogenous whole blood</td>
<td>10</td>
<td>1 ml/3 sec</td>
<td>pentobarbital (4)</td>
<td>spontaneous (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thiopental and chloralose (2)</td>
<td>controlled (2)</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>serum from incubated autogenous whole blood</td>
<td>10</td>
<td>1 ml/3 sec</td>
<td>pentobarbital (4)</td>
<td>spontaneous (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thiopental and chloralose (6)</td>
<td>controlled (1)</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>heparinized fresh autogenous whole blood</td>
<td>10</td>
<td>1 ml/9 min</td>
<td>pentobarbital (7)</td>
<td>spontaneous (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thiopental and chloralose (2)</td>
<td>controlled (3)</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>fresh autogenous whole blood</td>
<td>2</td>
<td>1 ml/3 sec</td>
<td>pentobarbital (8)</td>
<td>spontaneous (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>controlled (1)</td>
</tr>
</tbody>
</table>
Ture activity, and their EEG’s were not mar-
red by muscle artifact.

During injection, the EEG wave am-
plitude and frequency decreased, beginning af-
ster 3 to 6 ml of fluid had been injected. This
change became most pronounced by the end
of the injection. The EEG tracings of the
dogs injected with saline or dextran were
never completely flat, and recovery took
place over 20 to 60 sec. However, the trac-
ings of the dogs given blood or serum usually
did become isoelectric, and remained so for
1 to 2 min before recovery began. In one of
the latter dogs, suppression bursts \(^{18}\)
appeared at the end of the isoelectric period for
60 sec before other wave forms reappeared.

The cerebrospinal fluid pressure in the cis-
terna magna (Fig. 2) and the parietal sub-
dural pressure each showed an immediate
and rapid rise during the injection of fluid
into the chiasmatic cistern, from control lev-
els around 5 to 15 mm Hg to peak values of
170 to 475 mm Hg by the end of the injec-
tion. This was typically followed by a rapid
fall in intracranial pressure over approxi-
mately 30 sec and then a more gradual de-
cline, approaching control levels within 15
min after the injection.

These rapid 10 ml injections also caused
temporary systemic arterial hypertension
and bradycardia (Fig. 2). The rise in blood
pressure usually began after 5 to 7 ml of
fluid had been injected, reached a peak about
5 to 10 sec after the injection was finished,
and returned to normal over another 1 to 3
min. The pulse rate typically began to dimin-
ish from 5 to 25 sec after the injection,
reached a minimum after another 10 to 25
sec, and returned to control values after 1 to
2 min more. Almost half of the dogs injected
with saline, dextran, or serum had brief car-
diac arrhythmias associated with the injec-
tion, but no other abnormalities were noted
in their EKG’s. However, most of the EKG’s
of the dogs injected with blood showed in-
version of the T waves and/or elevation of
the ST segments, either during the injection
or shortly thereafter, usually lasting less than
15 min. One of these dogs had later EKG
tracings on the second, fourth, and seventh
postinjection days, and depression of the ST
segments was noted in all three tracings. The
U waves appeared on the fourth day, but
were gone on the seventh. Postinjection
blood pH, pO\(_2\), and pCO\(_2\) measurements
were essentially the same as preinjection val-
ues.

In the six dogs injected with saline or dex-
tran, no intracranial arterial spasm was pres-
ent on any of the postinjection arteriograms,
made between 10 min and 1 hr after injec-
tion. In contrast, 14 of the 18 dogs injected
with blood had intracranial arterial spasm on
the initial postinjection carotid arteriograms,
Experimental arterial spasm

performed from 10 min to 4 hrs after injection (Fig. 3). Carotid arteriograms repeated in six of the dogs at 1, 1, 2, 3, 4, and 11 days after injection showed persistent though diminishing spasm, but the spasm had disappeared at 2, 3, 4, 7, and 12 days in five of the dogs. The six dogs injected with fresh serum demonstrated no definite intracranial arterial spasm in arteriograms performed from 5 to 15 min after injection and again from 1 to 5 days later. However, all of the 10 dogs injected with serum from incubated blood showed spasm in the initial postinjection arteriograms (Fig. 4). Spasm was still present on subsequent arteriograms at 1 day in one dog and at 6 days in another. In the latter dog the spasm had disappeared by 8 days. Four other dogs had normal arteriograms from 2 to 4 days after injection.

Several of the dogs that received the rapid 10 ml injections into the chiasmatic cistern were found at postmortem examination to have hemorrhages in the diencephalon or within the ventricles (especially the posterior portions of the lateral ventricles).

Group 5

Slow Injection (10 ml Blood). The pressure within the cisterna magna was measured in one dog throughout the 90-min blood injection. This slowly rose from 6 to 28 mm Hg.

In most of the animals there was also a gradual diminution in the amplitude and frequency of EEG waves during the injection, with gradual recovery over 15 to 30 min.

In six of the eight dogs in which these parameters were measured, there were no significant changes in the systemic blood pressure, pulse rate, or EKG during injection. The other two dogs manifested bradycardia, with a fall in pulse from 205–210 to 170–180, and EKG abnormalities, with changes in the T waves in Leads I and II. (The blood pressure was not measured in the latter two dogs.) The blood pH, pO₂, and pCO₂ values were similar to those of the previous groups.

Cerebral arterial spasm was demonstrated in all of the initial postinjection films, made from 5 to 30 min after injection in nine dogs, and also at 50 and 150 min in one dog (Fig. 5). One animal had another carotid arteriogram on the fourth day after injection, and spasm was still present, but arteriograms in a different dog at 4, 7, and 12 days were normal.

Blood in the subarachnoid spaces, especially about the circle of Willis, was found in all nine dogs, sacrificed from 1 to 12 days after injection. In one dog there was a dien-
cephalic hemorrhage, and in another there were hemorrhages within the posterior portions of both lateral ventricles.

**Group 6**

**Rapid Injection (2 ml Blood).** Although four of these eight dogs were allowed to breathe spontaneously, none had a seizure or respiratory arrest during injection. The EEG remained unchanged in seven dogs, and in the eighth there was only minor slowing and decrease in wave amplitude beginning at the end of the injection and lasting for 80 sec.

Changes in systemic blood pressure and pulse rate were inconstant and minor. Likewise, there were no significant EKG changes initially, though a repeat EKG in one dog 2 days post-injection demonstrated ST depression and T wave changes. The blood pH, pO₂, and pCO₂ values were similar to those of the other groups.

The parietal subdural pressure in one dog rose from a control value of 6 mm Hg to a peak of 31 at the end of the injection, and then fell gradually to 14 over a period of 65 min. In another dog it rose from 13 to 30 mm Hg during the injection and then fell to 18 within 3 min. The changes in the CSF pressure in the cisterna magna in the latter dog were similar to those in the parietal subdural space. Cisternal pressure in a different dog rose from a control level of 5 mm Hg during the injection, reaching a peak of 12 mm Hg 15 sec later, where it stayed for the remaining 6 min of the tracing.

Spasm was present in the initial postinjection arteriograms of all eight dogs, from 10 min to 4 hrs after injection (Fig. 6). Subsequent arteriograms in one of the dogs showed persistent spasm on the second and third days after injection as well.

Postmortem examinations demonstrated that the 2 ml of blood injected into each of these dogs were confined to the subarachnoid spaces surrounding the circle of Willis (Fig. 7). There were no intraventricular or intracerebral hemorrhages.

**Discussion**

The rapid injection into the chiasmatic cistern of the dog of 10 ml of fresh or heparinized autogenous whole blood, or of 10 ml of serum from incubated autogenous whole blood, consistently produced intracranial arterial spasm with the following properties:

1. Spasm was an intradural phenomenon, affecting primarily the major arteries of the circle of Willis and not involving the extradural arteries such as the extradural segment of each carotid artery.

2. Spasm was most noticeable in the proximal anterior cerebral arteries (those closest to the point of injec-
Experimental arterial spasm

FIG. 5. Dog 84. Mentovertex right carotid arteriograms made 15 min before (left) and 150 min after (right) the slow injection into the chiasmatic cistern of 10 ml of heparinized fresh autogenous blood over a period of 90 min. By chance the right external carotid and internal maxillary arteries are not filled in the preinjection film (left). In the postinjection film (right) there is spasm of the arteries comprising the circle of Willis, especially the posterior communicating and basilar arteries (arrows). An EEG needle is also visible.

FIG. 6. Dog 107. Mentovertex right carotid arteriograms made 15 min before (left) and 10 min after (right) the rapid injection into the chiasmatic cistern of 2 ml of fresh autogenous whole blood. By chance the right external carotid and internal maxillary arteries are not filled in the preinjection film (left). In the postinjection film (right), there is marked spasm of the vessels comprising the circle of Willis, but not of the extracranial arteries, including the extra-dural portion of the right internal carotid artery.
tion). This was fortunate since these arteries were the ones most consistently visualized on canine mento vertex carotid arteriography. The site of the greatest amount of subarachnoid blood at autopsy could not be correlated with the location of the most severe spasm on the carotid arteriograms.

3. Spasm began within 10 min after injection and lasted from several hours to several days. We did not encounter the biphasic spasm response reported by other authors.3,10,16,19

4. Spasm was influenced very little by variations in blood pH, pO2, and pCO2, and did not appear to be altered by intravenous succinylcholine chloride.

5. Spasm was not necessarily associated with a change in the dog’s level of consciousness, although many of the dogs were lethargic and unsteady for a few days after an injection of blood or serum into the chiasmatic cistern.

In most of these respects, the intracranial arterial spasm in the present canine model resembles its human counterpart.28 It does not appear to last as long, however. This might be due to species differences, or to the absence of direct arterial injury and repeated episodes of subarachnoid hemorrhage in these dogs. In addition, there were no instances of delayed onset of spasm, although this is common among humans with subarachnoid hemorrhage.16,28

Associated with the rapid 10 ml injections were a number of phenomena that appeared to be due primarily to an increase in the intracranial pressure. These included transient systemic hypertension, bradycardia, flattening of the EEG, and various EKG changes.4,11,20 Hypothalamic intracerebral hemorrhages also occurred, probably because this area of the brain bore the brunt of the direct pressure effect of the injection. Prolonged changes in respiration25 did not occur, and pulmonary edema7 and secondary brain stem hemorrhages18 were not encountered, perhaps because the intracranial pressure fell relatively rapidly after it had been elevated suddenly by the injection.6

Intracranial arterial spasm could also be induced in the dog without raising the intracranial pressure to any marked degree, with relatively minimal effects on the systemic blood pressure, pulse, EEG and EKG, and with rare intracerebral or intraventricular bleeding. This could be accomplished either by the injection of just 2 ml of blood into the chiasmatic cistern, or by the slow injection of 10 ml of heparinized blood with a constant infusion pump over a 90-min period. Amounts of blood smaller than 2 ml probably will not produce cerebral arterial spasm; two dogs not reported above were injected with 0.1 and 1.0 ml of blood respectively, and spasm did not develop.

The purple-brown serum taken from blood incubated at 37° for 2 to 8 days produced intracranial arterial spasm, whereas the straw-colored serum from fresh blood had little or no effect. This suggests that a vasoconstrictor substance or substances were liberated into the serum as hemolysis, and perhaps clot lysis, occurred.28,29 Further experiments to analyze the role of the various blood components35 are in progress in this laboratory.
Experimental arterial spasm

Acknowledgments

The authors thank Mrs. J. L. Danford and Mr. Ezra Hayes for their technical assistance, and Drs. Emil L. Weber, William P. Wilson, and Joseph C. Greenfield, Jr., for their advice.

References


Received for publication December 1, 1969.
Address reprint requests to: Robert H. Wilkins, M. D., Department of Surgery, Duke University Medical Center, Durham, North Carolina 27706.