Development and Evaluation of an Experimental Model for the Study of the Cerebral Circulation in the Unanesthetized Goat

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Abstract: Development and Evaluation of an Experimental Model for the Study of the Cerebral Circulation in the Unanesthetized Goat

An animal model has been developed for the continuous measurement of total cerebral blood flow in the unanesthetized, unrestrained goat. We selected the goat because each internal maxillary artery, a branch of the external carotid artery, provides the total blood flow to each hemisphere via the rete mirabile. After the occlusion of the extracerebral vessels of the internal maxillary artery with thrombin, an electromagnetic flow transducer was chronically implanted on this artery, distal to the temporal artery, to measure hemispheric blood flow. Reproducible measurements of cerebral blood flow were obtained in ten unanesthetized goats for periods ranging between two weeks and five months. The mean cerebral blood flow for the ten goats was 133 ± 5 ml/min/100 gm tissue at an average mean aortic pressure of 91 ± 3 mm Hg, heart rate of 79 ± 3 beats/min, arterial P\textsubscript{O}\textsubscript{2} of 31.4 ± 1.0 mm Hg, P\textsubscript{a} of 76.6 ± 1.7 mm Hg, and pH of 7.48 ± 0.02. The present experimental preparation allows cerebrovascular hemodynamics to be evaluated under near-normal conditions and is suitable for physiological and pharmacological studies in normal and abnormal states.

Introduction

The accurate measurement of cerebral blood flow remains a challenge for the researcher and the clinician. In the experimental laboratory, most of the methods used require general anesthesia and, very often, extensive surgery,\textsuperscript{1,2} conditions far removed from the normal situation present in awake animals with an intact circulation. In human subjects, the methods commonly employed, such as inert tracer and indicator-dilution techniques, permit only intermittent determinations to be made because steady-state conditions must be present for 10 to 20 minutes.\textsuperscript{3} Therefore, dynamic changes in cerebral flow cannot be delineated. Further problems in the measurement of cerebral blood flow stem from two facts. First, the determinations are frequently complicated by the lack of exclusion of extracerebral blood flow and, second, in most species more than one artery supplies each cerebral hemisphere.\textsuperscript{4} The present study describes the development of a new experimental model utilizing the unanesthetized goat in which virtually the entire circulation to a cerebral hemisphere can be continuously and accurately monitored on a beat-to-beat basis for up to several months.
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Methods

ANATOMICAL CONSIDERATIONS
We selected the goat because of its unique arterial supply to the brain. Each internal maxillary artery, a branch of the external carotid artery, provides the total blood flow to each hemisphere via the rete mirabile; in the goat, vertebral arteries do not contribute to the cerebral blood supply. In addition, no extracranial internal carotid artery exists. Figure 1 shows an acrylic polymer cast (Batson's #17 Anatomical Corrosion Compound, Polysciences, Inc., Rydal, Pennsylvania) of the arteries of the goat's head and illustrates the inflow to the rete by the internal maxillary arteries. When the rete is situated intracranially, as in the goat, a considerable potential communication exists across the midline with the rete of the other side. Under physiological conditions, however, blood from one rete does not cross to the other. We demonstrated this lack of a physiological communication in five anesthetized goats in which we injected 100 μCi 1181-labeled albumin macroaggregates into the left maxillary artery through a catheter placed in the temporal artery. The animals were killed within ten minutes by an overdose of intravenous pentobarbital, the whole brain was removed, and the distribution of radioactive macroaggregates was determined using a gamma scintillation camera (Nuclear Chicago Pho/Gamma III Camera, Chicago, Illinois). The contralateral cerebral hemisphere was virtually free of macroaggregates (fig. 2 left), indicating the unilateral localization of blood flow to the cerebral hemispheres in the goat and confirming earlier results of Andersson, who infused colored suspensions into the carotid arteries to map the distribution of the goat's cerebral blood flow. However, when one of the internal maxillary arteries is occluded, this physiological separation is disrupted and the contralateral internal maxillary artery provides the blood to both brain hemispheres through the rete communications. Thus, figure 2 (right) shows that the macroaggregates have been distributed to both hemispheres after they had been injected into the left maxillary artery, the right maxillary artery having been ligated one hour earlier. The number of counts per minute was similar in each hemisphere, suggesting that both sides were equally perfused despite ligation of the right internal maxillary artery.

The internal maxillary artery supplies the rete via two branches, the ramus anastomoticus, which joins the rete in its lateral and posterior portions, and the arteria anastomotica, which joins the rete anteriorly. The ophthalmic, ethmoidal and buccinator arteries are given off distal to the arteria anastomotica and constitute the main sources of extracerebral blood flow (figs. 1 and 3). The outflow from the rete goes via the distal remnant of the internal carotid artery into the circle of Willis. The circle of Willis in the goat is similar to that of man except that the blood flows in a caudal direction in the basilar artery, which has only insignificant communications with the vertebral artery (fig. 1).

SURGICAL PROCEDURE
An electromagnetic flow transducer placed on the internal maxillary artery measures blood flow destined not only for the brain but, also, flow to the orbit and nose (fig. 3, A). Therefore, the technique described here was devised to exclude the extracerebral component of internal maxillary flow so that true blood flow to the brain could be...
measured chronically by an electromagnetic flow transducer placed on this vessel.

Anesthesia was induced in 20 female goats, ranging in weight from 25 kg to 35 kg, by the intravenous administration of 2% sodium thiopental; supplemental doses were given as necessary for maintenance. After orotracheal intubation, artificial respiration with room air was instituted using a Harvard respirator. A plastic tube was placed in the stomach to prevent gas accumulation and postoperative vomiting and aspiration. Under sterile conditions the internal maxillary artery and the distal part of the external carotid artery were exposed through an incision along the ramus mandibulae and a portion of the ramus was resected to allow adequate exposure. Ligatures were then placed on the internal maxillary artery immediately distal to the origin of the ramus anastomoticus and on the temporal and dental arteries (fig. 3, B); the facial nerve and parotid gland were left intact. An electromagnetic flow transducer that had been calibrated previously in vivo (Biotronex, Silver Spring, Maryland) was placed on the internal maxillary artery. To obtain a zero flow baseline, a balloon occluder was placed on the external carotid artery, as close to the temporal artery as possible (fig. 3, A). Despite the fact that the occluder was proximal to the flow transducer, stable and reproducible zero flows were obtained because the internal maxillary artery was kept filled by the retrograde transmission of pressure from the opposite side via the rete, which occurs when normal blood flow is temporarily blocked by expansion of the balloon occluder.

However, anatomical considerations indicated and preliminary experiments had demonstrated that merely ligating the internal maxillary artery did not prevent extracerebral blood flow via the ophthalmic, ethmoidal, and buccinator arteries, which now received their blood supply as a result of retrograde flow through the arteria
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Schematic representation of the intracerebral and extracerebral branches of the internal maxillary artery of the goat. Arrows indicate the direction of blood flow. A: normal conditions, B: after ligation of the temporal and dental arteries and the internal maxillary artery distal to the ramus anastomoticus, C: same as B plus injection of 1,000 N.I.H. units of thrombin into the internal maxillary artery distal to its ligature. ID = inferior dental artery, E = electromagnetic flow transducer, O = balloon-type occluder, cross-hatched area = left rete, stippled area = extracerebral vessels deprived of blood, dashed line = skull. Other symbols as in figure 1.

FIGURE 3

TABLE 1

Measurements Obtained in Unanesthetized Goats

<table>
<thead>
<tr>
<th>Goat no.</th>
<th>CBF cc/min/100 gm</th>
<th>MAoP mm Hg</th>
<th>HR beats/min</th>
<th>PCO2 mm Hg</th>
<th>PO2 mm Hg</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>141 ± 5</td>
<td>100 ± 5</td>
<td>70 ± 6</td>
<td>30.6 ± 1.3</td>
<td>78.5 ± 36</td>
<td>7.52 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>116 ± 4</td>
<td>105 ± 7</td>
<td>70 ± 3</td>
<td>33.8 ± 1.4</td>
<td>75.4 ± 24</td>
<td>7.55 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>125 ± 10</td>
<td>75 ± 7</td>
<td>92 ± 3</td>
<td>30.6 ± 1.0</td>
<td>70.1 ± 19</td>
<td>7.57 ± 0.03</td>
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<tr>
<td>4</td>
<td>121 ± 7</td>
<td>90 ± 3</td>
<td>80 ± 7</td>
<td>30.0 ± 1.3</td>
<td>85.5 ± 2.3</td>
<td>7.48 ± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>130 ± 7</td>
<td>80 ± 5</td>
<td>60 ± 6</td>
<td>25.0 ± 1.6</td>
<td>84.0 ± 2.0</td>
<td>7.45 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>146 ± 14</td>
<td>105 ± 4</td>
<td>82 ± 7</td>
<td>35.1 ± 1.5</td>
<td>74.0 ± 3.2</td>
<td>7.50 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>150 ± 5</td>
<td>90 ± 4</td>
<td>80 ± 4</td>
<td>29.7 ± 1.5</td>
<td>76.3 ± 0.9</td>
<td>7.46 ± 0.02</td>
</tr>
<tr>
<td>8</td>
<td>164 ± 6</td>
<td>85 ± 6</td>
<td>82 ± 10</td>
<td>32.7 ± 1.8</td>
<td>74.2 ± 1.2</td>
<td>7.38 ± 0.04</td>
</tr>
<tr>
<td>9</td>
<td>126 ± 6</td>
<td>80 ± 5</td>
<td>95 ± 3</td>
<td>31.2 ± 1.4</td>
<td>79.8 ± 2.2</td>
<td>7.49 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>110 ± 9</td>
<td>100 ± 3</td>
<td>80 ± 5</td>
<td>35.2 ± 1.4</td>
<td>68.6 ± 2.4</td>
<td>7.45 ± 0.02</td>
</tr>
<tr>
<td>Mean</td>
<td>133</td>
<td>91</td>
<td>79</td>
<td>31.4</td>
<td>76.6</td>
<td>7.48</td>
</tr>
<tr>
<td>SE</td>
<td>± 5</td>
<td>± 3</td>
<td>± 3</td>
<td>± 1.0</td>
<td>± 1.7</td>
<td>± 0.02</td>
</tr>
</tbody>
</table>

The data are expressed as mean values (± SEM) based on at least five measurements in each goat obtained on different days. CBF = cerebral blood flow, MAoP = mean aortic pressure, and HR = heart rate.

Because of their inaccessibility these vessels could not be approached surgically. To obliterate these vessels a new technique was devised that consisted of injecting 1,000 to 2,000 N.I.H. units of thrombin (Thrombin, Topical, Parke Davis, Detroit, Michigan) dissolved in 1 ml of saline into the internal maxillary artery beyond the ligature that had been placed on this vessel. This maneuver produced an almost immediate thrombosis in these vessels (fig. 3, C), as evidenced clinically by the production of ipsilateral blindness. Confirmation of the effectiveness of this technique for the exclusion of the extracerebral vessels was obtained in four goats in which cineangiograms were performed; these studies demonstrated nonvisualization of the extracerebral vessels. Further proof of obliteration was gained in three additional goats in which 2 ml
of metallic mercury were injected under pressure into the internal maxillary artery at the moment of sacrifice. Mercury filled at rete, circle of Willis, basilar artery and the ethmoidal vessels of the contralateral side, but did not fill the extracerebral vessels of the ipsilateral side because they had been thrombosed two weeks before by the injection of thrombin.

A polyethylene catheter was placed in the temporal artery with its tip beyond the ligature for measurement of internal maxillary artery pressure and for injection of contrast material (Angio-Conray) in some experiments. This catheter, together with the leads from the flow probe and the occluder, were led out subcutaneously and secured to the goat's horn. Aortic pressure was obtained from a chronically implanted catheter introduced through the femoral artery, and heart rate was measured from the aortic pressure pulse with a ratemeter. Flow measurements were made using a Biotronex electromagnetic flowmeter (Model BL-620). Blood flow, aortic pressure, and heart rate were recorded on an Electronics for Medicine photographic recorder. After surgery each animal received 600,000 units of penicillin I.M. and was placed in a large, warm pen.

**FIGURE 4**

Representative tracings of left cerebral blood flow (LCBF) (pulsatile and mean), aortic pressure (AoP), and heart rate (HR) from a goat on the day of surgery and at several times postoperatively. Zero flow is obtained by expanding the occluder as illustrated in the middle of each panel. Electrical baseline was adjusted during occlusion in lower right panel.
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Recovery occurred rapidly and after 24 to 30 hours they were able to eat, drink, walk and behave normally. The various measurements were made with the goat in a large cage without restraints, except for a wooden stock fitting loosely around the neck that limited forward and backward motion.

Arterial blood was analyzed periodically for pH, P_{CO_2}, and P_{O_2} (Instrumentation Laboratories, Model 123, Watertown, Massachusetts). Complications attributable directly to the surgical procedure were pneumonia in two goats and a wound infection in two other goats. Because of the repeated occlusion of the external carotid artery required to obtain zero flow, local vascular damage and eventual thrombosis occurred in six goats, which necessitated termination of the study within the first two postoperative weeks.

Results
Technically successful preparations that allowed studies to be carried out for periods ranging from two weeks to five months were obtained in ten goats, and the data from these animals are summarized in table 1. Values obtained from individual animals were highly reproducible and the range encompassed by the entire group was relatively narrow. In addition, the physiological levels of heart rate, arterial pressure, and blood gases reflect the fact that these unanesthetized animals were in a basal state when the determinations were made. We noted invariably that the cerebral blood flow at the time of surgery was markedly lower than the values recorded when the animal had recovered fully from the effects of general anesthesia and surgery (fig. 4). In our total experience, the intraoperative cerebral blood flow ranged between 55% to 65% of that present at the time of full recovery.

Observations made at the time of surgery, immediately before and after the injection of thrombin, allowed us to quantify the percentage of internal maxillary artery blood flow that normally goes to the brain, on the one hand, and to the extracerebral areas, on the other hand. It was determined that 50% to 70% goes to the brain and the remainder supplies areas such as the orbit and nose (fig. 5).
**Discussion**

Definitive delineation of the physiological and pharmacological mechanisms that control cerebral blood flow has not been possible because of the lack of an experimental model that would permit the effects of various interventions to be assessed on a beat-to-beat basis in an unanesthetized animal. The present study was undertaken to develop such a standardized preparation. Because of its unique cerebral blood supply, the goat was chosen as the optimum experimental animal, and after many preliminary trials, reproducible and reliable cerebral blood flow measurements were obtained using a chronically implanted electromagnetic flow transducer. Inasmuch as studies are carried out after full recovery, the acute effects of anesthesia and surgery are obviated. Therefore, the preparation allows cerebrovascular hemodynamics to be evaluated under near-normal conditions.

The significant decrease in cerebral blood flow produced by anesthesia and surgery is illustrated in figure 4 and is consonant with the work in cats reported by Sokoloff and Kety.8 The pharmacological effects of different anesthetics on cerebral blood flow have been emphasized recently.8 Besides allowing animals to be studied in the awake state, the preparation described here also permits the major portion of extracerebral blood flow carried by the internal maxillary artery to be eliminated. Since this extracerebral flow amounts to between 30% to 50% of total internal maxillary artery flow (fig. 5), the importance of eliminating this potential source of error when evaluating true brain blood flow becomes evident. However, it must be mentioned that we have observed in some goats that from one to three fine vessels sometimes arise from the ramus anastomoticus to supply regions of the posterior palate and maxilla. Because of their small size (0.5 mm in diameter or less), we do not think that they materially affect the validity of our results.

Thus, we have described an experimental model that allows the cerebral circulation to be studied in unanesthetized animals and, hopefully, will permit physiological and pharmacological interventions to be studied in normal and abnormal states.

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