Description of a Closed Window Technique for In Vivo Study of the Feline Basilar Artery

Gerrit J. Bouma, MD; Joseph E. Levasseur, MS; J. Paul Muizelaar, MD, PhD; Robin G. Wiatt; and Hermes A. Kontos, MD, PhD

Recent interest in the regulatory functions of large cerebral arteries has led to many studies addressing the specific reactivity of these vessels. Current data originate mainly from in vitro experiments, as in vivo studies of larger intracranial cerebral arteries have been cumbersome so far due to the lack of a suitable animal model. We provide a detailed technical description of a closed transclival window method for in vivo study of the basilar artery in cats. We present our experience with this preparation in 29 animals, which shows that the technique is feasible and allows repeated, accurate, and reproducible measurements of the basilar artery, although possible depressive effects of the anesthesia on vascular reactivity have to be taken into account. With hyperventilation, the basilar artery constricted by 12.2 ± 7.6% of the baseline diameter. The cerebral blood flow response to hypocapnia with this preparation was 2.0 ± 0.4%/torr Paco₂. An exudative clouding of the window occurred in some cats but had no apparent effect on vascular reactivity. We also discuss possible pitfalls in the surgical preparation. (Stroke 1991;22:522-526)

A traditional concept in vascular physiology is that the larger arteries are conduction vessels, whereas the smaller arteries are the site of modulation/regulation of vascular resistance and blood flow. Unlike observations in other vascular beds, however, there is increasing evidence that the large cerebral arteries, which account for 30-40% of the total cerebrovascular pressure gradient,1-4 have an important role in the regulation of the cerebral circulation.5 It has become evident that responses of the large cerebral arteries to sympathetic stimulation or neurohumoral stimuli may well differ or even oppose those of the smaller arteries and arterioles.3,6-8 This dissociation in vascular reactivity has also been reported with more aspecific physiological stimuli, such as changes in systemic arterial blood pressure9 and, more recently, hemodilution.10 Recent studies have suggested that caliber changes of the larger cerebral arteries serve to modulate microvascular pressure, even in the absence of changes in cerebral blood flow (CBF), possibly by an endothelium-dependent mechanism.5 Endothelium-dependent responses of the large cerebral arteries have thus far been studied almost exclusively in vitro and the results have not been consistent,6-11 whereas little is known about the in vivo reactivity of these vessels.12 One factor that may have hampered the progress of such studies is the lack of a suitable animal model for in vivo study of the large cerebral arteries. Direct transclival approach of the basilar artery in cats has been performed in the past,13,14 but a major problem with these preparations has been the open exposure, causing a loss of CO₂ from the cerebrospinal fluid (CSF), which may significantly affect the vascular responses.15

To overcome this problem, a closed transclival window model was developed, allowing direct observation and quantification of basilar artery width in cats while controlling the perivascular environment. In our laboratory, this method has been used successfully to study physiologic responses of the basilar artery to hematocrit changes (J.P. Muizelaar et al, unpublished observations), but the model is also well-suited for other applications in cerebrovascular physiology. The purpose of this paper is to provide a detailed description of the technique, with special attention to pitfalls in the preparation.

Materials and Methods

The window is made of clear acrylic and is designed to optimize the transmission of light onto the basilar artery (Figures 1 and 2A). The size of the window is maximized within the limits of the space available via the anterior cervical approach to the
clivus in cats. The window has a generally inward taper to achieve bottom surface dimensions of 3.7 mm x 7.2 mm. This configuration enhances the cone of light that reaches the base of the window. The bottom or clival surface is flanged, which allows the window to rest above and within the clival opening at a more or less equal distance from the basilar artery in each preparation. The top and bottom surfaces are polished with white rouge to a clear gloss finish. Along the axial component of the window, two holes are drilled to serve as conduits to flush the space under the window. The lateral surfaces are taped with cellophane tape to facilitate the removal of the dental acrylic so that the window can easily be recovered and reconditioned for reuse in another preparation.

All procedures involving cats were carried out in accordance with the Public Health Service Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. Adult cats of either sex and mixed breed weighing 2.5-4.0 kg were anesthetized with 30 mg/kg i.v. sodium pentobarbital. Catheters were inserted into the femoral artery and vein, and arterial blood pressure was monitored via a Statham pressure transducer (Glen Burnie, Md.) Additional pentobarbital was given as needed to maintain a constant blood pressure and miotic pupils. A tracheotomy was performed through a midline cervical incision. Ventilation was regulated with an animal respirator (Harvard Apparatus, South Natick, Mass.), and skeletal muscle paralysis was achieved with 5 mg/kg gallamine triethiodide. The respiratory rate and volume were adjusted so as to maintain expiratory PCO2 (47210A capnometer, Hewlett-Packard Co., Waltham, Mass.) at 30 mm Hg. Arterial blood gases were obtained at regular intervals (158 pH/blood gas analyzer, Corning Medical and Scientific, Medfield, Mass.). Rectal temperature was monitored with an electric thermometer and kept at 37-38°C using an electric heating pad. In most cats, CBF was monitored by a thermal diffusion flow probe (model 6000-01, Flowtronics Inc., Phoenix, Ariz.) as described by Carter and Atkinson placed on the left cortex and covered with bone wax and dental acrylic in a watertight fashion.

The clivus is exposed by blunt dissection between the carotid sheath and the trachea. After division of the superficial transverse vein and the hyoid bone, the trachea and esophagus are laterally displaced by a self-retaining retractor. An adequate airway is maintained by placing the tracheostomy low in the neck. Compression of the carotid artery is avoided, and to the descending vagus nerve the artery is left intact. The longus colli muscles are removed using electrocautery so that the clivus becomes exposed. From this point, an operating microscope is used. All soft tissue is carefully removed from the clivus. The surface is cleaned and dried with gauze, and a thin layer of cyanoacrylate glue is applied. Using a microdrill and microrongeurs, a small rectangular osteotomy in the clivus is made to fit the bottom surface of the window. The dura is opened and excised with microscissors; this is usually possible without cutting the arachnoid membrane around the basilar artery. After secure hemostasis using bipolar cautery, the acrylic window described above is fitted into the opening and kept firmly in place while a small amount of fluid dental acrylic is placed around it.
After hardening of the acrylic cement, the space under the window is filled carefully with artificial CSF.17

Upon completion of the preparation, a Leitz compound microscope with a ×3.8 dry objective (Rockleigh, N.J.) was focused on the basilar artery (Figure 2B). Vessel diameter was measured using a Vickers image splitter (Woburn, Mass.) attached to the microscope. At each time, the width of the basilar artery was measured at three separate points, and the arithmetic mean of these three measurements was taken as the vessel’s diameter at that time. Before the measurements were started, 30–60 minutes were allowed for stabilization of the vessel diameter and other physiologic parameters. Baseline values for CBF, basilar artery diameter, and blood gases were obtained. To assess cerebrovascular reactivity, the cats were hyperventilated to an end-tidal PCO₂ of 18 mm Hg and all measurements were repeated. Next, normocapnia was reestablished and the measurements were repeated once more. Since it was a priori uncertain how the preparation would affect the basilar artery, it was felt inappropriate to use changes in this vessel’s diameter with hypocapnia as a criterion to determine whether overall cerebrovascular reactivity was preserved. Instead, CBF criteria were used, and cerebrovascular reactivity was defined as intact if there was at least a 1.5% change in CBF per torr change in PaCO₂.

Results
Thus far, we have carried out this technique in 29 cats. The duration of the complete preparation averaged 3.5 hours. In eight cats the preparation was considered unsuccessful because of 1) CSF leakage
due to poor adherence of the dental acrylic to the clivus (three cats), 2) severe bleeding from the caudal end of the cavernous sinus due to uncareful drilling in the rostral part of the clivus (two cats), 3) bleeding from an accidentally damaged branch of the basilar artery during opening of the dura (one cat), and 4) bleeding under the window after it had been implanted from the bone edge or from a dural vessel (two cats). Six of these eight failures occurred among the first 10 animals, indicating that inexperience with the technique played a role. In this stage, several modifications to the window were made, resulting in its final design as described above.

In 21 cats serial measurements of basilar artery diameter were obtained; in 19 of these CBF was also monitored. In most animals some degree of local spasm of the basilar artery was usually present upon completion of the procedure, but this resolved spontaneously within 30–60 minutes. Mean±SD baseline basilar artery diameter was 507±85 μm. The standard deviation of repeated measurements of the same vessel under steady-state conditions every 30 seconds during a 5-minute period by two independent observers was 5 μm, independent of vessel size.

The responses of CBF and basilar artery diameter to hyperventilation are shown in Table 1. Mean±SD constriction of the basilar artery with hypocapnia was 12.2±7.6% of baseline. The response of CBF to hyperventilation was greater (mean±SD 2.0±0.4%/torr Paco2), indicating that cerebral vasoreactivity was preserved. There was no quantitative relation between the degree of basilar artery constriction and the reduction in CBF.

In three cats the stability of the preparation was examined by repeated measurement of basilar artery diameter every 45–60 minutes during a 3-hour period under steady-state conditions after normal vasoreactivity to hypocapnia had been established. In all three animals, the basilar artery constricted by 10–15% with hyperventilation, returned quickly to baseline thereafter, and remained stable for the next 3 hours. Thus, the vessel appeared not to be affected by the window.

In eight cats, after 2 or 3 hours an exudative deposit under the window developed, gradually fogging the image and impairing measurements. The occurrence of such a reaction did not bear any relation to the length of the experimental preparation or the presence of any bleeding, nor did it affect basilar artery diameter.

Discussion

One issue that needs to be addressed first is whether a closed window preparation is really necessary for in vivo study of the basilar artery. The rationale behind controlling the perivascular pH with in vivo study of cerebral arteries is that decreases in perivascular pH due to a loss of CO2 from the CSF through the exposed surface causes vasoconstriction and diminished vascular reactivity. The pH sensitivity of larger cerebral arteries has been the subject of controversy, however. According to Kapp et al,13 pH changes of the perivascular bathing fluid would not significantly affect the basilar artery, presumably due to the much thicker arachnoid sheath that surrounds larger arteries compared with smaller arteries and arterioles. Nevertheless, this finding is in contradiction with many other data. Navari et al15 found that the reduction of vasoreactivity in open compared with closed preparations was independent of vessel size. Wahl et al16 also found larger arteries to be pH sensitive but noted that the fluid of lower pH had to penetrate the Virchow-Robin space to attain this effect. In addition, our data showing an approximately 10% decrease in basilar artery diameter with hypocapnia, thus confirming earlier reports,19 support the contention that the basilar artery is pH sensitive since it has been well demonstrated that the reactivity of cerebral arteries to CO2 is mediated via changes in extracellular pH.18,20 Moreover, the observations of Kapp et al13 were made in an open preparation, which in itself may have blunted the vascular responses to local pH variations. One may therefore conclude that with in vivo studies of large cerebral arteries, control of the perivascular pH is required. Previously described methods to control the perivascular environment, such as superfusing with buffered fluid or layering of mineral oil on the exposed surface, do not prevent a decrease in perivascular pH19 and have the additional disadvantage that controlled changes in the perivascular environment cannot be easily introduced.

The described clival window technique is feasible, allows reproducible measurement of the basilar artery with a reasonable degree of accuracy, and does not impair vascular reactivity. Once the window is fixed in place, the basilar artery can be studied for several hours. Changes in the perivascular environment can easily be introduced by flushing through the inlet and outlet ports of the window. In this way the effect of topical applications of vasoactive agents or drugs can be studied.

This technique is suitable for acute experiments only because the invasive nature of the preparation requires maintained anesthesia throughout the experiment, which obviously depresses the responsiveness of cerebral arteries.21 This is a distinct but unavoidable problem inherent to in vivo study of the basilar artery, and certainly the effects of the anesthetics used have

| TABLE 1. Physiologic Measurements Before and After Hyperventilation in Cats |
|---------------------------------|-----------------|-----------------|
| Time                           | Paco2 (mmHg)   | Basilar artery  |
|                                | (n=21)         | diameter (μm)   |
| Before (baseline)              | 31.9±4.0       | 507±85         |
| Hyperventilation               | 21.4±3.2       | 446±92*        |
| After ("normal" ventilation)   | 32.7±2.8       | 511±81         |

Values are mean±SD.

*p<0.01 different from before by paired t test.
to be taken into account. However, anesthetics in the concentrations used in normal surgical anesthesia appear to affect only the magnitude, and not the direction, of the vascular responses.21

Another problem associated with this technique is the development of an exudative deposit after a few hours, which we observed in some cats. Although the nature of this reaction, which is also known to occur with the acute-type cranial window,17 is not fully clear to us and while it did not seem to impair vasoactivity, it has led us to design our experiments such that the observation period is not extended beyond 3 hours.

The experimental preparation is rather lengthy and carries some pitfalls, which may lead to a high failure rate. With the use of microsurgical instruments and bipolar coagulation as well as meticulous attention to certain details, however, this failure rate can be reduced to an acceptable level. As outlined above, the most common problems are bleeding and CSF leakage. A frequent source of bleeding during the surgical preparation in the present series was venous bleeding from the caudal part of the cavernous sinus during drilling of the clivus or due to forced separation of the dura from the bone by the use of excessive bone wax. The chance of bleeding under the surgical preparation in the present series was considerable.

Leakage of CSF may be prevented by the application of a thin layer of cyanoacrylate glue onto the clivus, and may contribute to new investigations in this nature of this reaction, which is also known to occur with acute-type cranial window,17 is not fully clear to us and while it did not seem to impair vasoactivity, it has led us to design our experiments such that the observation period is not extended beyond 3 hours.

The experimental preparation is rather lengthy and carries some pitfalls, which may lead to a high failure rate. With the use of microsurgical instruments and bipolar coagulation as well as meticulous attention to certain details, however, this failure rate can be reduced to an acceptable level. As outlined above, the most common problems are bleeding and CSF leakage. A frequent source of bleeding during the surgical preparation in the present series was venous bleeding from the caudal part of the cavernous sinus during drilling of the clivus or due to forced separation of the dura from the bone by the use of excessive bone wax. The chance of bleeding under the surgical preparation in the present series was considerable.

Leakage of CSF may be prevented by the application of a thin layer of cyanoacrylate glue onto the clivus, and may contribute to new investigations in this nature of this reaction, which is also known to occur with acute-type cranial window,17 is not fully clear to us and while it did not seem to impair vasoactivity, it has led us to design our experiments such that the observation period is not extended beyond 3 hours.

acknowledgments

The authors thank Jana Dunbar for making the photographs and Amy Richardson for the technical assistance.

references

8. Tamaki K, Heistad DD: Response of cerebral arteries to sympathetic stimulation during acute hypertension. Hypertension 1986;8:911-917
19. Faraci FM, Mayhan WG: Role of large arteries in regulation of blood flow to brain stem in cats. J Physiol (Lond) 1987;387:115-123

key words • animal models • basilar artery • cats
Description of a closed window technique for in vivo study of the feline basilar artery.
G J Bouma, J E Levasseur, J P Muizelaar, R G Wiatt and H A Kontos

doi: 10.1161/01.STR.22.4.522

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/22/4/522

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/