Transoral, transsphenoidal microsurgical exposure of the pituitary gland and infundibulum in the rhesus monkey

Description of operative technique

GEORGE T. TINDALL, M.D., JOHN PATTON, M.D., AND JIMMY D. NEILL, PH.D.

Department of Surgery, Division of Neurosurgery and Department of Physiology, Emory University School of Medicine, Atlanta, Georgia

A transoral, transsphenoidal microsurgical technique for exposing the pituitary stalk and gland of the rhesus monkey is described and illustrated. The technique has proved to be a safe and practical method for performing a variety of pituitary operations in a total of 37 animals. The procedures have included complete hypophysectomy, posterior lobe resection, pituitary stalk section, collection of portal venous blood, collection of pituitary venous blood, and placement of lesions in the organum vasculosum of the lamina terminalis.

KEY WORDS • pituitary • hypophysectomy • pituitary stalk • hypophyseal portal system

In an effort to determine the concentration of luteinizing hormone releasing hormone (LRH) in the long portal venous system and to correlate these concentrations with simultaneously determined plasma luteinizing hormone (LH) concentrations in the female rhesus monkey during menstrual cycles, a transoral, transsphenoidal microsurgical approach to the pituitary and infundibulum was developed in our laboratory. This operative technique has provided a safe and practical method for performing a variety of surgical procedures in and about the pituitary gland of the rhesus monkey, including complete hypophysectomies, posterior lobe resections, pituitary stalk section with insertion of a Silastic barrier, collection of portal venous and pituitary venous blood, and placement of lesions in the organum vasculosum of the lamina terminalis (OVLT).

The purpose of this report is to describe and illustrate the operative technique. The endocrine results of our investigations have been reported elsewhere.

Operative Method

Transpalatal Transsphenoidal Approach

Because of the depth and the small size of the operative field, the operating microscope is essential for the performance of this operative procedure. We used a Zeiss operating microscope with a × 20 eyepiece and a 225 mm lens. Additional illumination was provided by a Wehmerlite Fiber optic
FIG. 1. Position of rhesus monkey in special head holder with mouth retractor in place. An oral endotracheal tube is shown on the left side of the animal's mouth. The skull is rigidly fixed to the four threaded rods, each of which is tipped with a small pointed stainless steel pin that penetrates the scalp and outer table of the skull. By tightening these rods, the head is held immobile, an essential step in the operative approach since considerable magnification is used throughout the operation.

FIG. 2. Midline sagittal section of the head of a rhesus monkey to illustrate diagrammatically the transoropharyngeal, transsphenoidal operative approach to the pituitary gland and stalk. The synchondrosis, which lies immediately anterior to the floor of the sella turcica and is illustrated just above the arrow, is a valuable landmark as it guides the operator to the floor of the sella. Inset: An enlargement of the pituitary gland within the sella turcica. The portal venous system on the anterior lobe and the clear line of demarcation between posterior and anterior lobes can be seen.

Surgical anesthesia is obtained using phencyclidine hydrochloride (Sernylan) intramuscularly in a dosage of 5 mg/kg for induction and 1 to 2 mg/kg/hour for maintenance. Atropine sulfate (10 µg/kg) is administered intramuscularly with the initial dose of anesthetic to decrease upper airway secretions and to stabilize the myocardium against a vaso-vagal reflex. After induction of anesthesia, a cuffed pediatric endotracheal tube is inserted, secured to the left side of the mouth by a suture, and left in place throughout the operative procedure. Since Sernylan does not depress respiration, mechanical ventilation is not necessary. An infusion of lactated Ringer's solution is made into a saphenous vein throughout surgery at a rate of 2 ml/kg/hour. Dexamethasone sodium phosphate (4 mg) and sodium methicillin (25 mg/kg) are administered intravenously at the beginning of surgery and at 6-hour intervals.

The animal's head is placed in a specially constructed four-point head-holder (Fig. 1) with the neck slightly extended (10° to 20°) and the head rotated toward the operator. A self-retaining retractor is placed inside the upper and the lower incisors and the mouth opened as widely as possible to allow access to the posterior oropharynx. The transpalatal, transsphenoidal operative approach is illustrated in Fig. 2, which represents a mid-sagittal section through the female rhesus monkey's head, and Fig. 3, which shows several steps in the operation.

The rugated surface of the maxillary mucosa merges posteriorly with the smooth mucosa with salivary gland ducts over the posterior portion of the hard and soft palate. The posterior border of the hard palate, which is formed by the premaxillary and palatine bones (Fig. 3 A), is easily palpated. The median palatine suture separates the maxillary and palatine bones of each side.

Using the cutting cautery (Bovie), a midline mucosal incision is made from the level of the second premolar anteriorly to the posterior edge of the hard palate. The horizontal plates of the two palatine bones

*Wehmerlite Fiber optic source manufactured by B. F. Wehmer, Medical Instrument Division, Inc., P.O. Box 1687, Lexington, Kentucky.
Pituitary surgery in monkeys

form a pointed posterior nasal spine. The mucosal incision is continued 1.5 cm posterior to the spine, taking care to remain in the midline since a laterally placed incision will enter the posterior nares.

The mucosa is undermined subperiosteally and reflected laterally until the medial edge of the greater palatine foramen is exposed. The nerves and blood vessels to the buccal mucosa and gingival margin pass through this foramen. This neurovascular bundle is cauterized and divided. A No. 23 needle inserted into each of these foramina and held laterally with a rubber band provides satisfactory retraction of the mucosa of the hard palate.

The exposed portion of the hard palate is drilled to less than 0.5 mm in thickness. Further removal of the bone is accomplished with a small bone curette carrying the removal laterally to the greater palatine foramen and medially to the nasal septum. With the aid of a small double-ended dissector, the nasal mucosa is dissected off the nasal septum and vomer. The nasal septum is removed with a small rongeur. Next, the nasal mucosa of each side is retracted away from the midline and held in place by No. 23 needles secured in the sphenoid bone and retracted laterally. This exposes the ventral surface of the sphenoid bone.

The sphenoid bone is drilled with a 2 mm diameter diamond burr in lightly applied strokes, moving slowly in an anterior to posterior direction (Fig. 3 D). Careful, slow drilling in this manner allows identification of the anterior and posterior basisphenoid synchondroses. These are readily recognized because of their light blue-gray color and cartilaginous consistency. Identification of the anterior synchondrosis is especially useful in locating the sella turcica (Fig. 2), as the latter can be exposed by drilling away the bone beginning immediately caudal to this synchondrosis. Extending the bone removal cephalad to the anterior synchondrosis will result in exposure of the optic chiasm, while an approach caudal to the posterior synchondrosis will expose the bifurcation of the basilar artery. Once the anterior and posterior synchondroses have been identified, drilling proceeds through the solid sphenoid bone until the white cortical bone of the floor of the sella turcica is reached. Drilling of the sellar floor continues until it is paper-thin and the dura mater overlying the pituitary gland can be seen clearly through the thinned bone (Fig. 3 E). Removal of the sellar floor is completed with a very fine bone curette. Next, the dura is opened in a stellate fashion and the circular sinus, which is situated immediately anterior to the pituitary gland and within the dura, is coagulated and divided. The cut margins of dura mater are retracted from the pituitary gland by applying the bipolar current to the cut dural edge. This provides good visualization of the light orange-colored anterior lobe and a small portion of the pale, cream-colored posterior lobe (Figs. 3 F and 4). At this point, the investigator has the option of performing a variety of procedures.

Pituitary Venous Blood Collection

Usually two small veins, one on each side, can be identified draining the inferior portion of the adenohypophysis into the corresponding cavernous sinus. A Leitz micromanipulator† is used to place the polyethylene tip of a collection cannula adjacent to a segment of the vein to be cut. After obtaining absolute wound hemostasis, the animal is injected with 3000 units of heparin intravenously, the pituitary vein is cut, and the blood aspirated with the collection cannula attached to a Harvard infusion-withdrawal pump.‡ The rate of withdrawal is adjusted to the rate of bleeding.

Posterior Pituitary Lobe Resection

After removal of the sellar floor, the dura is reflected off the dorsum sellae. The lower portion (4 to 5 mm) of the dorsum sellae is removed by drilling, after which the dura is opened inferiorly and posteriorly to the pituitary gland. With a small, thin retractor which is positioned with the micromanipulator, the anterior lobe is elevated, exposing a well defined cleavage plane between the anterior and posterior lobes. Transection of the posterior lobe is made at the junction of the white, pale posterior lobe with the light gray stalk.

†Micromanipulator made by E. Leitz, Inc., U.S. address: Rockleigh, New Jersey.
‡Harvard infusion-withdrawal pump manufactured by Harvard Apparatus, Inc., 150 Dover Road, Millis, Massachusetts.
FIG. 3. Steps in the operative procedure for exposure of the pituitary gland and stalk. A: After making a midline incision, the mucosa is retracted laterally, exposing the hard palate. B: The hard palate is carefully drilled away with small cutting burrs. C: The hard palate has been removed exposing the midline thin nasal septum of bone, and the intact nasal mucosa which is reflected and held laterally as described in text. D: The nasal septum has been removed and the sphenoid is drilled away with small diamond-tipped burrs. The anterior synchondrosis shown running transversely immediately above the burr aids in localization of the sella turcica. E: The sella floor has been removed exposing the dura mater over the pituitary gland. The midline dashed line indicates the dural incision. The slightly darker shaded area running transversely in an inverted V-shape indicates the circular sinus. F: The dura over the gland has been opened and the edges retracted by applying the bipolar coagulation forceps. The anterior lobe (A) can be clearly identified and separated from the posterior lobe (P). The dura containing the circular sinus has been divided and coagulated. The midline dashed line indicates the line of incision through the diaphragma sellae which is opened to expose the full length of the stalk and median eminence. G: The pituitary gland has been removed, the diaphragma sellae opened down to the point of entry of the stalk and median eminence. At this point, the collection cannula can be fitted over the stalk with a micromanipulator for collection of portal venous blood.

Pituitary Stalk Section

The stalk is exposed adequately by retracting the anterior lobe inferiorly and opening the diaphragma sellae. After transecting the stalk with fine microscissors, a small square of Silastic sheet (Durafilm) can be inserted between the cut end of the stalk and the gland to prevent regeneration of the portal venous system into the adenohypophysis.

Complete Hypophysectomy

Hypophysectomy in the rhesus monkey is similar to the procedure in humans. The gland is retracted inferiorly and the stalk divided as described above. The gland is then freed of its attachments laterally and inferiorly. A fine blunt dissector separates the gland from the sella and allows its removal intact, insuring a complete hypophysectomy.
Pituitary surgery in monkeys

FIG. 4. Transsphenoidal exposure of the rhesus monkey pituitary gland. After opening the dura mater (D.M.), the edges can be retracted by applying bipolar coagulation. Portal veins (P.V.) are visualized on the anterior lobe of the pituitary (A.P.). The clear demarcation between anterior lobe and posterior lobe (P.P.) is much sharper than it appears in this illustration. A pituitary vein (Pit. V.) is shown draining the anterior lobe into the cavernous sinus.

Portal Venous Blood (Stalk) Collection

The pituitary gland is freed of its attachments to the dura of the lateral walls of the sella turcica. Retraction of the pituitary inferiorly as described above exposes the stalk as it pierces the diaphragma sellae. Exposure of the entire stalk requires division of the anterior portion of the diaphragma sellae. Dissection of the arachnoid away from the stalk with angled micropicks exposes the portal veins coursing down the stalk. Branches of the superior hypophyseal arteries supplying the area of the median eminence can also be visualized. It is important to spare these branches as they are responsible principally for supplying the portal venous system. Other small arterial branches situated in the arachnoid membrane and also arising from the superior hypophyseal arteries can be identified running across the surface of the diaphragma sellae to join the inferior stalk and enter the sella turcica to supply the pituitary gland. It is essential to identify, coagulate, and divide these vessels as this prevents inadvertent contamination by arterial blood during the period that portal venous blood is being collected from the distal stalk. Cerebrospinal fluid accumulating around the infundibulum is removed by a separate small polyethylene catheter connected to suction.

After the stalk is exposed and wound hemostasis complete, the animal is heparinized and the stalk divided at its junction with the gland and the gland removed. A polyethylene tube with the end flared is then manipulated over the distal end of the bleeding stalk. The micromanipulator which holds the collection cannula is used to guide the cannula precisely over the cut stalk. Gentle suction on the cannula with a Harvard infusion-withdrawal pump (rate: 19 to 30 µl/min) will usually yield a satisfactory collection of blood.

Organum Vasculosum of the Lamina Terminalis Lesioning

The organum vasculosum of the lamina terminalis (OVLT) is situated immediately anterior to the optic chiasm and between the distal A-1 segments (Fig. 5) of the anterior cerebral arteries just before the latter join to form a common distal anterior cerebral artery.

Our research interest in the OVLT was stimulated by the previous demonstration of a high concentration of LRH in this structure. The OVLT is exposed by drilling through the sphenoid beginning at the anterior synchronodrosis (Fig. 2), which marks the anterior level of the sella turcica, and extending forward for a distance of 1.0 cm. The dura mater is opened and the anterior edge of the chiasm visualized. The distal A-1 segments of the anterior cerebral arteries pass forward to form a single anterior cerebral artery (Fig. 5). In the living animal, the OVLT is clearly identified as a rich vascular tuft. Lesioning is performed by cauterizing the OVLT with the bipolar forceps.
Wound Closure

Following completion of the surgical procedure, a small strip of Gelfoam and muscle removed from the thigh and soaked in antibiotic solution is placed over the sellar area. Surgicel is placed over the muscle and bonded to the surrounding tissue with one or two drops of cyanoacrylate. The nasal mucosa is allowed to fall back in place. The oral mucosa is reapproximated using interrupted 3-0 chromic catgut sutures. When closure is complete, a single sheet of Surgicel is placed over the suture line and sealed to the mucosa by the application of cyanoacrylate. The animal is then allowed to recover spontaneously from the effects of the Sernylan.

The endotracheal tube is removed when the animal begins making purposeful attempts to remove the tube.

Results

The operative approach described in this report has been used in a variety of different laboratory investigations of the pituitary gland in rhesus monkeys. Table 1 summarizes the various procedures and the results in terms of survival.

It should be emphasized that the purpose of all of these investigations was not necessarily long-term survival. The high surgical mortality of the procedure in those animals that had pituitary stalk blood collection carried out is not related to the operative technique but rather to the long duration of the operation (12 to 18 hours) and the extensive manipulation of the infundibulum during

Fig. 5. Transsphenoidal exposure of the organum vasculosum of the lamina terminalis (OVLT). Left: Anatomic specimen demonstrating the relationship of the OVLT to the optic chiasm and anterior cerebral arteries. OC: optic chiasm; ME: median eminence; ACA: anterior cerebral artery; AP: anterior lobe of the pituitary; CA: internal carotid artery. Right: Exposure of the OVLT and the anterior cerebral arteries at the site of junction of the distal A-1 segments to form a single anterior cerebral artery (ACA).

§Available in local hardware stores as Krazy-Glue or Super-Glue.
Pituitary surgery in monkeys

<table>
<thead>
<tr>
<th>Type of Operation</th>
<th>No. of Animals</th>
<th>No. of Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>pituitary stalk blood collection</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>selective posterior lobectomy</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>destruction of organo-vascularus of lamina terminalis (OVLT)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>sham OVLT lesion</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>pituitary venous blood collection</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>pituitary stalk section</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td><strong>37</strong></td>
<td><strong>20</strong></td>
</tr>
</tbody>
</table>

stall blood collection. In those 16 animals that had brief procedures carried out using this same approach (Groups II–VI, Table 1), 14 survived. The two deaths were both anesthesia deaths that occurred early in our series.

### Discussion

Several investigators have described various methods for hypophysectomy in rhesus monkeys. Among the first were Smith and Knobil, et al., who used a parapharyngeal approach through a lateral neck incision to reach the sphenoid bone and then the pituitary gland. Although this approach is entirely adequate, it does require a tracheostomy and it seems to us more cumbersome than the direct, shortest approach through the open mouth.

A subtemporal approach to the rhesus pituitary was described by Roth, who used the technique in a total of 28 animals. This procedure requires a craniotomy and does not provide optimum exposure of the stalk and gland. This method does not ensure completeness of pituitary ablation. More recently, Shintani, et al., have described a transorbital operative technique. This requires an orbital enucleation and drilling off of the medial orbital wall, which exposes the pituitary stalk from above as it pierces the diaphragma sellae. Refinements in this method have been described by Carmel, et al., but it remains a formidable procedure.

We believe that the technique described in this report is a relatively safe method for gaining access to the monkey pituitary gland. It requires minimal tissue destruction and no retraction of brain tissue. A portion of the hard palate, posterior nasal septum, and sphenoid bone are removed. Furthermore, it allows manipulation of the pituitary gland with minimal risk of injury to the pituitary stalk or hypothalamus. Finally, it provides an animal model for microsurgical experience that closely simulates the transsphenoidal microsurgical approach to the human pituitary.

### References


Address reprint requests to: George T. Tindall, M.D., Section of Neurological Surgery, Emory University Clinic, 1365 Clifton Road, N.E., Atlanta, Georgia 30322.