Improved method of canine decerebration

MISLAV TONKOVIC-CAPIN, MIRKO KROLO, ECKEWARD A. E. STUTH, FRANCIS A. HOPP, AND EDWARD J. ZUPERKU
Zablocki Department of Veterans Affairs Medical Center, and Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53295

Tonkovic-Capin, Mislav, Mirko Krolo, Eckehard A. E. Stuth, Francis A. Hopp, and Edward J. Zuperku. Improved method of canine decerebration. J. Appl. Physiol. 85(2): 747–750, 1998.—We describe an improved decerebration method for dogs that is suitable for studies of brain stem neurons in the absence of anesthesia. Previously reported techniques of canine decerebration often lead to respiratory and hemodynamic instability and lack of typical decerebrate rigidity. We have developed a precise, visually controlled, midcollicular brain stem transection technique that overcomes these problems. Our method results in only moderate blood loss while preserving carotid and basilar artery circulations. Consistent levels of brain stem transection routinely lead to stable postdecerebration hemodynamic parameters, allowing prolonged brain stem neuronal recordings. The same model should also be useful for a variety of studies involving other physiological systems in dogs in the absence of anesthesia and for studies of anesthetic effects.

dogs; brain stem neurons; respiratory neurons; midcollicular decerebration; decerebrate rigidity

Decerebration is the separation of the cerebral cortex and diencephalon from the lower brain stem and spinal cord by dissection of neural pathways between the red nuclei in the midbrain and the vestibular nuclei of the medulla (Fig. 1, top left). Decerebration is usually performed by midcollicular midbrain transection and leads to an almost immediate decerebrate rigidity (7) because of the unopposed excitatory effect of the vestibulospinal and pontine reticulospinal neurons on motor neurons that innervate the extensor muscles of the limbs.

Decerebrate animals are poikilothermic. However, vital centers in the pons and medulla remain functional, and most respiratory and circulatory reflexes are preserved. All conscious sensation is abolished, and the responsiveness to nociceptive stimuli is depressed. Therefore, the animals can be studied in the absence of anesthesia.

Despite a multitude of biological studies in dogs, there are few reported techniques for decerebration (1, 2, 6, 8). Commonly reported problems include lack of typical decerebrate rigidity, hemodynamic instability, excessive blood loss, air embolism, brain edema, and premature mortality.

The objective of this study was to obtain a hemodynamically stable, anatomically and functionally well-defined decerebrate canine preparation that overcomes the shortcomings of previous methods and allows for long, stable recordings from single respiratory brain stem neurons. The absence of general anesthesia allows for an anesthetic-free control state in studies of anesthetic effects on neurotransmission. The same decerebration model can also be used in a wide range of studies of other physiological systems in dogs without need for anesthesia, thus avoiding extrapolation of results from decerebrate animals of other species that could lead to erroneous conclusions.

METHODS

This research was approved by the Medical College of Wisconsin Animal Care Committee and conformed with standards set forth in the National Institutes of Health Guide for Care and Use of Laboratory Animals. Experiments were performed on eight adult mongrel dogs (weight 8–15 kg) that were used for medullary respiratory neuronal studies. Inhaled halothane anesthesia was induced, and the trachea was intubated with a cuffed tube. The lungs were mechanically ventilated with a 1.1–1.6% halothane concentration during surgery (minimum alveolar concentration in dogs; ~0.9%). Airway CO2 and halothane concentrations were continuously recorded with an infrared analyzer (POET II, Criticare Systems). The animals were monitored for signs of inadequate anesthesia, such as salivation, lacrimation, and/or increases in blood pressure (BP) and heart rate (HR). The depth of anesthesia was increased immediately if such signs were present.

The femoral artery was cannulated for continuous BP monitoring (Gould-Statham P231D transducer) and periodic arterial blood-gas sampling. A triple-lumen catheter was placed in the femoral vein for administration of drugs and for continuous infusion of NaCl solution (0.9%) at 5–10 ml·kg^-1·h^-1 as a maintenance fluid. In addition, 3 ml of the solution were infused to replace each milliliter of estimated blood loss. The animals were initially paralyzed with a bolus (0.1 mg/kg iv) of pancuronium bromide (Pavulon). Dexamethasone (0.25 mg/kg iv) was given prophylactically every 6 h to minimize potential brain stem edema related to surgical trauma. Esophageal temperature was measured and was maintained within a target range of 37.5–38.5°C with a servo-controlled heating pad. The dogs were positioned in a Kopf (model 1530) stereotaxic apparatus. Bilateral dorsolateral neck dissections were performed to obtain recordings of phrenic nerve activity subsequent to bilateral cervical vagotomy. An occipital craniotomy was created to expose the
dorsal surface of the medulla for the respiratory neuronal studies.

Decerebration. After a midline skin incision was made from the spinal process of the axis to the nasion, the muscles and underlying connective tissue were cut along the external sagittal and nuchal crests, dissected bilaterally, and reflected to expose the parietal bones from the midline to 4 cm laterally and from the nuchal crests to 4.5 cm rostrally. A high-speed drill with burr was used to drill the margins of exposed parietal bones to form 3.5-cm-long × 3.5-cm-wide bilateral craniotomy windows (Fig. 1, top right). These windows were placed ~0.5 cm lateral from the sagittal crest and ~1.0 cm rostral from the nuchal crests to avoid disruption of the dorsal sagittal and transverse venous sinuses. Rongeur forceps were used to complete the craniotomy. Bone wax was applied to the cut bony edges to stop bleeding and to prevent potential air embolism. The dura was removed with scissors to expose the parietal and occipital lobes bilaterally. A bipolar coagulator (model 0441010; Burton Division, Cavort) with insulated, bayonet forceps (90-mm shaft, 2-mm tips; Edward Week) was used to coagulate small volumes of brain tissue. Coagulated tissue was suctioned with a tonsillar suction tip. Intermittent saline irrigation and gentle retraction of the medial surface of the cerebral hemisphere and the caudal surface of the occipital lobe by a blunt spatula were used as necessary to better visualize the brain tissue. With the sequential coagulation and suctioning of small amounts of the brain tissue, the caudal portions of the parietal and temporal lobes and the occipital lobe were removed bilaterally to expose the midbrain region (Fig. 1, bottom right).

Cotton-tipped applicators were used for blunt dissection of midline brain structures under the falx and above the brain stem to expose the great cerebral vein and its main branches. The vein was occluded with two metal clips and then transected to avoid accidental tearing, which could lead to air embolism. The applicators were also used to identify the midcollicular line, which runs just in front of the vermis and the commissure of the inferior colliculi. The origins of the oculomotor nerves were identified bilaterally on the ventral surface of the rostral mesencephalon by using the applicator to gently elevate (2–3 mm) the brain stem.

To facilitate brain stem transection in the desired plane, the edge of a spatula was placed by the side of the brain stem, from just behind the origin of oculomotor nerve ventrally and tilted toward the midcollicular line dorsally, to serve as a guide for the scalpel. The last few millimeters of the ventral
brain stem were cut gently to avoid damage to the cavernous sinuses. This step usually led to arterial bleeding from the cut caudal communicating arteries on the ventral edges of the transected brain stem, 2- to 4-mm rostral from their connection to the basilar artery and the pons. Bleeding was stopped by gentle bipolar coagulation and gauze packing. An absorbable hemostatic material [oxidized regenerated cellulose (Surgicel); Johnson & Johnson Medical] and saline-soaked gauze were gently placed on the transected brain stem and brain surfaces. After the decerebration procedure was completed, halothane was discontinued, and the dogs were tested for the presence of decerebrate rigidity before any additional neuromuscular blockade with pancuronium, which was given to avoid motion artifacts related to respiratory pump muscles during respiratory neuronal recordings.

Recordings. BP, HR, moving time average of phrenic nerve activity (phrenic neurogram), and concentrations of airway CO₂ and halothane were recorded on a Grass model 7D polygraph. Data were collected for analysis of cardiopulmonary stability during steady-state conditions just before the start of brain surgery (−1 h), at completion of the brain stem transection (0 h), 1 h after decerebration (1 h), and every 1.5 h thereafter. Postdecerebration data, during subsequent halothane exposures to investigate anesthetic effects on respiratory brain stem neurons, were excluded from the stability analysis. A minimum of 30 min was allowed for halothane washout before data collection. A repeated-measures, one-way ANOVA was used to test the significance of time-dependent changes after the decerebration procedure and halothane washout. P values < 0.05 were considered statistically significant. Values are expressed as means ± SE.

RESULTS

The duration of the decerebration procedure was 1 ± 0.1 h. Estimated total blood loss during decerebration was −10 ml/kg of body weight (range: 7–15 ml/kg). Predecerebration mean values (at −1 h) for BP, HR, and respiratory rate (RR) were 84 ± 6 mmHg, 116 ± 3 beats/min, and 15 ± 2 breaths/min, respectively, during 1.1% end-tidal (ET) halothane anesthesia at an ET P CO₂ (P ET CO₂) of 41 ± 3 Torr (Fig. 2). The immediate postdecerebration values (at 0 h) for BP, HR, and P ET CO₂ were not significantly changed (the phrenic neurogram was not recorded during and immediately after decerebration). At 2.5 h postdecerebration, when halothane washout was complete and P ET CO₂ averaged 57 ± 3 Torr, as prescribed by our neuronal study protocol, BP and HR had increased to 126 ± 8 mmHg and 174 ± 11 beats/min, respectively. Statistical comparisons of mean values relative to those at 2.5 h indicated that these parameters remained unchanged (P > 0.05; Fig. 2, top, time period C). RR decreased from 15 ± 2 breaths/min predecerebration to 8 ± 1 breaths/min at 2.5 h. Four hours after decerebration, RR increased, relative to the

![Fig. 2](http://jap.physiology.org/)

**Fig. 2.** Individual (numbered thin lines) and mean data (thick lines) for blood pressure (top left), heart rate (top right), respiratory rate (bottom left) and end-tidal CO₂ partial pressure ([P ET CO₂], bottom right) before (−1 h; A) and after decerebration procedure (0–12 h; B and C). ETHAL, end-tidal halothane. *P < 0.05 during steady-state period (C) relative to values at 2.5 h.**
Decerebrate rigidity was observed in all seven dogs tested. No significant changes in BP, HR, RR, or motor responses were noted during clamping of the paws or cheek skin after decerebration and complete halothane washout. The brain stem appeared to be without edema or herniation, and arterial pulsations were markedly diminished compared with nondecerebrate animals, allowing for stable respiratory unit recordings, typically for up to 10 h postdecerebration. After completion of the respiratory neuronal study protocols, the dogs were killed by intravenous bolus of KCl solution. The brain stems were removed for confirmation of the transection level. The precise locations of the transections were verified histologically, as illustrated by the representative example of a paramedian sagittal section at the level of the inferior colliculus (Fig. 1, bottom left).

DISCUSSION

In previously described decerebration methods, the transection levels were only partially visualized or assumed on the basis of empirical transection angles, and the actual planes were determined postmortem. These methods commonly resulted in cardiorespiratory instability and/or lack of typical decerebrate rigidity. Our method of decerebration with isolation and visualization of the mesencephalon allows for precise transection at the desired level, leading to consistent results.

Small anatomic differences in the transection level of decerebration can profoundly affect postural changes (5). In addition, if parts of the hypothalamus are included caudal to the transection plane, the animals are capable of producing a ragelike reaction, i.e., rapid panting, urination, defecation, and large increases in arterial pressure and HR. These results can occur spontaneously or can be triggered by mild tactile or other stimuli (3, 4). We observed hyperdynamic cardiovascular and respiratory changes consistent with a ragelike reaction during our pilot decerebration studies without direct visualization of the midcollicular line and oculomotor nerve origins. In these pilot studies, macroscopic examination of the brain stems revealed that the transection plane frequently went through the superior colliculi dorsally and/or the mamillary bodies ventrally, leading to a lack of decerebrate rigidity because of inclusion of the red nuclei and/or cardiorespiratory instability when parts of hypothalamus, such as the mamillary bodies, were included. These results illustrate the importance of direct visualization of both the midcollicular line at the dorsal surface and also the space between the pons and the origin of the oculomotor nerves at the ventral surface of the brain stem.

BP and HR did not significantly change during or immediately after decerebration (from −1 to 0 h). These parameters increased until 2.5 h postdecerebration, most likely because of the combined effects of halothane washout and a deliberate increase in PETCO2 to ~60 Torr, as required by our neuronal study protocol. At 2.5 h and thereafter, BP and HR remained stable until the end of the experiments. RR decreased at 1–4 h after decerebration but then increased significantly until 10 h postdecerebration. The initial decrease in the rate was probably caused by a combined effect of decerebration and withdrawal of halothane anesthesia. The later increase in the rate may partially be caused by a small, gradual, but statistically not significant, linear increase in body temperature from 38.1 ± 0.5 at 2.5 h to 38.9 ± 0.5°C at 10 h postdecerebration. This gradual increase in temperature is likely caused by loss of thermoregulation, as a result of decerebration, in conjunction with a warm laboratory environment used as an aid in controlling body temperature.

Our improved method of decerebration has the following advantages. 1) The brain stem is transected consistently in the desired midcollicular plane. 2) Cardiopulmonary parameters remain relatively stable over long periods of time. 3) Decerebrate rigidity is routinely present. 4) Ragelike reactions are not observed. 5) The carotid circulation is preserved, which allows chemoreceptor and baroreceptor reflexes to be studied. 6) The dural sinuses and basilar artery circulations are preserved. 7) Blood loss is only ~10 ml/kg of body weight, which is <15% of the animal’s blood volume. 8) The perioperative mortality rate is low (0 of 8 dogs). 9) Brain stem swelling is absent, allowing prolonged neuronal recordings from the brain stem. 10) The brain structures rostral to the transection, including diencephalon and pituitary gland, may be studied because they are not removed and their circulation is preserved.

The authors are grateful to Jack Tomlinson for expert surgical assistance and thank Sarah A. Botsford and Mary J. Henning for generous help in preparing the histological sections.

This work was supported by the Department of Veterans Affairs Medical Research Funds and the Department of Anesthesiology of the Medical College of Wisconsin, Milwaukee, WI.

Address for reprints requests: E. J. Zupenko, Research Service 151, Zablocki VA Medical Center, Milwaukee, WI 53295 (E-mail: ezupenko@mcw.edu).

Received 9 December 1997; accepted in final form 31 March 1998.

REFERENCES


