Cerebral Blood Flow and Metabolism following Pancuronium Bromide in Newborn Lambs

JAQUES BELIK, L. CRAIG WAGERLE, AND MARIA DELIVORIA-PAPADOPOULOS

*Departments of Physiology and Pediatrics, University of Pennsylvania School of Medicine,
Philadelphia, Pennsylvania 19104*

ABSTRACT. The purpose of this study was to evaluate cerebral blood flow and metabolism following pancuronium bromide paralysis in healthy newborn lambs. Cerebral blood flow and cerebral metabolic rate for O_2 and glucose were measured along with blood pressure and blood gases before and again at 15 and 60 min following pancuronium paralysis in seven newborn lambs. Pancuronium bromide paralysis had no effect on any of these parameters either at 15 or 60 min of paralysis. Total cerebral blood flow, cerebral metabolic rate for O_2 , and cerebral metabolic rate for glucose were 87 ± 6 ml/min/100 g, 258 ± 10 μ mol O_2 /min/100 g, and 53 ± 10 mmol glucose/min/100 g, respectively. Neither was any change in regional cerebral blood flow noted. In spite of being connected immediately to the ventilator, however, some animals experienced a transient increase (average = 32%) in blood pressure, that was not associated with an increase in end tidal CO_2 . The data suggest that pancuronium paralysis in healthy awake newborn lambs does not lead to any alteration in cerebral blood flow or metabolism. (*Pediatr Res* 18:1305-1308, 1984)

Abbreviations

CBF, cerebral blood flow
CMRO₂, cerebral metabolic rate for oxygen
CMR_{glucose}, cerebral metabolic rate for glucose
IVH, intraventricular hemorrhage

Neuromuscular blocking agents are used in infants in neonatal intensive care units to induce respiratory muscle paralysis. Among the nondepolarizing muscle relaxants, pancuronium bromide is the most commonly used agent, based on fewer side effects following its administration. A recent study, however, in preterm infants reported an increase in cerebral blood velocity measured by Doppler technique following paralysis with curare or pancuronium bromide (Pavulon) (9). Inasmuch as derangement of cerebral blood flow appears to be linked to the pathogenesis of intracranial hemorrhage encountered in the human premature brain (8), there is reason to postulate a relationship between cerebral blood flow changes following the use of skeletal muscle relaxants and intracranial hemorrhage in neonates.

While the effect of muscle relaxants upon the cerebrovascula-

ture of newborn animal models has not been studied, it has been shown in adult rats that there is an increase in cerebral blood flow and cerebral oxygen consumption following paralysis (1). The purpose of this study, therefore, was to evaluate the cerebral blood flow and metabolism following pancuronium bromide paralysis in healthy newborn lambs.

MATERIALS AND METHODS

Animal preparation. Studies were performed on newborn lambs less than 1 wk of age. Two to 3 days prior to the experiment, ketamine HCl, 10 mg/kg, was administered intramuscularly and additional 10-mg doses were given as necessary during the surgical procedure. Following subcutaneous infiltration with lidocaine HCl (1%), polyvinyl catheters (No. 5 French) were placed into the inferior vena cava for the administration of fluid and drugs, into the left brachial and femoral arteries for monitoring blood pressure and withdrawal of reference blood samples, and directly into the left cardiac ventricle through the femoral artery for injection of microspheres. Sagittal sinuses were chronically catheterized with a 22-gauge stainless steel cannula with an over-the-needle Teflon catheter.

Experimental protocol. On the day of the study, the animals were fed as usual. The larynx was visualized with the aid of a laryngoscope and an endotracheal tube was advanced and secured with tape to the mouth. The lambs were placed in a sling, padded, and wrapped for warmth and allowed to rest for approximately 1 h after all the catheters had been checked for patency. During this period, all animals became accustomed to the new environmental conditions and did not appear to be distressed by the endotracheal intubation. Following this period and after initial control measurements (0 min) were obtained, pancuronium bromide (Organon, W. Orange, NJ; 1 mg/ml), 0.1 mg/kg, was infused into the inferior vena cava and the animal immediately was connected to a mechanical ventilator.

A slow intravenous infusion of pancuronium bromide, 0.1 mg/kg/h, in normal saline (10 ml/h) was started following the initial bolus injection. Continuous monitoring of the end tidal CO_2 allowed us to adjust the ventilator settings to maintain $Paco_2$ unchanged from the initial values. The fractional inspired oxygen concentration was adjusted to maintain the PaO_2 constant. Animals were handled in accordance with the recommended actions for the Guiding Principles in the Care and Use of Animals, Department of Health, Education, and Welfare.

Arterial blood pressure, sagittal sinus pressure, and rectal temperature were continuously recorded on a Grass polygraph. End tidal CO_2 was measured throughout the experiment. Arterial and sagittal sinus blood pressures were measured with Statham P23 Db pressure transducers. Rectal temperature was monitored with a model 43 TA telethermometer (Yellow Springs Instruments, Yellow Springs, OH) and end tidal CO_2 was measured by an

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Requests for reprints should be addressed to: Jaques Belik, M.D., c/o Maria Delivoria-Papadopoulos, M.D., Department of Pediatrics, Hospital of the University of Pennsylvania, 2nd Floor Maloney Building, 3400 Spruce Street, Philadelphia, PA 19104.

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infrared CO₂ analyzer (LB2, Beckman Instruments, Fullerton, CA) sampling from the endotracheal tube.

Simultaneous arterial and sagittal sinus blood samples (1.5 ml) were obtained together with each CBF measurement at control (0 min), 15 min, and 60 min following pancuronium paralysis. Blood samples were analyzed in duplicate for pH, PaO₂, and PaCO₂ by conventional electrodes (Radiometer, London Co., Cleveland, OH). Oxygen content was determined with the use of a micro-oxygen content analyzer (Lex-O₂-Con-K, Lexington Instruments Corp.). Serum glucose was measured in duplicate in a glucose analyzer (Beckman Instruments, Fullerton, CA). In addition, microhematocrits were obtained following each measurement in a standard microcentrifuge. CMRO₂ and CMR_{glucose} were calculated as the product of cerebral blood flow and the respective arteriovenous difference for each substrate.

Measurement of cerebral blood flow. Blood flow to the brain was measured by injection of microspheres (5), 15- μ m diameter, into the left ventricle over a period of 15–30 s. The microspheres were labeled with ¹⁴¹Ce, ⁸⁵Sr, and ⁴⁶Sc (3M Co., St. Paul, MN). Stock solutions of microspheres were sonicated for 5 min and shaken vigorously on a Vortex mixer for 2–4 min. Approximately 0.7 to 0.9 million microspheres were placed in specially prepared injection vials (5) and shaken on the Vortex mixer prior to injection. The vial containing the microspheres was attached to the left ventricular catheter and the contents injected by flushing 3–5 ml of warmed saline solution through the vial. For 10 s prior to, during, and 60 s after the injection of microspheres, reference arterial blood samples were withdrawn from the left brachial artery into heparinized glass syringes.

At the end of each experiment, the animal was killed with an intravenous administration of euthanasia solution (T61, American Hoechst) and the brain was removed and sectioned into right and left cerebrum, cerebral gray matter, cerebral white matter, caudate nucleus, hippocampus, thalamus-hypothalamus, mid-brain, cerebellum, pons, and medulla. Each brain region was weighed and placed into counting vials. The counting vials were filled with tissue to a height of less than 2.5 cm to minimize geometrical counting error. Reference blood samples were placed in glass counting vials and distilled water was added to lyse the red blood cells and allow the microspheres to settle to the bottom of the vial. Tissue and reference blood samples were counted for 5–10 min with a three-channel gamma counter (model 8000, Beckman Instruments, Inc., Fullerton, CA). Energy windows were set at 125–170, 460–550, and 820–1060 keV for ¹⁴¹Ce, ⁸⁵Sr, and ⁴⁶Sc, respectively. Nuclide separation was performed using standard techniques (5). Blood flow to the brain and its regions was calculated with the formula: $Q = At \cdot Qr / Ar$ whereas Q is blood flow to tissue (ml/min), At and Ar are the activity (counts/min) in the tissue, and reference blood samples, respectively, and Qr is rate of withdrawal of the blood sample. Blood flow to each region was divided by its weight, multiplied by 100 and reported per 100 g of tissue. Cerebral vascular resistance was calculated as the ratio of mean arterial blood pressure to CBF.

Statistical analysis. Statistical analysis was performed by one-way analysis of variance with repeated measurements. Differences between means were determined by paired *t* test with Bonferroni correction when necessary. All differences were considered significant when $p < 0.05$.

RESULTS

Seven animals were studied under this protocol. Paralysis was observed in all animals following intravenous administration of pancuronium bromide. The hematocrit, pH, PaCO₂, arterial, and sagittal sinus oxygen content are illustrated in Table 1. Pancuronium administration had no effect on any of these parameters and the hematocrit did not change during the whole experiment. During steady state measurements, heart rate and blood pressure did not change following paralysis. Heart rate was 217 ± 17 at control, 232 ± 17 at 15 min, and 269 ± 25 beats/min at 60 min ($p = \text{NS}$). Blood pressure was 77 ± 2 at control, 82 ± 4 at 15 min, and 79 ± 5 mm Hg at 60 min. Following pancuronium administration and in spite of connecting the animal immediately to the ventilator, some animals experienced a transient increase in blood pressure as illustrated in Figure 1. This hypertensive state was not associated with an increase in end tidal CO₂. The average transient increase in systolic blood pressure for the whole group was 32% following paralysis.

Figure 2 illustrates the cerebral blood flow and cerebrovascular resistance at control, 15 min, and 60 min following paralysis. A significant change in either parameter was not seen following pancuronium bromide administration. Analysis of the regional blood flow revealed a blood flow of 83 ± 6 to the cerebrum, 103 ± 9 to the midbrain, 92 ± 7 to the thalamus, 52 ± 5 to the hippocampus, 84 ± 5 to the caudate, 196 ± 7 to the cerebellum, 88 ± 6 to the pons, and 95 ± 5 ml/min/100 g to the medulla at 0 min. No significant changes in blood flow to any specific regions following paralysis were observed. The cerebral oxygen consumption following paralysis is depicted in Figure 3. Except for one animal that experienced an unexplained drop in oxygen consumption at 15 min, the values were unchanged following pancuronium bromide paralysis. Arterial glucose concentration was 7.5 ± 0.4 mmol/liter at control, and was not statistically different at 15 and 60 min following paralysis. The CMR_{glucose} was 53 ± 10 at 0 min, 57 ± 10 at 15 min, and 60 ± 12 mmol/min/100 g at 60 min ($p = \text{NS}$). Sagittal sinus pressure was 5.4 ± 0.6 at 0 min, 6.0 ± 0.3 at 15 min, and 6.5 ± 0.3 mm Hg at 60 min following paralysis ($p = \text{NS}$).

DISCUSSION

Contrary to what has been described in premature human neonates (9) and adult rats (1), cerebral blood flow in the newborn lamb does not increase following pancuronium bromide paralysis. Carlsson *et al.* (1) studied rats paralyzed with *d*-tubocurarine following catheter placement surgery and nitrous oxide anesthesia withdrawal. There was an increase in CBF and CMRO₂ in the unanesthetized paralyzed group that could be blocked by propranolol priming or previous adrenalectomy. Immobilization stress with subsequent catecholamine release was postulated to be the mechanism to induce changes in cerebrovascular tone and metabolism. The present study design greatly differs from others inasmuch as this constitutes a chronic preparation where the animals did not experience any stress prior to paralysis. Although immobilization stress was proposed as the mechanism to induce changes in cerebral oxygen consumption and flow, it is conceivable that the observed changes were related to surgical stress and pain following anesthesia withdrawal. In

Table 1. Arterial blood gases following pancuronium bromide infusion in newborn lambs*

Time	HCT (%)	pH	PaCO ₂ (torr)	Arterial O ₂ content (mM)	Sagittal sinus O ₂ content (mM)
Control (0 min)	33 ± 2	7.45 ± 0.01	36.6 ± 1.6	6.3 ± 0.4	3.2 ± 0.1
15 min	33 ± 2	7.42 ± 0.01	40.3 ± 1.4	6.5 ± 0.4	3.5 ± 0.2
60 min	33 ± 2	7.44 ± 0.01	37.4 ± 2.0	6.3 ± 0.4	3.1 ± 0.2

* Values are mean $\pm$ SE. PaCO₂ at 0 min represents measurements taken immediately prior to infusion of pancuronium bromide.

Fig. 1. Arterial blood pressure recordings from three lambs during bolus injection of 0.1 mg/kg pancuronium bromide into the inferior vena cava.

CEREBRAL VASCULAR RESISTANCE

CEREBRAL BLOOD FLOW

Fig. 2. Effect of pancuronium bromide paralysis on cerebral blood flow and cerebrovascular resistance in seven newborn lambs. Measurements at 0 min represent control values taken immediately prior to beginning infusion of pancuronium bromide.

CEREBRAL O_2 CONSUMPTION

Fig. 3. Effect of pancuronium bromide paralysis on cerebral oxygen consumption in seven newborn lambs. Measurements at 0 min represent control values taken immediately prior to beginning infusion of pancuronium bromide.

surge had occurred in these animals. Therefore, the discrepant results, as compared to those of Carlsson *et al.*, could be explained by the absence of stress reactions in the animals of the present study. It might also be postulated that either brain maturation makes the newborn less likely to manifest a response as compared to mature animals or that species differences may influence stress reactions to immobilization. In humans, administration of *d*-tubocurarine to a conscious adult did not lead to any changes in electroencephalogram, electrocardiogram, and blood pressure (13).

Apart from the effect of immobilization stress upon cerebral blood flow, one has to consider the possible direct effect of the muscle relaxant agents utilized. *d*-Tubocurarine is known to cause histamine release and sympathetic ganglion blockade (4). As opposed to these effects, pancuronium has been shown to induce adrenergic transmitter release from the postganglionic nerve endings (2). Furthermore, at least under thiamyl anes-

the present study, we did not measure plasma catecholamine levels; however, sustained changes in heart rate and mean arterial blood pressure were not observed following pancuronium administration, making it unlikely that a significant catecholamine

sia, pancuronium induces a significant rise in free epinephrine concentration (17). Therefore, if the muscle relaxants were to be directly implicated in the pathogenesis of increased cerebral blood flow, one would expect a greater effect upon catecholamine release when pancuronium as opposed to *d*-tubocurarine is administered. Varma *et al.* (14), in anesthetized adult dogs, has shown a different physiologic response to these two muscle relaxants. In that study, cerebral blood flow was not determined, but a 63% increase in carotid blood flow followed *d*-tubocurarine administration while no change occurred in another group receiving pancuronium bromide.

In sick premature neonates, cerebral blood flow velocity, as determined by the Doppler technique, was noted to increase following paralysis with curare or pancuronium (9). Furthermore, intracranial pressure as measured by a transducer applied to the anterior fontanel increased significantly more upon elevation of peak inspiratory pressure and leg-raising as compared to values obtained prior to muscle relaxant administration (10). These observations were emphasized by the author as suggestive of a direct effect of muscle relaxants upon the cerebrovascular tone in the neonates with a loss of cerebral autoregulation. Unfortunately, blood gas results were not reported in the above study. A certain degree of hypoxia and hypercarbia are almost universally present when respiratory paralysis is clinically utilized and are well known factors associated with an increase in cerebral blood flow (16). Thus, the reported changes in these premature infants' cerebral blood velocity could have been directly related to changes in PaCO_2 and PaO_2 and not to loss of cerebral autoregulation. In fact, studies in newborn lambs under paralysis have shown that the CBF response to hypoxia is not different than the reported values for nonparalyzed awake animals, supporting an intact cerebral responsiveness to hypoxia (15).

Abrupt changes in arterial blood pressure have been associated with IVH in premature neonates. Transient increases in blood pressure state associated with hypercarbia have been documented to precede IVH in neonates with pneumothorax (6). The transient hypertension observed immediately following paralysis in our animals probably reflects a hypoxic phenomenon. Hypoxia following administration of muscle relaxants has been previously observed in neonates (11) and is probably related to the reported changes in diaphragmatic motion and alveolar ventilation leading to ventilation perfusion abnormalities (3, 12). Based on the response observed immediately following pancuronium administration, we speculate that the risk of hypoxia and perhaps hypercarbia following paralysis is the greatest in this initial period. Such abrupt changes in blood gases could lead to an increased incidence of IVH in premature neonates receiving muscle relaxants independent of any direct effect of the drugs upon the cerebral vascular tone.

These data suggest that pancuronium paralysis in healthy awake newborn lambs does not lead to any alteration in cerebral blood flow or metabolism contrary to what has been previously reported in other species. Following paralysis, however, a short-lasting increase in blood pressure is observed which may be associated with a transient ventilation perfusion mismatch. It is concluded that no direct effect of this drug upon cerebrovascular tone or metabolism could be appreciated in newborn lambs.

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