Carbon Dioxide-Induced Anesthesia Has No Effect on Brain Biogenic Amine Concentrations in Mice

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Ensuring a humane death for laboratory animals used in research experiments is important to the experimenter. To aid researchers in such decisions, the American Veterinary Medical Association (AVMA) convenes panels to set guidelines for selection of appropriate methods of euthanasia. The AVMA Panel on Euthanasia suggests that several criteria be considered in choosing an appropriate method of euthanasia, the principal criterion being the ability to induce loss of consciousness and death without causing pain or distress (1).

In experiments involving mice in which the integrity of the brain tissue is of concern, the animal is commonly killed by manual cervical dislocation, followed by decapitation. Manual cervical dislocation, when properly conducted, is a humane method of euthanasia for smaller animals such as mice. In larger animals, this technique is generally accompanied only by use of mechanical dislocation, owing to a greater muscle mass in the cervical area (1). This method is preferred over other methods because of its ability to induce rapid death while maintaining the brain tissue in a chemically uncontaminated state. However, previous research indicates cervical dislocation can be stressful for the animal and the experimenter. In particular, stress is associated with the physical restraint required to manipulate the conscious animal (2). Furthermore, there are safety concerns to the experimenter (e.g., being bitten) when manipulating a conscious animal prior to physical euthanasia. Data also indicate that electrical activity in the brain persists for 13 sec after cervical dislocation (3), perhaps indicating that pain receptors are activated in the animal. The AVMA Panel on Euthanasia, therefore, recommends that, when practical, animals be rendered unconscious prior to physical euthanasia, using anesthesia.

The rapid anesthetic effects of carbon dioxide (CO₂) are well established (1). In an attempt to ensure a humane method of euthanasia, use of CO₂-induced anesthesia prior to cervical dislocation followed by decapitation has increased recently (2). Controversy regarding the use of anesthesia exists, nevertheless; there is continuing concern about the effects of anesthetics on neurochemical parameters (4). Due to the modulus of research on the effects of CO₂ on brain neurochemistry, the researcher is left with a concern that CO₂ may contaminate neurochemical measures.

Carbon dioxide is a quick-acting and inexpensive anesthetic that does not introduce exogenous chemicals into the body, but works as a CNS depressant drug, increasing respiratory rate until unconsciousness occurs (5, 6). Although the exact mechanism governing CO₂'s anesthetic effects is not completely understood, it has been hypothesized that inhalation of CO₂ may induce anesthesia through a narcotic effect and by lowering brain intracellular pH (5). Carbon dioxide-induced anesthesia does not have an effect on cholinergic markers in brain tissue in decapitated rats (7). Furthermore, serum concentrations of tropic hormones in decapitated rats are not adversely affected or increased by CO₂ use prior to euthanasia, indicating that brief exposure to CO₂-induced anesthesia might alleviate the stress of restraint associated with the euthanasia procedure (2). Research has been conducted to date examining the effects of CO₂-induced anesthesia on measurement of biogenic amine concentrations in the mouse brain. The aim of the study reported here was to examine these effects, using high-performance liquid chromatography (HPLC) with electrochemical detection, which is a sensitive method for detecting biogenic amines in brain tissue (8). In particular, three neurotransmitters—norepinephrine, dopamine, and serotonin—were evaluated in four commonly studied brain regions: the frontoparietal cortex, hippocampus, striatum, and cerebellum. These regions are the principal areas of localization and innervation from the widespread biogenic amine systems (9, 10).

All animal procedures were approved by the Wellesley College Animal Care and Use Committee. Twelve specific-pathogen-free, 6-month-old female BALB/cByJ mice (The Jackson Laboratory, Bar Harbor, Maine) were studied. Mice were housed in a facility that has animal care and use programs accredited by AAALAC, International. Mice were kept on pine shavings in polycarbonate cages 15 x 26 x 12 cm (individual cages) or 21 x 44 x 15 cm (group cages) with wire lids. Individually and group-housed mice were evenly distributed between the two experimental groups. The animal room was maintained between 20 to 22°C on a 12/12-h light/dark cycle, with lights on at 0600 h. Mice were fed Harlan Teklad rodent food (no. 8640; Harlan Teklad, Madison, Wis.) and water ad libitum.

Mice were randomly assigned to one of two test groups. Each mouse remained in its home cage in the animal room and was brought to another room immediately before euthanasia. Mice of the first group (n = 6) were introduced individually into an acrylic chamber, 29 x 50 x 28 cm, that had been charged for 15 sec with a 70% CO₂/30% O₂ gaseous mixture from a compressed gas cylinder. Mice remained in the chamber for 30 sec or until they lost consciousness. They were then removed from the chamber and, after cervical dis-
location, were decapitated. The second group of mice (n = 6) were subjected to cervical dislocation and decapitation without prior anesthesia.

The brain was removed rapidly and dissected immediately while on ice. The frontoparietal cortex was dissected bilaterally, followed by the hippocampus, the striatum, and the cerebellum. Dissected specimens were frozen immediately on dry ice; complete dissection took 2 to 3 min. Specimens were stored at -70°C until the time of the HPLC electrochemical detection assay.

Concentrations of the biogenic amines were measured, using HPLC coupled with electrochemical detection (Bioanalytical Systems, Inc., West Lafayette, Ind.). A model 480 HPLC system was used, consisting of a CO-5 flow cell and a PM-80 solvent delivery pump. The chromatographic column was a reverse phase Microsorb-MV C18 column, with an LC4C electronic controller and a glassy carbon electrode (applied voltage: 650 mV; range: 50 nA; filter: 0.1 Hz). To prepare the mobile phase, 9.6 g of sodium acetate, 0.6 ml of phosphoric acid, 10 mg of EDTA, and 275 mg of octyl sulfate were diluted in 500 ml of HPLC grade water. After the solution was thoroughly stirred, 100 ml of acetonitrile was added; the mobile phase was again stirred and brought to a final volume of 1 L. The pH was adjusted to 3.1. The mobile phase was then filtered through a 0.2 μm nylon membrane, and degassed under a vacuum.

Biogenic amine external standards, including norepinephrine, dopamine, and serotonin, were prepared to a final concentration of 1.25 × 10^{-4}M in 50 mM perchloric acid (HClO4). An internal standard of 1.25 × 10^{-4}M 3,4-dihydroxybenzylamine (DHBA) was used to monitor sample degradation. All standards were obtained from Research Biochemical International (Natick, Mass.). Brain specimens were removed from -70°C and placed on ice. Ice-cold 50 mM HClO4, which contained 1.25 × 10^{-4}M DHBA, was added to the tissue in a 20 ml/mg ratio. The HClO4 was added to the cerebellar specimens in a 10 ml/mg ratio because of the large size of these specimens. Sonification of the specimens was done for 15 to 20 sec to disrupt the tissue. The homogenate was centrifuged for 15 min at 14,000 X g to precipitate the protein. The supernatant was carefully removed and was transferred into an autosampler microvial. Twenty microliter samples were injected onto the column by use of the autosampler. Data were collected and analyzed, using Gilson 712 System Controller software.

A repeated measures analysis of variance (ANOVA) was performed on the HPLC data for each of the three neurotransmitters, examining treatment as the main effect and regional differences as the repeated measure. The significance level was set at P < 0.05.

Norepinephrine concentration did not differ significantly between animals anesthetized with CO2 prior to decapitation and those decapitated without prior anesthesia (F[1,10] = 0.155, P > 0.05). Norepinephrine concentration did vary significantly by region, with the highest value appearing in the cortex and the lowest value appearing in the striatum (F[3,30] = 14.825, P < 0.0001) (Figure 1).

Dopamine values did not differ significantly between animals anesthetized with CO2 prior to decapitation and those decapitated without prior anesthesia (F[1,10] = 0.909, P > 0.05). However, dopamine values differed significantly by region (F[3,30] = 119.446, P < 0.0001) (Figure 2); they were highest in the striatum and virtually absent in other regions.

Similarly, serotonin values did not differ significantly between animals anesthetized with CO2 prior to decapitation and those decapitated without prior anesthesia (F[1,10] = 0.331, P > 0.05), yet serotonin values differed significantly by region (F[3,30] = 5.207, P < 0.01) (Figure 3). The striatum and the hippocampal regions had higher serotonin values than did the cortical and cerebellar regions.

Minimizing animal pain and distress should always be a consideration in determining the method of euthanasia used. For experiments in which the integrity of the brain tissue is also a concern, the method must also allow for the maintenance of chemically uncontaminated brain tissue. These criteria leave few options for methods of euthanasia. Euthanasia should result in rapid unconsciousness followed by a cessation of cardiac or respiratory activity, which will eventually lead to a loss of brain function. The procedure should minimize anxiety and stress in the conscious animal (1). Cervical dislocation followed by decapitation is a method that is often chosen. However, this method can be stressful to the animal and to the experimenter due to the handling and physical restraint required to perform, and the safety concerns that are associated with, these techniques (1, 2). Furthermore, in educational experiments there could be stress to a student observing this form of euthanasia on a conscious animal, due to its aesthetically displeasing nature. The stress of prolonged restraint can be reduced somewhat by anesthetizing the animal prior to decapitation.

High concentrations of CO2 can rapidly lead to death in the animal due to mucosal irritation and ventilatory stimulation (11). The AVMA recommends that a CO2 concentration of 60 to 70% will allow a rapid anesthetic effect while alleviating adverse long-term ventilatory effects (1). In the study reported here, the animals were anesthetized for 30
Figure 2. Dopamine concentrations (pmoles/0.25 mg tissue) in various mouse brain regions after decapitation with prior anesthesia or decapitation after a 30-sec exposure to carbon dioxide. Each bar represents the mean ± SEM for six mice in each group. See Figure 1 for explanation of abbreviations.

sec (until the mice were observed to be immobile) in a precharged chamber with the recommended concentration of 70% CO₂.

The use of CO₂ anesthesia in euthanasia protocols for neurochemical experiments has conventionally been avoided due to concerns that CO₂ anesthesia may alter brain chemistry. Findings from this study indicate that brief exposure to CO₂-induced anesthesia does not significantly alter concentrations of biogenic amines in the mouse brain. Values reported here agree with those previously reported from other studies of brain catecholamine values without CO₂-induced anesthesia (8).

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References