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Brain and body temperature homeostasis during sodium pentobarbital anesthesia with and without body warming in rats

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Abstract

High-speed, multi-site thermorecording offers the ability to follow the dynamics of heat production and flow in an organism. This approach was used to study brain–body temperature homeostasis during the development of general anesthesia induced by sodium pentobarbital (50 mg/kg, ip) in rats. Animals were chronically implanted with thermocouple probes in two brain areas, the abdominal cavity, and subcutaneously, and temperatures were measured during anesthesia both with and without (control) body warming. In control conditions, temperature in all sites rapidly and strongly decreased (from 36–37 °C to 32–33 °C, or 3.5–4.5 °C below baselines). Relative to body core, brain hypothermia was greater (by 0.3–0.4 °C) and skin hypothermia was less (by ~0.7 °C). If the body was kept warm with a heating pad, brain hypothermia was three-fold weaker (~1.2 °C), but the brain–body difference was significantly augmented (−0.6 °C). These results suggest that pentobarbital-induced inhibition of brain metabolic activity is a major factor behind brain hypothermia and global body hypothermia during general anesthesia. These data also indicate that body warming is unable to fully compensate for anesthesia-induced brain hypothermia and enhances the negative brain–body temperature differentials typical of anesthesia. Since temperature strongly affects various underlying parameters of neuronal activity, these findings are important for electrophysiological studies performed in anesthetized animal preparations.
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Keywords: Barbiturates; General anesthesia; Thermorecording; Brain metabolism; Heat production; Heat loss

1. Introduction

General anesthesia is a procedure commonly used in neurobiological research, including studies of neuronal activity and responsiveness, alterations of blood flow, and body temperature regulation. While it is known that most drugs used for general anesthesia inhibit brain metabolism, decrease cerebral blood flow, and induce body hypothermia [4,6,17,22,26], several aspects of brain–body temperature homeostasis during general anesthesia remain unclear. Although data obtained in acute experiments suggest that anesthesia also results in robust brain hypothermia [5,18,29,32], the extent and time-course of brain temperature changes and their relation to body core temperature during the development of and recovery from anesthesia have not been thoroughly studied. By studying the relative changes in brain and body core temperature during an anesthetic state, it becomes possible to clarify whether brain hypothermia reflects primarily inhibition of brain metabolism and thus has an intra-brain origin, or occurs due to body cooling related to general inhibition of metabolism. Since anesthesia results in peripheral vasodilatation and enhanced heat dissipation to the external environment [26], anesthesia should affect skin temperature, which reflects both the state of vessel tone and arterial blood temperature [1]. This parameter is studied little in relation to brain and body core temperatures during the development of and recovery from anesthesia. Finally, although body warming is commonly used during experimental anesthesia in animals to counteract heat loss and increase body core temperature, it remains unknown how this procedure affects brain temperature.

To clarify these issues, temperatures were simultaneously recorded from the brain, body core, and subcutaneously in

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awake, unrestrained rats during sodium pentobarbital-induced anesthesia, a procedure commonly used in animal research and a prototype of general anesthesia used in humans. Using a within-animal design, measurements were performed in the same animals during anesthesia in two conditions: at normal environmental temperatures (23 °C) and when body temperature was maintained at normal (37.5 °C) levels by external body warming. By tracking temperature responses in different brain and body sites with a short collection interval one should be able to map the dynamics of heat generation, flow, and loss within the organism.

For brain recording sites, we chose two structures, which differed in their functions, dorso-ventral location, and basal temperatures. The ventrally located medial preoptic area of the hypothalamus (MPAH) is a structure considered to be the primary thermointegrative center [25] and has a high basal temperature. The more dorsally located hippocampus (Hippo) was chosen as a control structure, which has significantly lower basal temperatures [9]. Because of the requirements of long-term monitoring in freely moving rats, core body and skin temperatures were assessed by miniature thermocouple sensors that were chronically implanted in, respectively, the retroperitoneal space and subcutaneously in the forehead area.

2. Methods

2.1. Subjects and surgery

Six male Long–Evans rats (Taconic, Rockville, MD), 3–4 months in age and 440±30 g in weight, housed individually (12-h light cycle beginning at 7:00) with free access to food and water, were used. Protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication 865–23) and were approved by the Animal Care and Use Committee, NIDA-IRP. Under Equithesin anesthesia (3.3 ml/kg), each rat was implanted with four thermocouple probes in two brain sites and two peripheral locations. The probes were prepared from twin copper and constantin wires (diameter 125 μm) obtained from Physitemp Instruments (Clifton, NJ, USA) and described in detail elsewhere [8]. Briefly, the insulation was removed 200–400 μm from the tip of each wire, the tips were soldered together, and re-insulated with polyester micro-shrink tubing (~20 μm thickness after shrinking) and epoxy. The wires were connected to copper and constantin pins and fixed in a plastic connector with epoxy. Brain probes were stereotaxically implanted, using coordinates of Paxinos and Watson (1996) [21], in the medial preoptic area of the hypothalamus (MPAH; −1.1 mm AP, 1.8 mm L, 8.1 mm deep at 10°) and the hippocampus (Hippo; −3.6 mm AP, 2.0 mm L, 3.4 mm deep). The third probe was implanted subcutaneously (skin) along the skull's center line approximately 9–12 mm in front of bregma. This location minimizes thermocouple probe movement and recording artifacts typical to other moving locations (i.e., tail and body external surfaces). The fourth probe was implanted into the retroperitoneal space between the peritoneum and trunk muscular wall (body core) approximately 5 cm from the bottom of abdominal cavity via a ~12 mm skin incision between the rectum and tail base. In this case, thermocouple wires, except the very tip, were covered by a plastic thermo-isolated material. Connecting wires were fed subcutaneously from the tail base to the head assembly. As with the location of the subcutaneous probe, this recording site prevented movement of the thermocouple probe and thus artifacts in recording. All four probes were secured with dental cement to three stainless steel screws threaded into the skull. During experiments, a four-channel cable connected the probes on the head mount, via electric commutator, to a computer running temperature collection software (Thermes-16, Physitemp Instruments, Clifton, NJ).

2.2. Experimental protocol

All rats were given 3 days recovery following surgery plus 1 day of habituation (6 h) prior to testing. On test days, rats were given a 2-h habituation to the testing environment. A single intraperitoneal (ip) injection of sodium pentobarbital (50 mg/kg in 0.4 ml saline) was given on 2 separate test days with 1 day of rest in between. During this resting session, rats received a single ip injection of saline. All rats received body warming during one drug session, half with the first injection and half with the second injection. Body warming was provided by a flat heating pad (22×17 cm) with a rectal thermal probe feedback system set at 37.5°. The probe was inserted about 4 cm into the rectum and the pad was placed under the rat's body 10 min after the pentobarbital injection. The rat was positioned on the heating pad such that the head and neck were off the edge. The rectal probe and heating pad were removed at the first sign of movement. All recordings took place during the light phase of the rat's cycle (10:00–16:00) in a Plexiglas chamber (32×32×32 cm) placed inside of a sound-attenuating box. Environmental temperatures were maintained at 23±0.5 °C and their stability was controlled by an additional thermal probe placed inside of the experimental chamber.

2.3. Histology

The day following the last test, rats were euthanized by pentobarbital overdose and brains were removed for subsequent histological verification of the location of brain thermal probes. The location of the recording sites was determined from cryostat cut, 45 μm slices mounted on glass slides.

2.4. Data analysis

Temperature changes were presented in three ways: as absolute change, as relative change, and as differences between recording sites (brain–body and brain–skin differ-