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IN VIVO EFFECTS OF PENTOBARBITAL AND HALOTHANE ANESTHESIA ON LEVELS OF ADENOSINE 3′,5′-MONOPHOSPHATE AND GUANOSINE 3′,5′-MONOPHOSPHATE IN RAT BRAIN REGIONS AND PITUITARY

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Abstract—The effects of two general anesthetics, pentobarbital and halothane, on *in vivo* levels of cyclic AMP and cyclic GMP were examined in seventeen brain regions and the pituitary in the rat. Ventilation was controlled to produce normal values of arterial pH, pCO_2 and pO_2, to eliminate changes in cerebral perfusion and oxygen delivery which occur as a result of the respiratory depressant effect of these drugs. Arterial pressure was monitored and colonic temperature was maintained within normal limits. Pentobarbital was given as a single i.p. injection of 80 mg/kg. Control animals received an equivalent volume of vehicle solution. Induction of halothane anesthesia was accomplished by placing the animals in a jar flushed with 3% halothane in air. After 3 min the animals received 2% halothane in air via a nose cone. Control animals for this experiment were placed in an air-filled jar. Experimental and control animals were killed by microwave irradiation 1 hr after the start of anesthesia. Both drugs decreased levels of cyclic GMP in virtually all regions. The largest changes occurred in the cerebellum, where cyclic GMP dropped to 7.4 per cent of control with pentobarbital and to 9.8 per cent of control following halothane. Levels of cyclic AMP significantly increased in the cerebellum, brainstem and hypothalamus after halothane, by 58, 49 and 65 per cent, respectively. Both pentobarbital and halothane markedly increased cyclic AMP levels in the pituitary (to 784 and 270 per cent of control values, respectively). These results show that halothane and pentobarbital, which modify synaptic transmission, selectively alter cyclic AMP and cyclic GMP levels in specific brain regions and the pituitary.

Inquiry into the neurochemical basis of the action of general anesthetics and other central nervous system depressant drugs has led a number of investigators to examine the effects of these drugs on levels of the cyclic nucleotides adenosine 3′,5′-monophosphate (cyclic AMP) and guanosine 3′,5′-monophosphate (cyclic GMP). Both cyclic AMP and cyclic GMP are found in the CNS in relatively high concentrations, and both compounds have been suggested as mediators of synaptic transmission [1, 2]. At present, a large body of evidence points to alterations in synaptic transmission as being responsible for much of the phenomena of general anesthesia [3, 4].

Biebuyck *et al.* [5] found that whole brain levels of cyclic AMP were increased approximately 2-fold after 1 hr of anesthesia with halothane, ketamine or morphine in rats ventilated to control pCO_2 at normal values. Nahrwold *et al.* [6] reported no significant change in cyclic AMP in mouse cortex, cerebellum or spinal cord during halothane anesthesia, except for a decrease in cyclic AMP in the cortex at concentrations of halothane of 2.4% or greater. These higher doses of halothane were associated with hypoxemia and attendant acidosis.

Barbiturates produce centrally mediated behavioral effects but their mode of action remains unknown. Neurochemical studies in rats during acute barbiturate administration have shown changes in the turnover of the catecholamines, serotonin and acetylcholine [7]. These changes might cause alterations in the post-synaptic activities of the cyclic AMP and cyclic GMP systems. Cyclic GMP levels in the cerebellum have been shown to decrease with acute administration of pentobarbital [8, 9] and our laboratory has reported cyclic GMP decreases in many brain regions in rats maintained chronically on barbiturates [10].

Mueller *et al.* [11] have demonstrated an inverse correlation between cerebellar cyclic GMP and pCO_2 as ventilation was varied. Their studies illustrate the need to verify that arterial blood gas values are in the normal range before the effects of a drug on brain cyclic GMP levels can be reliably assessed.

Microwave irradiation minimizes post-mortem changes in the levels of cyclic nucleotides by providing rapid tissue inactivation while leaving the brain in a condition suitable for regional dissection. Using this method, this laboratory has been able to measure *in vivo* levels of cyclic AMP and cyclic GMP from rat brain regions [10, 12].

The aim of this study was to provide data on regional changes in cyclic GMP and cyclic AMP produced by two general anesthetics with quite dif-

ferent properties under conditions where arterial pressure, pO_2, pCO_2, pH and body temperature were known to be within the normal physiologic range.

MATERIALS AND METHODS

Animals. Male albino rats (300–353 g), WRC stock from the Walter Reed Army Institute of Research colony, were used in all experiments.* The animals had free access to food and water, and were maintained in a 12-hr light–dark cycled room. Experiments were performed between 8:30 a.m. and 4:30 p.m. Control and anesthetized animals were alternately killed within each experiment.

Solutions. Pentobarbital was obtained from Abbott (North Chicago, IL) and contained pentobarbital (50 mg/ml) in an aqueous vehicle containing 40% propylene glycol and 10% alcohol. Halothane was obtained from Halocarbon (Ontario) Ltd.

Administration of anesthesia. Pentobarbital was given as a single intraperitoneal injection of 80 mg/kg. Control animals received an equivalent volume of vehicle solution and were then returned to their home cages. After pentobarbital, the animals were placed on a warming blanket, a colonic thermistor probe was inserted, and colonic temperature was maintained between 37 and 38°. Tracheostomy was performed and the animals were ventilated with room air using a Harvard rodent respirator via a 14-gauge Medicut cannula. In all animals, ventilation was begun within 6 min of the pentobarbital injection. An incision was made to expose the femoral artery, which was cannulated with polyethylene tubing and flushed with a solution of heparin in 0.9% NaCl. The arterial line was connected to a Statham model P-37 transducer, and periodic measurements of arterial pressure were made. At 30 and 55 min after the start of anesthesia, a 0.6-ml aliquot of arterial blood was withdrawn into a syringe rinsed with a solution of heparin, and analyzed immediately for pCO_2, pO_2 and pH, using a Corning model 161 blood gas analyzer. After each sample was removed, the animals received a slow injection of 0.6 ml of 0.9% NaCl solution through the arterial catheter.

Induction of halothane anesthesia was accomplished by placing the animals in an 8-liter jar which had been previously filled and continually flushed with 3% halothane in air. Control animals were placed in an air-filled jar for 3 min and returned to their home cage. After loss of righting reflex and response to stimulation (about 3 min), the animals were placed on the warming blanket and they received 2% halothane in air via a nose cone while tracheostomy and placement of colonic temperature probe were performed. After placing a 14-gauge Medicut cannula into the trachea, the halothane concentration was reduced to 1% in air, and ventilation was controlled with a Harvard rodent respirator for the remainder of the 60-min anesthetic. In all animals, controlled ventilation was begun within 9 min from the beginning of halothane administration. Halothane was delivered and the concentration was varied using a calibrated Fluotec Mark II vaporizer. Arterial cannulation and blood gas sampling were performed as described for the animals receiving pentobarbital.

Microwave irradiation. At 59 min after the start of induction of anesthesia the temperature probe was removed, and the animals were disconnected from the arterial pressure transducer and the ventilator, and were positioned in a plexiglass holder. Preliminary experiments demonstrated that the measured physiologic variables remained within normal limits for over 1 min after the animal was removed from the ventilator. Control animals were taken from their home cages and placed in the plexiglass holder. The holder was inserted into a hole in the short-circuiting endplate of a WRC 430 waveguide exposure chamber in such a manner that the longitudinal axis of the rat head was perpendicular to the microwave E field. The animals were killed 60 min from the start of anesthesia by microwave irradiation at 2450 MHz. The power source and waveguide were modified to achieve greater uniformity and efficiency of inactivation [13, 14]. Animals were exposed for approximately 5 sec with 2.5 kW forward power (1–3% reflected).

Sample preparation. Following microwave irradiation, the heads were cooled briefly on dry ice for ease of handling. The brain was then carefully removed and the desired regions were dissected as described previously [15]. The tissue pieces were weighed, placed in 50 mM sodium acetate buffer (pH 6.2) and then sonicated with a Heat Systems model 185 for 5–30 sec, depending on tissue size, at a power setting of 50 W. The sonicates were centrifuged at 25,000 *g* for 15 min. The supernatant fractions were stored at −70° until assayed.

Cyclic nucleotide assay. Cyclic AMP and cyclic GMP levels were determined by a slight modification of the radioimmunoassay described by Steiner *et al.* [16]. The reaction volume of 0.5 ml differed in that it contained 200 μg of rabbit gamma globulin (Schwarz/Mann, Orangeburg, NY). After 16 hr at 4°, separation of free and bound cyclic nucleotides was accomplished by addition of 1.5 ml of ice-cold 50 mM sodium acetate (pH 6.2) with 16% Carbowax 6000 (Schwarz/Mann) and 1 mg/ml rabbit gamma globulin, followed by centrifugation at 4° for 10 min at 2000 *g* with subsequent aspiration of the supernatant fraction.

For measurement of the cyclic nucleotides in the smaller brain regions, a further modification of the method described by Harper and Brooker [17] was employed. Standards and brain samples were acetylated at the 2'0 position in 50 mM sodium acetate (pH 4.8) using a freshly prepared 2:1 mixture of triethylamine/acetic anhydride. Following the acetylation procedure, the radioimmunoassay proceeded as noted above.

The data were analyzed by computer using a nonlinear four parameter logistic model weighted for non-uniformity of variance [18]. The respective sensitivities (minimal detectable amounts) for cyclic AMP and cyclic GMP were 0.10 and

* In conducting the research described in this report, the investigators adhered to the *Guide for Laboratory Animal Facilities and Care*, as promulgated by the Committee of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

0.025 pmole/assay tube for the routine assay and 3 fmoles for the acetylated assay. Phosphodiesterase treatment of tissue extracts reduced cyclic AMP and cyclic GMP to undetectable levels, representing a reduction of greater than 95 per cent for cyclic AMP and 80 per cent for cyclic GMP in each region.

RESULTS

Physiologic variables. All animals receiving pentobarbital or halothane were found to have pO_2, pCO_2, pH and mean arterial pressure within the physiologic range, as seen in Table 1. The only borderline value, and the only pO_2 less than 65 Torr, was a pO_2 of 56 in one animal in the halothane group. This animal had no concomitant acidosis, hypotension, or other evidence of hemodynamic or respiratory compromise and hence was included in the study.

The difference in mean arterial pressure between the pentobarbital and the halothane groups (Table 1) is a reflection of the greater degree of depression of myocardial contractility seen with halothane.

Effects of pentobarbital on regional cyclic GMP and cyclic AMP levels. Pentobarbital significantly decreased levels of cyclic GMP in fourteen of the seventeen brain regions examined (Fig. 1), as well as in the pituitary (Fig. 2). In most of these regions, the cyclic GMP levels were decreased by over 50 per cent. The most dramatic decreases were seen in the cerebellum (to 7 per cent of control levels), the pineal (to 13 per cent of control) and in the cortical areas (24–33 per cent of control).

Table 1. Physiologic variables*

Variables	Pentobarbital	Halothane
pO_2	72.0 (69–76)	84.2 (56–95)
pCO_2	38.7 (35–43)	38.0 (36–42)
pH	7.417 (7.39–7.45)	7.423 (7.35–7.46)
MAP	122 (95–135)	83 (67–104)

* Gas tensions and mean arterial pressure (MAP) are expressed in Torr. Values represent the mean of the measurements made on each of the six animals in each grouping during min 55 of anesthesia. The range of the six measurements is in parentheses.

A striking increase of more than 7-fold in pituitary cyclic AMP was found after 1 hr of pentobarbital anesthesia (Fig. 2). Other statistically significant changes occurred in the frontal cortex where cyclic AMP levels dropped by 28 per cent and in the amygdala where cyclic AMP levels were increased by 27 per cent. All other regions showed small and statistically insignificant changes with pentobarbital.

Effects of halothane on regional cyclic GMP and cyclic AMP levels. Halothane significantly decreased levels of cyclic GMP in fifteen of the seventeen brain regions measured (Fig. 3) and in the pituitary (Fig. 2). Again, the decreases in the cerebellum and pineal

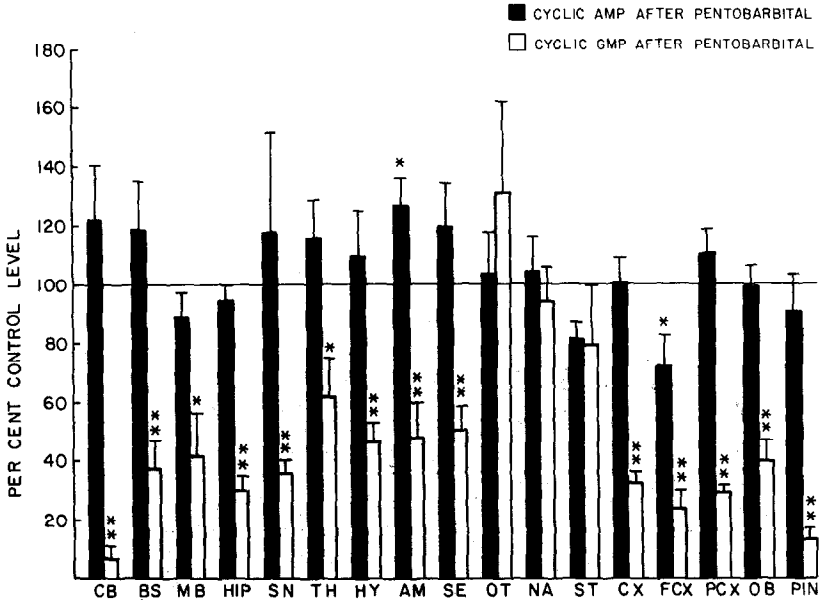

Fig. 1. Levels of cyclic AMP and cylic GMP in rat brain regions after 1 hr of pentobarbital anesthesia expressed as percent of control. Each value is the mean ± S.E.M. of six animals. Control levels of (cyclic AMP, cyclic GMP) for each brain region, expressed as pmoles/mg wet wt, and regional abbreviations are: CB, cerebellum (0.463, 0.505); BS, brainstem (0.397, 0.089); MB, midbrain (0.554, 0.097), HIP, hippocampus (0.450, 0.032); SN, substantia nigra (0.636, 0.047); TH, thalamus (0.498, 0.026); HY, hypothalamus (0.730, 0.049); AM, amygdala (0.361, 0.035); SE, sep*al region (1.643, 0.041); OT, olfactory tubercle (1.022, 0.044); NA, nucleus accumbens (0.543, 0.049); ST, striatum (0.536, 0.039); CX, cortex (0.639, 0.049); FCX, frontal cortex (0.734, 0.060); PCX, pyriform cortex (0.457, 0.031); OB, olfactory bulb (0.652, 0.097); and PIN, pineal (1.288, 0.082). Student's *t*-test was performed on absolute levels (two-tailed). Key: (*)$P < 0.05$, and (**)$P < 0.005$ (significantly different from control level).

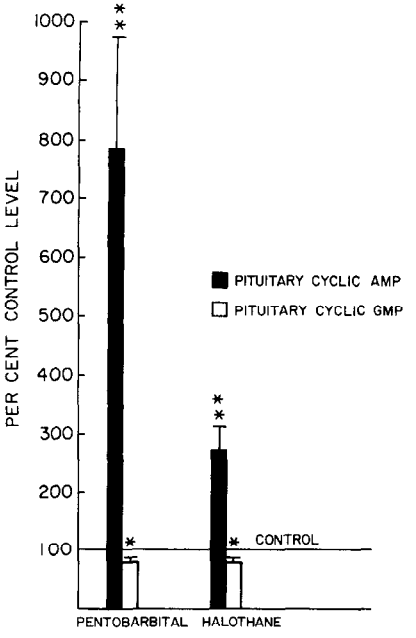

Fig. 2. Levels of cyclic AMP and cyclic GMP in rat pituitary after 1 hr of pentobarbital or halothane anesthesia expressed as per cent of control. Each value is the mean ± S.E.M. of six animals. Control levels are expressed as pmoles/mg wet wt: cyclic AMP, 1.194 (pentobarbital), 1.070 (halothane) and cyclic GMP 0.055 (pentobarbital) and 0.077 (halothane). Key: (*) $P < 0.05$, and (**) $P < 0.005$ (significantly different from control level).

were most marked (to 10 and 12 per cent of control levels, respectively).

Halothane elevated cyclic AMP levels in the pituitary to 270 per cent of control levels after 1 hr of anesthesia (Fig. 2). Significant increases (with levels reported as percentage of control) were also observed in the cerebellum (158 per cent), brainstem (149 per cent) and hypothalamus (165 per cent). In the striatum, levels were significantly decreased to 75 per cent of control levels.

DISCUSSION

Both pentobarbital and halothane anesthesia greatly decreased levels of cyclic GMP in most of the brain regions examined. These data are consistent with observations that sedative or depressant drugs generally decrease rat brain cyclic GMP levels, especially in the cerebellum which has been most extensively studied [8–10, 19–22]. Conversely, cerebellar cyclic GMP has been found to increase after administration of stimulant drugs [19, 23]. In addition to such drug-induced changes in cyclic GMP levels, we have also reported that locomotor activity itself may affect levels of cyclic GMP in some brain regions. Cerebellar cyclic GMP levels increase markedly after 5 min of activity in a running wheel [24] and decrease significantly following 15 min of forced immobilization. Thus, some of the decrease in cyclic GMP levels observed after 1 hr of anesthesia may be linked to the decreased level of motor activity in the anesthetized animals.

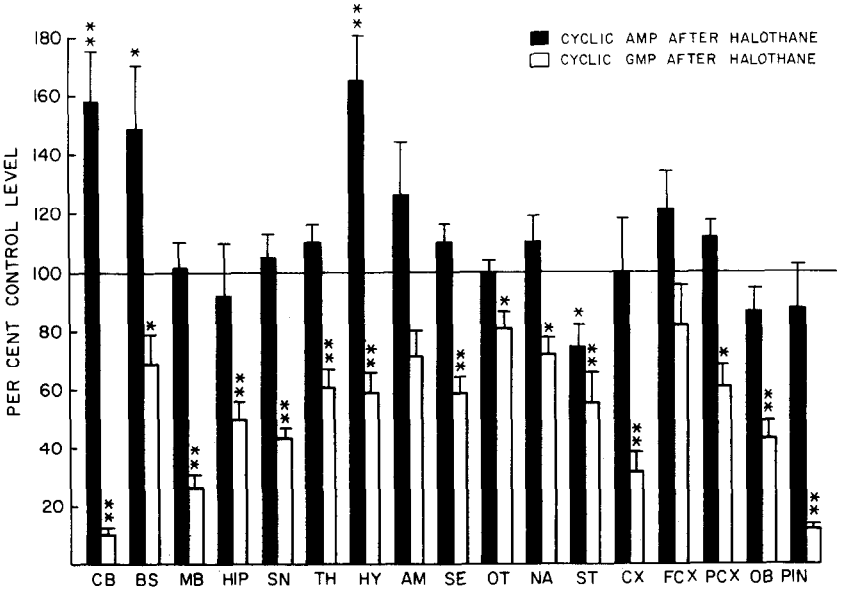

Fig. 3. Levels of cyclic AMP and cyclic GMP in rat brain regions after 1 hr of halothane anesthesia expressed as per cent of control. Each value is the mean ± S.E.M. of six animals. For brain region abbreviations, see Fig. 1. Control levels of (cyclic AMP, cyclic GMP) are expressed as pmoles/mg wet wt: CB (0.304, 0.823), BS (0.351, 0.117), MB (0.582, 0.120), HIP (0.568, 0.029), SN (0.742, 0.077), TH (0.536, 0.018), HY (0.674, 0.052), AM (0.543, 0.061), SE (0.904, 0.061), OT (0.718, 0.069), NA (0.678, 0.065), ST (0.528, 0.052), CX (0.385, 0.050), FCX (0.680, 0.044), PCX (0.729, 0.061), OB (0.905, 0.107) and PIN (1.236, 0.181), (*) $P < 0.05$, (**) $P < 0.005$ [Student's t-test (two-tailed) on absolute levels].

The anesthetics could also affect levels of cyclic GMP in particular brain regions by modification of pre- or post-synaptic transmission in specific neuronal pathways. For example, pentobarbital has been shown to enhance inhibitory GABAergic transmission [25, 26], and an inverse relation between GABA and cyclic GMP has been demonstrated in the rat cerebellum [27]. More work of this type is required to elucidate the interplay of anesthetics, neurotransmitters and cyclic GMP at the cellular level.

The changes in cyclic AMP observed were less widespread throughout the brain and specific to a few regions, especially the pituitary. We did not observe changes in cyclic AMP levels in a previously reported chronic sodium barbital model in which pituitary cyclic AMP was not measured. The lack of significant alterations in cyclic AMP levels after pentobarbital in any regions except the amygdala (27 per cent increase) and the pituitary (680 per cent increase) is thus consistent with the chronic data [10]. The pituitary cyclic AMP system appears to be remarkably responsive. We have recently reported large pituitary cyclic AMP increases after phosphodiesterase inhibition with RO 20-1724 or after the dopamine agonist apomorphine [23]. Catecholamine-sensitive adenylate cyclases have been found in the anterior and posterior pituitary [28, 29]. Reserpine has also been found to increase pituitary cyclic AMP *in vivo* [30]. Possibly, the pentobarbital-induced cyclic AMP rise reported here is mediated via alterations in activity in the tubero-infundibular dopamine system, or other neuropeptide-neurotransmitter systems linked to the pituitary.

Alternatively, pentobarbital might increase pituitary cyclic AMP via increased release of adenosine as recently reported by Cohn and Cohn [31] for whole brain.

The pattern of cyclic AMP response after halothane was qualitatively different than that after pentobarbital. Again, pituitary cyclic AMP was increased, although to a lesser extent than seen after pentobarbital. Possibly changes in transmitter turnover in the hypothalamo-pituitary regions are responsible. Halothane has also been reported to directly increase adenylate cyclase activity in the uterus [32], and this anesthetic might affect adenylate cyclase activity directly in the pituitary as well as in the other brain regions (hypothalamus, cerebellum and brainstem) where cyclic AMP increases were observed.

Although cyclic AMP in the pituitary was less increased after halothane than after pentobarbital, cyclic AMP levels in several brain regions were increased after halothane but not after pentobarbital. These findings are consistent with the reported increases in whole brain cyclic AMP after halothane [5]. However, as this regional study demonstrates, the increases in cyclic AMP after halothane are not uniform but restricted to specific brain regions.

In summary, these results show that two anesthetics which modify synaptic transmission selectively alter cyclic GMP and cyclic AMP levels in specific brain regions and the pituitary. The neuronal pathways involved in each instance and the exact molecular mechanisms remain to be defined.

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