

Cyclic Adenylate and Hypothalamic Regulatory Functions* (34012)

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Adenosine 3',5'-monophosphate (cyclic AMP) may be implicated in the release of acetylcholine at motor nerve endings under certain conditions (1) and, by analogy, it may participate in the secretion of other neurohumoral agents and hypophyseal releasing factors in the hypothalamus. This portion of the brain contains structures, sensitive to reproductive hormones, which control both physiological and behavioral events (2). The actions of numerous hormones on peripheral tissues are mediated by the cyclic nucleotide (3). Various nerve tissue preparations respond to catecholamines, indolalkylamines, and histamine by increasing levels of cyclic AMP (4-6). In exploring some of the possibilities suggested by these earlier findings, we have implanted cyclic AMP and N⁶, O²-dibutyryl adenosine 3',5'-monophosphate (db cyclic AMP) into the hypothalamus of rats.

Materials and Methods. Implants were prepared by mixing db cyclic AMP monopotassium salt¹ with half its weight of cholesterol acetate and packing approximately 0.5 mg of the mixture into the tips of 22-gauge, thin-walled, stainless-steel tubes. For controls, 5'-adenylic acid (AMP) was neutralized with K₂CO₃, lyophilized, and then mixed with cholesterol acetate in the same way. In later experiments, sodium salts of db cyclic AMP, cyclic AMP, and AMP were used. The acid form of db cyclic AMP was obtained by elution from an anion exchange column (Bio-Rad AG1-X10) and then neutralized with NaHCO₃. The salt was found to contain no potassium in excess of the blank by flame

photometry. Female Sprague-Dawley rats (Camm Research Institute, Inc., Wayne, New Jersey) were anesthetized with ether for the stereotaxic placement of the implants, which were securely fixed to the skull with dental cement. Penicillin (75,000 units) was injected intramuscularly. The location of the tip of the implant was verified in each instance by histological examination of fixed and stained sections. Body temperatures were measured by means of a thermistor inserted 3 cm beyond the anal orifice. Rats were individually caged with access to measured amounts of Purina Laboratory Chow.

Results and Discussion. Experiments designed to explore the possibility that cyclic AMP is an integral part of hypothalamic systems under estrogen control were performed with adult female rats. Animals which had been spayed a minimum of 14 days previously were given a midline implant of db cyclic AMP at de Groot coordinates (7) A: 7.5, depth: -1.5. The castrate animals were tested for sexual responsiveness 2 times daily with a minimum of 10 mounts per test. On Day 3 after db cyclic AMP implant a large pellet of progesterone was added subcutaneously and testing continued for 4 more days. None of the castrate animals showed any lordosis responses before or after the addition of progesterone. Histological verification of implant sites indicated all were localized within the neural region previously shown to be estrogen-sensitive and essential to the induction of the lordosis response (8). Thus, db cyclic AMP cannot substitute for estradiol at those neural tissues where estrogen can induce sexual receptivity.

Intact adult female rats showing regular estrous cycles as determined by changes of vaginal cytology were implanted with db cyclic AMP or AMP at A: 6.0 midline, depth: -3.0. Daily vaginal smears were made to de-

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¹ Nucleotides were obtained from Calbiochem, Los Angeles, California; and cholesterol acetate was obtained from Sigma Chemical Company, St. Louis, Missouri.

termine whether the estrous cycle had been altered. Eight of ten rats with db cyclic AMP implants at the level of the arcuate nucleus showed a lengthening of the estrous cycle; five animals became pseudopregnant (*i.e.*, a cycle of 13 to 15 days). None of the five animals bearing control implants at the arcuate showed any disruption of the estrous cycle. By applying the Fisher exact probability test to the comparison of cycle lengthening between the db cyclic AMP and AMP groups, a value of $p = 0.007$ was obtained. Thus, db cyclic AMP at the level of the arcuate nucleus can disrupt the feedback control between brain, pituitary, and ovary.

With immature rats (body weight 95–130 g), it was noted that hyperphagia and an accelerated body weight gain occurred in the 3 days after db cyclic AMP implant into the mammillary region of the hypothalamus (weight gain, $\bar{x} = 19.0$ g; $n = 3$) as compared to implants elsewhere in the hypothalamus either of db cyclic AMP (weight gain, $\bar{x} = 5.5$ g; $n = 6$) or of AMP (weight gain, $\bar{x} = 3.0$ g; $n = 5$). Differences between the group with mammillary implants and the two control groups were significant at the 0.01 level in the t test. The body weight gain during days 4 to 14 was similar for all three groups with mean values of 36.7, 31.5, and 36.2 g.

In an earlier experiment each of the six immature female rats bearing implants of the potassium salt of db cyclic AMP in the arcuate or anterior hypothalamic area exhibited hyperthermia and the striking hyperactive and aggressive behavior recently reported by Krishna *et al.* (9). None of six rats with implants of the potassium salt of AMP was affected. The increased body temperature provided a convenient means of checking further the specificity of the cyclic nucleotide effects. Implants of the sodium salts of db cyclic AMP, cyclic AMP, AMP, and of cholesterol acetate were placed in female rats (125–155 g) in the small portion of the anterior hypothalamus bounded by de Groot coordinates A: 5.6 to A: 6.8, ventral to -2,

and within 1 mm of the midline. Five of eight animals with db cyclic AMP had a body temperature of 38.5° or higher during the first day while only 1 of 11 rats with implants of AMP showed a similar elevation. Four of seven animals with cyclic AMP also had an elevated body temperature. Values obtained by means of the Fisher exact probability test were $p = 0.023$ for the db cyclic AMP group, and $p = 0.047$ for the cyclic AMP group when compared with the AMP group. It is clear that cyclic AMP, independent of butyryl moieties or potassium ion, can lead to elevated body temperature. Therefore, it is likely that the prolongation of the estrous cycle and the initial hyperphagia also can be attributed to the cyclic AMP portion of the material implanted. These studies suggest that cyclic AMP may be involved in regulation of a variety of homeostatic mechanisms which are integrated at the level of the hypothalamus.

Summary. N⁶, O²-dibutyryl adenosine 3',5'-monophosphate implanted into various hypothalamic sites in immature rats results in hyperthermia, hyperphagia, and increased locomotor activity. Prolongation of the estrous cycle occurs in adult female rats. The cyclic nucleotide does not substitute for estradiol in inducing sexual receptivity.

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