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Intracranial Cycloheximide: Effects on Maternal Behavior in the Postparturient Rat

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HO, G. K., D. QUADAGNO AND H. MOLTZ. *Intracranial cycloheximide: effects on maternal behavior in the postparturient rat.* PHARMAC. BIOCHEM. BEHAV. 2(4) 455–458, 1974. – Cycloheximide (Cyclo), 100μg/μl saline infused bilaterally into the preoptic area (POA) of female rats on the fourth day postpartum, significantly suppressed maternal behavior for a period of some 72 hr. At the same time, females subjected to infusions of Cyclo in the caudate nucleus, as well as those receiving as s.c. injection of the drug, continued to show high levels of maternal behavior. Since, in addition, estrogen-progesterone induced lordosis was unaffected by POA infusion when the infusion occurred 24 hr after estrogen administration, the view was advanced that (a) maternal behavior was selectively suppressed, (b) the suppression was produced through biochemical rather than through cytotoxic action, and (c) continuing protein synthesis, particularly in the POA, is essential for the maintenance of such nurtural responses as nursing, nest building, licking, and retrieving.

Maternal behavior Cycloheximide Protein inhibition

SUCH ANTIBIOTICS as actinomycin D (Act-D) and cycloheximide (Cyclo), when implanted or infused into the preoptic area (POA), act to block steroid-induced sexual receptivity in the ovariectomized rat [2, 9, 11]. Since both drugs inhibit protein formation – Act-D by suppressing DNA-dependent mRNA [3] and Cyclo by direct action at the ribosomal level [10] – the conclusion was drawn that estrogen facilitates lordotic behavior by acting on selected loci in the brain to promote the *de novo* synthesis of protein [11]. The purpose of the present study was to determine whether protein synthesis is also involved in the expression of maternal behavior.

Evidence has recently been advanced to indicate that the hormones accompanying the termination of pregnancy underlie the induction of maternal behavior in the primiparous rat [5,13]. In this connection, it has been shown that a regimen of estrogen and prolactin injected against a background of progesterone withdrawal will induce in the nulliparous female all those nurtural responses her puerperal counterpart characteristically displays toward young.

But while the induction of maternal behavior appears to be hormone dependent, its maintenance does not [4]. Thus, once the behavior is evinced – once nursing, nest building, licking and retrieving are expressed – it will survive adrenalectomy and ovariectomy [4,12], as it will the inhibition of prolactin release through the injection of ergocornine [8]. In brief, it would seem that, upon being activated hormonally, the neuronal mechanisms underlying maternal behavior are sustained, thereafter, by the stimulative properties of the young.

One question that arises here concerns the role of protein synthesis in the maintenance if not in the induction of maternal behavior. Perhaps such synthesis, while initially facilitated through hormone action, is subsequently sustained through pup stimulation. If that is the case and, if in turn, continued synthesis is essential for maintenance, then cycloheximide should act to suppress ongoing nurtural activity. Accordingly, we decided to infuse this glutarimide antibiotic into the POA beginning on Day 4 following parturition. The POA was chosen as the site of infusion since a graduate student (working in the laboratory of

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H.M.) showed that the medial aspect of the POA, possibly through its connections with the lateral hypothalamus, is critical for the continued expression of nursing, nest building, and retrieving [7].

METHOD

Pregnant Sprague-Dawley females, about to give birth for the first time, were housed in plastic maternity cages measuring 25.0 cm wide x 50.8 cm long x 16.5 cm deep. Each animal was maintained under a reversed day-night cycle (lights on at 0200 and off at 1400 hr) and provided with food and water ad lib. Within 12 hr after delivery, every litter was reduced to 6 in number and each female, in turn, assigned at random to one of the following treatment groups.

Cyclo-POA (N=8) – On the afternoon of the fourth day following delivery, the females of this group received bilateral infusions into the POA of 50 μg of cycloheximide to a total of 100 μg in 4 μl of saline.

Cyclo-CN (N=9) – These females also received bilateral infusions (100 μg) cycloheximide on Day 4, but into the caudate nucleus (CN) rather than into the POA.

Cyclo-SC (N=5) – For this group, a total of 100 μg of the antibiotic was injected sc.

Saline-POA (N=5) – As with the females just mentioned, those of the present group were also treated on the fourth day following delivery. Here, however, the saline vehicle alone was infused bilaterally into the POA.

All infusions were carried out under ether anesthesia using a 22-ga stainless steel cannula attached to a microsyringe to deliver the solutions. Into each solution, India ink was mixed to serve as a histological marker. Following DeGroot [1] the coordinates used for the POA infusion were 7.0–8.0 mm anterior from the vertical zero plane, 0.5–1.2 mm lateral from the midline and 1.0 mm below the horizontal zero plane. For the CN, the boundaries were taken as 7.4–8.2 mm anterior from the vertical zero plane, 2.0–4.0 mm lateral from the midline, and 1.0–3.0 mm above the horizontal zero plane.

For 4 days prior to treatment and for 5 days following treatment, each female was observed for 30 min at 0900 hr and again at 2100 hr. Scores of nursing, nest building, licking, and retrieving were recorded using criteria discussed previously by Moltz and Robbins [6]. Due to pup mortality, it was necessary to proffer additional young to several of our females to maintain their litters at full complement.

As one way of assessing the nonspecific effects of cycloheximide – arising perhaps from the general toxicity of the drug – 3 additional groups of postparturient females were used. Each had her litter removed two days after delivery and each was then given 3 μg estradiol benzoate on Day 2 and again on Day 3, followed by 0.5 mg progesterone on Day 5. For one of these groups, cycloheximide was infused into the POA on Day 4; for another, only the saline vehicle was infused; and for the third, no infusion at all was carried out. On Day 5, some 5 hr after the injection of progesterone, each female was placed with a sexually vigorous male and a lordosis quotient was computed (LQ = number of lordosis responses/20 mounts by the male x 100).

It is important to note that some 24 hr intervened between the second injection of estrogen and the infusion of cycloheximide. As Quadagno, Shryne and Gorski [9] and Quadagno, Ho and Hough (to be published) point out in the case of actinomycin D and cycloheximide, respectively, the POA is particularly sensitive to protein inhibition during the first 12 hr after estrogen administration, and that by 24 hr, this sensitivity largely disappears. Consequently, if, after an estrogen-to-drug interval of 24 hr, a significant inhibition in sexual activity is seen, it could be attributed, not as much to protein inhibition, as to the cytotoxicity of the drug.

RESULTS

Prior to treatment, all animals were fully maternal, displaying nursing, nest building, licking, and retrieving, in characteristic fashion. Following treatment, a different picture emerged, as represented in Fig. 1. Bilateral infusion of cycloheximide into the POA severely depressed maternal behavior for a period of some three days, whereas systemic administration of the drug or its infusion into the caudate nucleus had no effect at all. A slight depression in maternal behavior was seen in the Saline-POA females, but this was evidenced for only one day and, upon comparison with the Cyclo-CN and Cyclo-SC groups, proved statistically insignificant ($\chi^2 = 0.91; p > 0.05$). Additional chi-square analyses revealed that during each of the first 3 days following treatment, the percentage of Cyclo-POA females displaying all four maternal responses was significantly lower than the comparable percentages in the remaining three groups ($p < 0.01$).

The behavior of individual females is of course not shown in Fig. 1 nor is the differential survival of their litters. However, in this connection it can be pointed out that for at least 36 hr following infusion, 6 out of 8 Cyclo-POA females failed to display any maternal interest at all in their young, whereas the females of every other group were observed either to be fully maternal or to exhibit at least 3 of the 4 recorded nurtural responses. As a result of this initial post-treatment neglect on the part of the Cyclo-POA animals, 15 of their young died, while only one pup in the remaining 3 groups was found dead. Additionally, 3 mothers in the Cyclo-POA group cannibalized their young, a behavior exhibited by none of the other females.

However, following this cycloheximide-induced depression in maternal attention, recovery of the behavior did occur. By the fourth post-treatment day, for example, 5 of our 8 Cyclo-POA females were once again observed to be fully maternal and by the fifth day 2 more were seen to reconstruct their nests and then retrieve, lick, and nurse their young. Subsequently, the young of these 7 females survived to weaning.

In contrast to its effect on maternal behavior, the infusion of cycloheximide into the POA did not produce any observable inhibition of lordosis. Specifically, the LQ scores for our Cyclo-POA, Saline-POA, and Control females were 76.67, 83.33, and 85.00 respectively, with a one-way analysis of variance revealing no significant differences.

Histological examination showed that, in all but 2 females initially assigned to the Cyclo-POA group, the tip of the cannula was well within the boundaries of the area intended. These 2 animals were excluded from the study. For the Cyclo-CN group, no animals were discarded since each cannula was found to be accurately placed.

DISCUSSION

The use, as in the present study, of centrally-

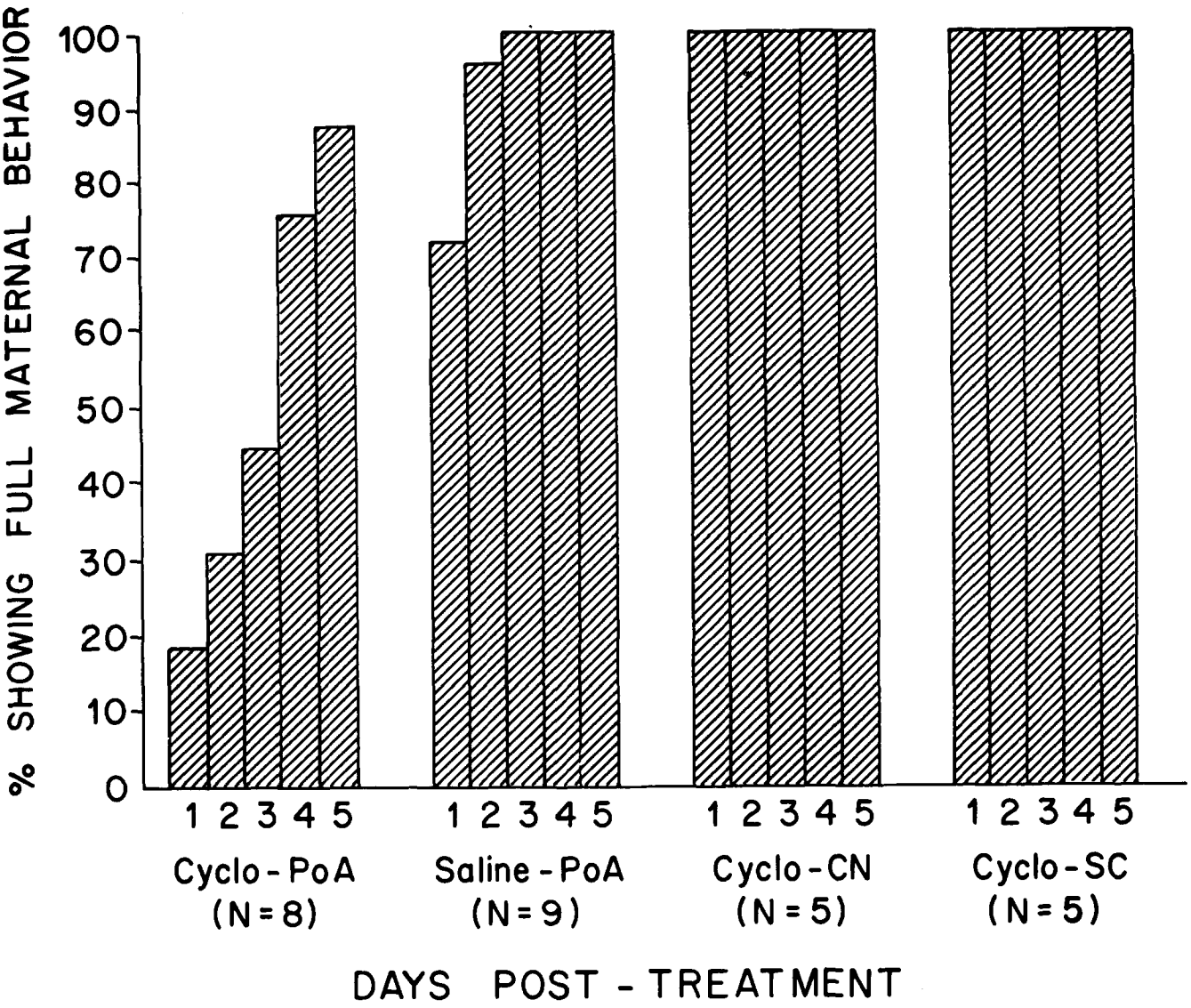

FIG. 1. Percentage in each group showing full maternal behavior.

administered cycloheximide raises two related questions. (a) Was the observed behavioral suppression due to protein inhibition, or was it due to some cytotoxic action of the drug, resulting perhaps in cell death at the site of infusion; [9] and (b) whether biochemically or cytotoxically induced, was the effect specific to maternal behavior, or were other, unrelated, behaviors also involved? Although more data are needed here to provide definitive answers, it is possible nonetheless to advance some tentative conclusions. First, it appears that POA-infused cycloheximide probably acted along reversible biochemical rather than along irreversible cytotoxic pathways, as evidenced by the reappearance of maternal behavior after an initial period of marked suppression. And second, at least some specificity of effect did in fact occur in the present experiment, since maternal but not lordotic behavior was inhibited under the conditions employed. Of course, with respect to this latter point, it is obviously impossible to examine all behaviors in an effort to determine whether just one was disrupted. However, the possibility that unrelated behaviors were affected through general debilitation can safely be ruled out, for all our animals ate and drank in a normal manner and none showed any weight loss following treatment. Indeed, in the latter regard, our several groups were virtually identical, with the Cyclo-POA females, for example, having an average weight of 350 g on day 3 posttreatment and the Saline-POA an average weight of 353 g. Moreover, as already emphasized, during the very time that our Cyclo-POA animals exhibited the most marked depression in maternal behavior, other females, similarly infused with the drug, were exhibiting high lordotic quotients. (Since the completion of the present experiment, 5 additional Cyclo-POA females were run, each being injected with estrogen and progesterone while allowed to remain with their own litters. When tested 24 hr after POA-infusion (Day 1 posttreatment), 4 of these females showed lordotic behavior,

while none showed maternal behavior. Thus, in one and the same animal, cycloheximide differentially affected sexual and maternal behavior, which is further evidence, of course, for its specificity of action).

The data of the present experiment are the first to suggest that continued protein formation in the hypothalamus is essential for the maintenance of maternal behavior in the postparturient rat. Since the maintenance of this behavior, as distinct from its induction, does not require endocrine facilitation [4, 8, 12], it becomes of interest to speculate as to what postparturient conditions do promote protein synthesis and how such synthesis, in turn, mediates the continuing nurtural involvement of the mother. Some insight into these questions has been provided by research recently completed in one of our laboratories (H.M., in collaboration with M. Steele, D. Rowland, and A. Halaris) on changes in the concentration of hypothalamic norepinephrine (NE) during pregnancy and lactation. What was found was a significant elevation of NE during the postpartum period, the period of active concern with young. We conceive of this increase in NE to have been induced initially by hormonal changes accompanying the termination of pregnancy and, thereafter, as being sustained, not by further endocrine facilitation, but simply by the sight, sound and odor of the young. We futher assume that high levels of this putative neurotransmitter reflect high impulse traffic and that such traffic, within the hypothalamus, is essential for maternal behavior. Given these assumptions, the suppressive effects of cycloheximide can be explained briefly as follows. In inhibiting protein formation, cycloheximide inhibits, among other physiological processes, those enzymes essential for the continued synthesis of NE and hence the continued interest of the mother as expressed in nursing, nest building, licking, and retrieving. This leads to the prediction that pharmacologic agents which interfere specifically with the formation of hypothalamic NE - or with noradrenergic receptor activity in the same locus – will act to suppress maternal behavior in the otherwise highly maternal postparturient rat.

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