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Effects of Central Norepinephrine Depletion on the Initiation and Maintenance of Maternal Behavior in the Rat[1]

PHYLLIS ROSENBERG, ANGELOS HALARIS AND HOWARD MOLTZ[2]

Committee on Biopsychology, Department of Behavioral Sciences and Psychiatry
University of Chicago, Chicago, IL 60637

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ROSENBERG, P., A. HALARIS AND H. MOLTZ. *Effects of central norepinephrine depletion on the initiation and maintenance of maternal behavior in the rat.* PHARMAC. BIOCHEM. BEHAV. 6(1) 21–24, 1977. – The catecholaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), was used to test the hypothesis that increased transmission across selected noradrenergic synapses is involved in the initiation of maternal behavior. Specifically, 6-OHDA was infused intraventricularly either two days before parturition or four days after parturition. Control animals were infused with the vehicle alone. Among prepartum animals, NE depletion of more than 30 percent of control levels interfered with the initiation of maternal behavior. Among lactating animals, similar degrees of NE depletion had no significant effect on the maintenance of maternal behavior. Thus, NE appears to be involved in the initiation of maternal behavior, but not in the maintenance of the behavior once that behavior is established.

Norepinephrine initiation Maternal behavior 6-Hydroxydopamine

RECENT findings in our laboratory [4] indicate that, during the period immediately following parturition in the rat, the concentration of hypothalamic norepinephrine (NE) decreases while that of its major metabolite, 3-methoxy-4-hydroxy-phenylglycol (MHPG), increases. This is to be compared with low hypothalamic NE metabolism during most of pregnancy. The possible significance of such an increase in hypothalamic NE metabolism during the early postpartum period has been interpreted by Moltz *et al.* [4] as a possible neurochemical prerequisite to the onset of maternal behavior. Moreover, additional evidence for this view has been provided by the finding that parallel increases in the metabolism of hypothalamic NE have also been found in the maternally-behaving nulliparous animal but not in control females that do not respond to foster young [5].

To test further the hypothesis that increased transmission across selected noradrenergic synapses is involved in the initiation of maternal behavior, the catecholoaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), was administered intraventricularly to primiparous rats two days before the onset of parturition. To test whether NE is also involved in the maintenance of maternal behavior once that behavior is established, 6-OHDA was similarly infused four days after parturition.

METHOD

Animals

The animals were 42 primiparous Wistar female rats, between 120–180 days of age, reared and mated in the authors' laboratory from a stock obtained from the Camm Research Institute, Wayne, NJ. The females were maintained on a day–night cycle of 12/12 (lights on at 8:00 a.m.), and from the time of weaning were isolated from lactating animals with young.

Surgery

On Day 13 of gestation the females were anesthetized with intraperitoneal injections of sodium pentobarbital (Nembutal – 40 mg/kg), and placed in a Kopf stereotaxic. Guide cannulae, fashioned from 22 ga stainless steel tubing were implanted unilaterally 1 mm above the left lateral ventricle according to the stereotaxic atlas of DeGroot (A-P = 6.6 mm, Lat = 1.1 mm, DV = 4.0 mm) and secured to the skull with dental cement. A dummy cannula consisting of 28 ga filled wire was inserted into the guide cannula and remained in place at all times except during infusion. After surgery the females were placed in individual wire mesh cages and were allowed 1–2 weeks to recover.

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Drugs

Four groups were included in the design. Two groups of females received either unilateral infusions of 6-OHDA (n = 14) or the vehicle alone (n = 8) two days prior to the expected date of parturition (Prepartum Groups). The remaining two groups received similar infusions of 6-OHDA (n = 10) or vehicle alone (n = 10) on postpartum Day 4 (Postpartum Groups). 6-OHDA (6-hydroxydopamine hydrobromide, Regis Chemical Co.) was dissolved in 0.9% saline solution to which 0.5% ascorbic acid was added to prevent auto-oxidation. The drug was administered immediately upon solution at a dosage of 200 μg per 20 μl of vehicle fluid, (6-OHDA expressed as the salt).

The females were lightly anesthetized under ether during infusion. Each injection was delivered at a rate of 10 μl per min through a 28 ga injector cannula attached by silastic tubing to a 10 μl Hamilton Syringe. The injector cannula was constructed so that when inserted into the guide cannula, it extended 1 mm beyond the end of the guide cannula into the ventricle. In order to administerer the complete dosage, the injector cannula was removed 30 sec after the first 10 μl were delivered, quickly refilled, and reinserted into the guide cannula and the remainder of the drug immediately administered. Sixty sec later the injector cannula was removed and the dummy cap was again in place.

Testing

Two days before parturition all females, regardless of group membership, were placed in individual observation cages (8.58 x 7.80 cm) equipped with a front panel of clear Plexiglas to provide an unobstructed view of the interior. Food and water were available ad lib and paper strips were provided as nest material. The females were observed for the occurrence of parturition, and on the day of birth litters were counted and weighed and the quality of the maternal nest assessed according to methods previously described [5]. The litters were then culled to six and rotated so that each female received 6 pups from a litter other than her own. This was usually accomplished by simply switching the pups between a 6-OHDA and corresponding vehicle-treated animal. A standard maternal behavior test was conducted in which the litter of 6 pups was scattered about the cage and latency to retrieve, lick and crouch over the young was observed throughout the next hour and at random intervals thereafter for the next 24 hr. Retrieving was judged to have occurred if the pups were returned to the nest area. Crouching and licking were scored positively if a female was seen in a nursing-like posture over the young and the young in turn were clean and warm. Testing continued for the next 7 days in Prepartum animals and for 10 days in Postpartum animals, beginning at 10 a.m. each morning. At this time the litters and mothers were weighed, the quality of the maternal nest scored, and behavior tests were conducted. Pups in a litter were replaced only when found dead so that the litters were maintained at 6 live pups.

Assay

Between seven and nine days after infusion, the females were guillotined and the hypothalamus quickly dissected and stored in liquid nitrogen until a sufficeint number was collected for assay. Care was taken to sacrifice each female at approximately the same time of day, that is, at 1:00 p.m. Hypothalamic tissue was assayed for the presence of norepinephrine (NE) and dopamine (DA) following the methods of Anton and Sayre [1] modified according to Barchas *et al.* [2] and described in detail elsewhere [5].

RESULTS

Infusions of 6-OHDA two days prior to birth had no disruptive effects on the timing of parturition nor on the immediate survival of the young. 6-OHDA-treated females gave birth at the expected time (Day 23) to litters which did not differ from corresponding vehicle-treated females either in size or weight. Moreover, the infusion procedure itself did not appear to disrupt parturition as evidenced by the fact that the gestation day, litter size, and litter weights of females receiving infusions prior to parturition were within the same range as those observed in females which were untreated until four days after giving birth, $F(1) = 3$, $p>0.05$.

On postpartum Days 1–7, the litters of 6-OHDA Prepartum females were markedly different from those of control females. As Fig. 1 shows, litters of 6-OHDA Prepartum females either gained less weight per day or showed weight losses as compared with litters of corresponding vehicle-treated animals, $t(20) = 2.20$, $p<0.05$. Among the litters of 6-OHDA Prepartum females, a total of 50 pups was found either dead or missing during the 7-day postpartum test period. Only one pup died in the litters of vehicle-treated females. Moreover, among the surviving pups reared by 6-OHDA Prepartum mothers, weight gains were either absent or depressed as compared with pups reared by vehicle-infused mothers.

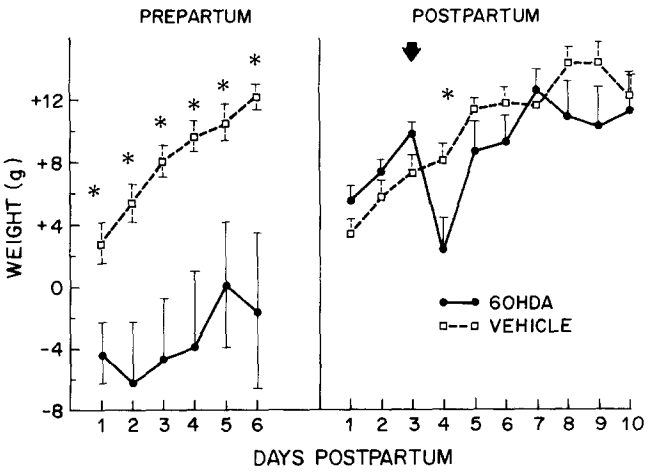

FIG. 1. Daily weight changes of litters reared by females receiving infusions of 6-OHDA or vehicle either 2 days prior to parturition (Prepartum) or on postpartum Day 4 (Postpartum). Arrow indicates day of infusion. * = $p<0.05$.

To assess whether the reduced survival of 6-OHDA litters was in fact due to a disruption in maternal behavior rather than to an interference with the process of lactation, the daily maternal-behavior scores of 6-OHDA Prepartum and corresponding vehicle-treated females were compared. As Fig. 2 shows, females treated with 6-OHDA two days prepartum built significantly poorer maternal nests than corresponding vehicle-treated animals, particularly during

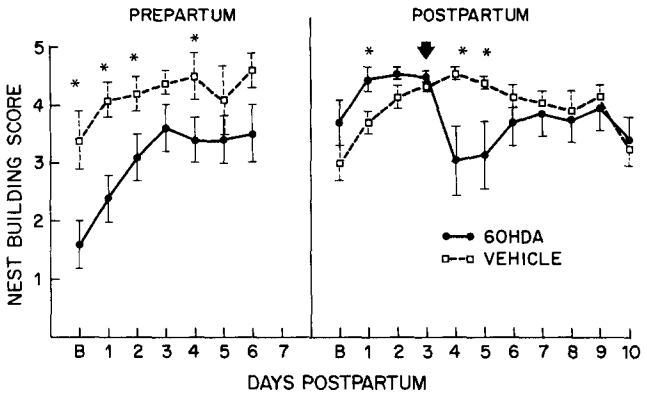

FIG. 2. Daily nest building scores for females receiving infusions of 6-OHDA or vehicle either 2 days prior to parturition (Prepartum) or on postpartum Day 4 (Postpartum). Arrow indicates day of infusion. * = $p<0.05$.

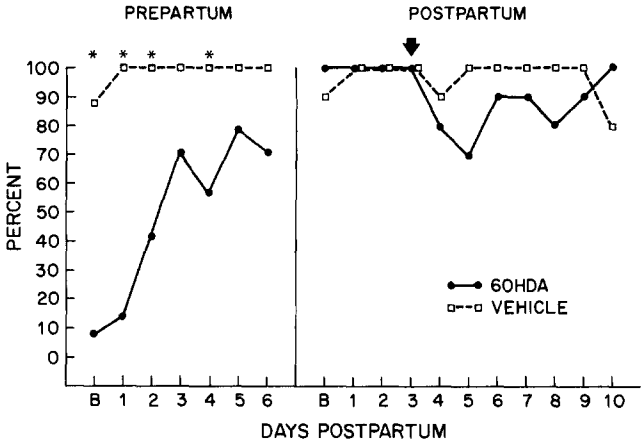

FIG. 3. Percentage of females receiving infusions of 6-OHDA or vehicle either 2 days prepartum or 4 days postpartum that were found nursing their young during each daily observation period. Arrow indicates day of infusion. * = $p<0.05$.

the first 3–5 days of the postpartum period, $t(20) = 2.20$, $p<0.05$. Moreover, daily observations of crouching behavior indicated that 6-OHDA Prepartum females adopted little if any nursing posture toward their pups, so that the majority of their litters had no access to the nipple or the warmth of their mothers (Fisher test, $p<0.05$, see Fig. 3). No disruption in crouching behavior was observed in corresponding vehicle-treated females. In contrast, the retrieving behavior of 6-OHDA Prepartum females was not significantly disrupted during standard retrieving tests.

In contrast to prepartum treatment with 6-OHDA, postpartum treatment had only a transient effect on maternal behavior, an effect confined mainly to the day following infusion. By the second day after infusion, weight gains of litters and maternal behavior scores were indistinguishable between the two groups treated postpartum.

Posttest fluorimetric assays for NE and DA revealed that 6-OHDA treatment administered either in late pregnancy or on Day 4 postpartum resulted in approximately 30–60% depletion of hypothalamic NE as compared with NE levels in corresponding vehicle-treated animals (see Table 1). In view of the behavioral differences between the pre- and postpartum drug infused animals, it is important to emphasize that both sustained the same degree of NE depletion. Hypothalamic values for DA did not differ between 6-OHDA- and vehicle-treated animals (Table 1).

TABLE 1

HYPOTHALAMIC NE AND DA CONCENTRATIONS FOR FEMALES RECEIVING INTRAVENTRICULAR INFUSIONS OF 6-OHDA OR VEHICLE BEFORE (PREPARTUM) OR AFTER (POSTPARTUM) PARTURITION

	Prepartum		Postpartum	
	6-OHDA	Vehicle	6-OHDA	Vehicle
NE (ng/g)	61.8	143.2	47.1	128.3
(SE)	(5.2)	(11.7)	(1.6)	(3.2)
DA (ng/g)	170.1	178.0	131.9	142.4
(SE)	(14.1)	(22.7)	(25.2)	(38.3)

DISCUSSION

Intraventricular infusions of 6-OHDA significantly disrupted maternal behavior when administered to the preparturient female, but not when given to the female that had successfully nursed her young for 4 days prior to infusion. Moreover, this differential behavioral effect in the face of the same degree of NE depletion indicates that the disruption was not due to general debilitation. These results are consistent with the hypothesis that noradrenergic activity in selected neural structures may be involved in the initiation of maternal behavior; at the same time such activity does not appear to be involved in the maintenance of maternal behavior. However, a number of questions still remain. First, it is not clear from the present study which specific neural structures mediate the noradrenergic initiation of maternal behavior. The hypothalamus is clearly implicated as a likely target area based on the observation that changing hypothalamic levels of NE are closely linked to the initial appearance of the behavior [4,5]. However, the involvement of cortical and limbic structures cannot be ruled out.

Another question that might be raised relates to the observation that some maternal responsiveness in prepartum 6-OHDA-treated females, namely, nest building and crouching, appears four or five days following initial infusion of 6-OHDA. The late appearance of these responses may suggest that noradrenergic activity has only a short-lived effect on the initiation of maternal behavior or, more likely, that the eventual appearance of maternal behavior is due instead to the developing supersensitivity of remaining noradrenergic synapses for NE [3].

Finally, the failure of 6-OHDA to disrupt maternal behavior in the lactating females even while substantially depleting the hypothalamus of NE suggests that the maintenance of maternal behavior is independent of hypothalamic NE activity, possibly relying instead on dopaminergic or serotonergic systems. However, another explanation can be offered. It is well known that the lactating females reacts differently from her nonlactating counterpart to a variety of stimuli, including responsiveness to ether stress, shock-induced fighting [7] and re-

sponsiveness to pentobarbital-induced disruption of temperature regulation [6,8]. The failure of 6-OHDA to disrupt maternal behavior in the lactating females may also be due to altered responsiveness. In this case the hormonal and neural events of lactation may alter the sensitivity of post-synaptic receptors to noradrenergic stimulation. That is, the lactating female may be so highly sensitive to such stimulation that what little NE remains after 6-OHDA infusion is sufficient to support maternal behavior. Additional research is necessary to clarify this possibility.

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