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Changes in the Metabolism of Hypothalamic Norepinephrine Associated with the Onset of Maternal Behavior in the Nulliparous Rat[1]

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ROSENBERG, P., L. LEIDAHL, A HALARIS AND H MOLTZ. *Changes in the metabolism of hypothalamic norepinephrine associated with the onset of maternal behavior in the nulliparous rat* PHARMAC. BIOCHEM. BEHAV. 4(6) 647–649, 1976 – Both norepinephrine (NE) and its major metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), were assayed both in the hypothalamus of nulliparous rats that had behaved maternally toward foster young and in the hypothalamus of those that had failed to behave maternally. It was found that the maternally-behaving animals had both lower concentrations of NE and higher concentrations of MHPG as compared with their nonresponding counterparts. These data parallel those reported for the puerperal female and suggest that the onset of maternal behavior may be mediated by increased transmission across hypothalamic noradrenergic synapses

Norepinephrine 3-Methoxy-4-hydroxyphenylglycol Hypothalamus Maternal behavior Parturition

RECENT work in our laboratory [9] involved the measurement of norepinephrine (NE) and that of its major metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), in rat hypothalamus during selected stages of pregnancy and lactation. It was observed that shortly after parturition the concentration of NE decreased sharply while that of MHPG showed a correspondingly sharp increase. Speculating as to the possible significance of such an increase in hypothalamic norepinephrine metabolism, Moltz *et al* [9] suggested that it might be related to the onset of maternal behavior.

It is well known that the parturient rat exhibits such maternal responses as nursing, licking, and retrieving almost immediately upon the emergence of the young [8,15]. Less well known, perhaps, is the fact that many nulliparous females will also behave maternally when given the opportunity to associate continuously with young, a procedure known as sensitization or concaveation [4, 14, 18]. Although, in the case of the nulliparous female, several days are usually required for the behavior to appear, nonetheless when it does appear, it is very similar to that exhibited by the parturient female [13,14] Accordingly the question arose as to whether an increase in the metabolism of hypothalamic NE similar to that found in the puerperal animal can also be found in the nulliparous animal once she responds to foster young. This is an important question, because, unlike her puerperal counterpart, the nullipara that begins to behave maternally is not at the same time experiencing the endocrine events that accompany lactation and parturition. Indeed she presents an entirely different endocrine profile. Consequently if we were to find similar changes in amine metabolism in the 2 preparations, then such changes could be linked that much more closely to the behavior itself. Moreover, to control for the possibility that the behavior as such is critical – and that any observed amine changes are not merely the result of exposure to young – we compared nulliparous females that responded maternally with females that were in association with young for the same length of time and under the same conditions, but which nonetheless failed to respond.

METHOD

The subjects were adult virgin Wistar rats reared in our laboratory under a day–night cycle of 12/12 (lights on at 8·00 a.m.). From the time of weaning they were housed in groups of 10 and isolated from lactating animals with young. Between 90 and 130 days of age, these females were placed in individual cages (8.58 × 7.80 cm), each 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. On the first day of observation at 10:00 a.m., 3 healthy foster pups, 1–5 days of age, were proffered to each female. These young were allowed to remain with the

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female until the following day at which time a new litter of the same age was substituted. Three times daily, beginning immediately after presentation of young, and then at 4 and 24 hr, respectively, observations were made for evidence of maternal behavior, that is, for evidence of retrieving, licking, and crouching. 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 to be in a nursing-like posture over the young and the young in turn were clean and warm. In addition, the quality of the maternal nest was assessed according to the methods of Numan [10] and Quadagno and Rockwell [12].

When an animal had displayed the full spectrum of maternal behavior for 3 consecutive days she was guillotined and the hypothalamus quickly dissected and stored in liquid nitrogen until a sufficient number was collected for assay. Care was taken to sacrifice each female at approximately the same time of day, that is, at 10 00 a.m.

Temporally coupled or yoked to each experimental female was a female that showed no maternal behavior whatsoever, despite having been housed with foster young for the same length of time and under the same conditions. These control females were of course also sacrificed for assay.

Different animals were used in assaying NE and MHPG· a total of 90 for NE and 77 for MHPG. For the assay of NE, individual hypothalami were weighed, homogenized in 0.4 N perchloric acid and centrifuged. Following pH adjustment to 6.5 and centrifugation to remove the salt, the supernatant was passed onto Amberlite (CH-50) columns according to the method described in Moltz *et al.* [9]. NE was then eluted from the columns in 1 N hydrochloric acid and was determined spectrofluorometrically by the method of Anton and Sayre [1] modified according to Barchas *et al* [2].

MHPG was assayed using the method of gas liquid chromatography developed by one of us (A H.) for hypothalamic tissue as described in Moltz *et al* [9]. Individual hypothalami (40–50 mg in weight) were homogenized in 0 4 perchloric acid and centrifuged The supernatant was incubated overnight at 37°C with 0.1 ml glusulase (Endo Laboratories, Inc.). The sample was then extracted with 3 ml nanograde ethylacetate (Mallinckrodt) and a derivative formed by the addition of 0.1 ml pentafluoropropionic anhydride (Pierce Chemical Corp.). The sample was dried under a stream of nitrogen, redissolved in ethylacetate, and analyzed in a Gas Chromatograph (Packard Model A 7400).

RESULTS

Figure 1 shows the mean concentration of NE and MHPG for females that responded maternally and for females that failed to respond. As might be anticipated from inspection of the figure, analysis of variance yielded a significant F ratio for NE ($F(1,86) = 7.07$, $p<0.01$, as well as for MHPG $F(1,60) = 10.14$, $p<0.001$. Specifically, animals that behaved toward young by displaying nursing, nest building, and retrieving had both lower concentrations of hypothalamic NE and higher concentrations of hypothalamic MHPG as compared with those that did not.

DISCUSSION

Our major finding is that nulliparous female rats that responded maternally toward foster young had both lower

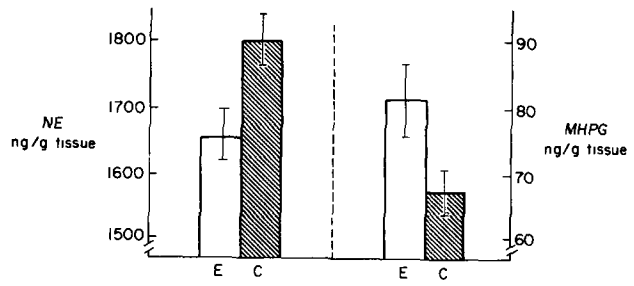

FIG 1. Mean Concentrations of NE and MHPG in Experimental (E) and Control (C) Animals Vertical lines represent ± SE.

concentrations of hypothalamic NE and higher concentrations of MHPG, its major metabolite, than did nonresponding females. This finding is similar to that reported for the puerperal animal which, it will be recalled, also shows a marked increase in NE metabolism [9]. The fact that both the puerperal female and her maternally-behaving nulliparous counterpart exhibit parallel changes in norepinephrine activity is consonant with the suggestion offered by Moltz *et al* [9] that the onset of maternal behavior may be mediated by increased transmission across hypothalamic noradrenergic synapses. However, 2 points need be raised in this connection.

First, there are data to suggest that biogenic amines, particularly in the hypothalamus, undergo sharp fluctuations in response to changes in steroid levels [3, 5, 6, 7, 16]. Although, as already mentioned, the maternally-behaving nulliparous female does not undergo the particular endocrine changes known to accompany parturition and lactation, there is the possibility that she may experience other endocrine changes, perhaps as a consequence of her exposure and response to young. In that case, fluctuations in the metabolism of hypothalamic norepinephrine may only be incidentally associated with the appearance of maternal behavior. With this in mind, we monitored changes in the steroid levels of maternally-behaving nulliparae by following their estrous cycles from the time of their initial introduction to young. No systematic disruption of cyclicity was observed (unpublished data). Moreover, it has already been shown that the onset of maternal behavior in the virgin animal is not associated with any particular phase of the estrous cycle [14].

Second, since our animals were sacrificed 3 days after the onset of maternal behavior, the results of this study do not indicate whether noradrenergic synapses are involved in the induction of the behavior. Moreover, recent evidence indicates that brain amine activity may fluctuate in response to behavioral changes as well as endocrine changes [17]. Thus, the present data do not answer the question of whether the observed increase in NE activity is a prerequisite or, conversely, a consequence of maternal responsivity.

In an effort to explore this question and to provide additional information regarding the involvement of NE in initiating the nurtural behavior of both the puerperal and nulliparous female, NE levels would have to be manipulated either pharmacologically or surgically prior to the onset of maternal behavior. Pharmacologically, by specifically inhibiting NE synthesis through the injection of diethyldithiocarbamate (DDC), for example. And surgically, by

sectioning the ventral NE pathway from the medulla and pons, the pathway that Olson and Fuxe [11] identified as innervating the basal and lateral regions of the hypothalamus and the preoptic area

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