NOTE
Lack of Effects of Melatonin Administration and Pinealectomy on the Milk Ejection Response in the Rat

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Synopsis

The effects of melatonin administration and pinealectomy on the milk ejection response were studied in the rat. The amounts of milk obtained by a litter during 1 hr suckling and during an additional 30 min suckling after 1 I.U. of oxytocin injection, following 7 or 8 hr separation from the mother on the 10th or 16th day of lactation, were considered as the naturally ejected milk and the residual milk and used as the index of the milk ejection response. Melatonin in doses of 0.8 or 1.6 mg was injected subcutaneously into the mother 2 hr before the suckling commenced. The amounts of the naturally ejected milk and the residual milk were not significantly influenced by these treatments compared with saline injected controls. The pinealectomized and sham-operated female rats were bred and the postpartum lactation was studied. No significant difference was observed between the 2 groups in the litter growth for 12 days from the 4th day of lactation, the total feed consumption of the mother during this period and also the milk ejection response determined on day 16 of lactation. Thus, the administration of melatonin and pinealectomy did not exert any significant effect on the milk ejection response under these experimental conditions. Also, pinealectomy did not affect the incidence of the vaginal estrous smear and the length of gestation period.

The mammalian pineal gland contains significant quantities of a variety of biogenic amines. The most pineal specific of these is melatonin (N-acetyl-5-methoxytryptamine). The biosynthesis of melatonin involves the N-acetylation of serotonin (5-hydroxytryptamine) and subsequent O-methylation. Although the acetylating enzyme is present in several tissues, the enzyme responsible for O-methylation has been found only in pineal (Axelrod and Weissbach, 1961), therefore melatonin is uniquely produced in the pineal gland. The concentration of serotonin is higher in the pineal gland than in any other organ in the rat (Bertler et al., 1964; Quay, 1963; Snyder et al., 1965). Moreover, a peptide with pressor, antidiuretic, rat and hen oxytocic activity was isolated from the bovine and swine pineal glands (Milcu et al., 1963; Pavel, 1965). In addition, the intravenous administration of protein extract of bovine pineal gland caused an increase in the oxytocic activity of the paraventricular nucleus in the dog and this increased concentration of oxytocin was considered to be due to the blocking oxytocin release (Milcou and Pavel, 1960).

Serotonin was reported to inhibit the milk ejection response when injected subcutaneously into the postpartum lactating rat, and it was suggested that serotonin might inhibit centrally the reflex release of oxytocin in response to the suckling stimulus (Mizuno et al., 1967). So far as the writers know, however, limited informations are available concerning the pineal gland and lactation. There is a

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recent report that pinealectomy did not influence the milk production (Nir et al., 1968). In view of the similarity in the chemical structure of melatonin to serotonin and the intimate association of these two amines in the pineal gland, it was thought desirable to examine the effect of melatonin administration as well as of pinealectomy on milk ejection response in the rat.

Materials and Methods

Virgin female rats, 4 months old, of the Wistar strain were used. They were kept in a temperature (25±1°C) and light (14 hr lighting) controlled animal room. The synthetic diet and water were always available.

Experiment 1.
The effect of melatonin on milk ejection was studied. Animals were bred, and after parturition the number of the young was reduced to 8 on the 4th day of lactation. The litter weight and the body weight and feed consumption of the mother were recorded daily. The milk ejection response was estimated on the 10th day of lactation. The difference in the weight of litter during the 1 hr suckling period after 8 hr separation from the mother was considered the amount of naturally ejected milk. To obtain the residual milk 1 I.U. of oxytocin (Pitocin, Parke Davis & Co.) was injected subcutaneously into the mother and the litter was allowed to suckle for an additional 30 min and weighed. The mothers were divided into 3 groups and injected subcutaneously with saline, 0.8 and 1.6 mg of melatonin in a volume of 0.2 ml, respectively, 2 hr before the suckling commenced. Melatonin was dissolved in ethanol, which was diluted to 5% with saline.

Experiment 2.
Experimental animals were pinealectomized by the method described by Hoffman and Reiter (1965), and controls were sham-operated at the same time. Following operation, daily vaginal smears were made on all animals. They were mated after the 4th cycle of estrus.

Table 1. Effect of melatonin administration on milk ejection response in rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of rats</th>
<th>Body wt. of mother on day 10 of lactation (g)</th>
<th>Body wt. gain of litter from day 4 to day 10 of lactation (g)</th>
<th>Amount of milk during 1 hr suckling (g)</th>
<th>Amount of residual milk for 30 min after oxytocin (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, saline</td>
<td>6</td>
<td>314±4</td>
<td>76±6</td>
<td>6.5±0.8</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>Melatonin, 0.8 mg</td>
<td>8</td>
<td>322±9</td>
<td>78±2</td>
<td>6.6±0.9</td>
<td>1.8±0.5</td>
</tr>
<tr>
<td>Melatonin, 1.6 mg</td>
<td>4</td>
<td>313±5</td>
<td>82±5</td>
<td>5.9±0.3</td>
<td>2.7±0.4</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, Injected s.c. 2 hr before the suckling commenced.</td>
<td>b, Adjusted to 8 pups on day 4 of lactation.</td>
<td>c, Determined on day 10 of lactation.</td>
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</tbody>
</table>

Table 2. Effect of pinealectomy on lactation in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Body wt. of mother on day 16 of lactation (g)</th>
<th>Total feed intake of mother (g)</th>
<th>Body wt. gain of litter (g)</th>
<th>Amount of milk during 1 hr suckling (g)</th>
<th>Amount of residual milk for 30 min after oxytocin (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, Sham-operated</td>
<td>4</td>
<td>310±7</td>
<td>537±42</td>
<td>150±19</td>
<td>8.8±0.3</td>
<td>-0.2±0.2</td>
</tr>
<tr>
<td>Pinealectomized</td>
<td>4</td>
<td>303±8</td>
<td>489±23</td>
<td>149±8</td>
<td>9.5±1.1</td>
<td>0±0.9</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, Determined on day 16 of lactation.</td>
<td>b, Adjusted to 8 pups on day 4 of lactation.</td>
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</tbody>
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had recurred. After parturition, animals were treated similarly as in experiment 1 and the milk ejection response was determined on the 16th day of lactation by the same method as in experiment 1, except that the litter was separated for 7 hr. All litters were weaned on the 21st day of lactation. The vaginal smears were taken from the mothers to check the recurrence of estrus after weaning. They were mated again after 4 cycles of estrus and the growth of litter in the second lactation was further studied.

Results and Discussion

The result of experiment 1 is shown in Table 1. There was no significant difference in the increase of the litter weight from the 4th to 10th day of lactation among the 3 groups, and this indicates that the lactational performance of the mother was essentially the same in every group. No significant difference was found in the amounts of naturally ejected milk and of residual milk between the control and melatonin injected groups. The dose of melatonin used here is much larger than the amount that the rat pineal is capable of synthesizing per day (Wurtman et al., 1963). Melatonin crosses the blood brain barrier with little hindrance (Wurtman et al., 1964). Therefore, the present result indicates that melatonin did not exert any inhibitory effect on milk ejection either peripherally or centrally, and also suggests that the inhibition of milk ejection by serotonin (serotonin creatinine sulfa, 5 mg/kg) observed in the previous experiment (Mizuno et al., 1967) was not due to the secondary effect by its conversion to melatonin.

The effect of pinealectomy on the lactational performance is shown in Table 2. The litter weight gain and the total feed consumption of the mother for 12 days from the 4th day of lactation were not significantly different between the control and pinealectomized groups. No significant difference was made in the efficiency of milk ejection determined on the 16th day of lactation between the 2 groups. In the second lactation, the litter weight gain was 10% larger, whereas the feed consumption of the mother was 5% smaller in the pinealectomized group than in controls, but these differences were also not significant. These results that pinealectomy did not affect lactation are quite in agreement with those obtained by Nir et al. (1968), although pinealectomy was undertaken when the animal was 21 days of age in their experiment.

In recent years, there has been increasing evidence that pineal gland has some influence on the reproductive functions (Kitay, 1967). Therefore, the effect of pinealectomy on estrous cycle was examined as well. After pinealectomy, the cycle of vaginal smear was not significantly different from that in sham-operated controls. This was opposite to the reports that pinealectomy, performed either in the immature or in the mature stage, increased the incidence of vaginal estrous smears in rats (Albertazzi et al., 1966; Chu et al., 1964; Gittes and Chu, 1965), but agreed with others (Kincl and Banagiano, 1967). The length of gestation period and the recurrence of vaginal estrous smears after weaning were also not different between the control and pinealectomized groups.

In summary, the administration of melatonin or pinealectomy had no significant influence on the milk ejection response in the postpartum lactation in the rat under the present experimental conditions. However, some evidence has recently reported that
melatonin acts within the central nervous system to modify the metabolism of serotonin. Intraperitoneal administration of melatonin (150 µg) was followed by a rapid rise in the concentration of brain serotonin; the greatest effect was seen in the midbrain (Anton-Tay et al., 1968), which contained most of the cell bodies of the central serotonergic neurons (Dahlstrom and Fuxe, 1964). Furthermore, melatonin administration (1 mg/kg per day for 5 days, intraperitoneally) caused a significant increase in the serotonin concentration of the pars intermedia without influencing concentration of serotonin in the other regions of the pituitary (Piezzi and Wurtman, 1970). On the other hand, the subcutaneous injection of serotonin or its precursors was reported to inhibit the milk ejection response, whereas oxytocin or serotonin antagonist, BOL-148, to be able to overcome this inhibition (Mizuno et al., 1967). Therefore, further precise study is necessary to elucidate the physiological role of serotonin and melatonin in the oxytocin release and hence in the milk ejection reflex.

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References


