Inhibitory Effect of Oxytocin Administration on Lactation in Mice

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Synopsis

Lactating mice were injected subcutaneously with 0.125, 0.25 and 1 I.U. of oxytocin twice daily from day 4 to day 8 of lactation. A gradual decrease in litter growth was observed with increasing amounts of oxytocin. The average litter gains were significantly lower in oxytocin treated groups than in saline injected controls during the injection period. On the other hand, the average increases and % increases in the body weight of the mother were greater in oxytocin injected groups than in controls. The nursing behavior of the mother was not influenced by the treatments but a marked accumulation of milk was observed in the mammary glands of the oxytocin injected mother, while young vigorously suckled the teats. After the withdrawal of oxytocin treatment, the growth rate of litter did not recover and several young died of starvation in a group received larger doses of oxytocin. The body weight of the mother, which had been increased by oxytocin, was reduced to the control level. Quite similar results were obtained in another experiment which was carried out duplicatedly. Oxytocin administered either subcutaneously or intravenously, 0.125, 0.25, 0.5 or 1 I.U. twice daily, significantly inhibited the litter growth with increasing doses irrespective of different brands of oxytocic preparation. There was no significant difference in the inhibitory effect on litter growth between routes of administration at the same dose level. An inhibitory effect on litter growth tended to be heightened in a regimen of 9.00 a.m. ~ 2.00 p.m. administration of oxytocin than in a regimen of 9.00 a.m. ~ 6.00 p.m. When killed on day 15 of lactation, the milk accumulation was clearly noticed in the mammary glands of some animals in the oxytocin injected groups and the average weight of mammary glands was greater in most of the oxytocin injected groups than in controls. These results indicate that exogenously administered oxytocin exerted an inhibitory effect on lactation in mice, possibly on the process of milk ejection.

Oxytocin is reflexly released in response to suckling or milking stimulus and plays an important role in milk removal by causing contraction of mammary myoepithelial cells, which is defined as milk ejection. Although most of the milk present in the udder of a dairy cow can be removed by milking which elicits the physiological process of milk ejection, the remainder, i.e. residual milk, is retained in the udder. Oxytocin administration to an animal after milking can cause a further ejection of residual milk. Thus, oxytocin has been widely used for the estimation of the quantity of residual milk and for the minimization of biases due to the variations in the quantity of residual milk in the studies of secretion rate, and also used clinically for the relief of problems associated with lactation in women, i.e. engorgement, pain and etc. In addition to an increase in the yield by milking the residual milk after oxytocin administration, relatively long-term increases in the yield of both milk and butter fat which can be regarded as a galactopoietic effect...
have been observed following the regular administration of oxytocin to cows, goats and sheep (Benson and Fitzpatrick, 1966). In laboratory animals, the use of oxytocin was recommended to make litter growth to be a more satisfactory index of the intensity of milk secretion in rats (Kumaresan and Turner, 1966) and also an increase in the growth rate of the litters was reported when oxytocin was administered to lactating rats for 18 days after parturition (Johnson and Meites, 1957). However, in our lactation studies in mice an unexpected decrease in litter growth was observed when oxytocin was injected. The present paper describes an inhibitory effect of oxytocin on lactation, possibly on milk ejection process. A part of this study was reported in a preliminary from (Mizuno and Shiiba, 1967).

Materials and Methods

Virgin female mice, 3 months old, of the dd strain were bred in our laboratory and were used for experiments only when they were in their first lactation stage. Litters were reduced to 6 young on day 1 of lactation, i.e. the following day of birth of the litter. All the animals were housed in a temperature (25 ± 1°C) and light (14 hr. light, 10 hr. dark) controlled room and maintained on a synthetic diet (Oriental Yeast Co., Ltd., Tokyo) and water ad libitum. To assess the lactational performance, the body weight of the litters, and the body weight as well as food and water intakes of the mother were measured daily at 9.00 a.m. Injections of oxytocin were given from day 4 to day 8 of lactation.

In experiment 1, Pitocin (Parke Davis & Co., Detroit; synthetic oxytocin, 10 I.U./ml) was used as an oxytocin preparation. Mice were divided into 4 groups and were injected subcutaneously twice daily at 9.00 a.m. and 2.00 p.m. with 0.05 ml of saline (group 1) and 0.125 (group 2), 0.25 (group 3) and 0.5 I.U. (group 4) of oxytocin in 0.05 ml.

Experiment 2 was carried out to see the effects of the difference of preparations, routes and schedules of administration and was essentially the duplicates of experiment 1. Not only Pitocin but Atonin or Atonin-0 (Teikoku Hormone Mfg. Co., Tokyo), which was a purified oxytocin preparation from the bovine posterior pituitary gland and containing 10 or 5 I.U. per ml, was used as oxytocin. Injections were given either subcutaneously on the back or intravenously in the tail vein twice daily at 9.00 a.m. and 6.00 p.m. except in 3 groups in which Atonin-0 was given at 9.00 a.m. and 2.00 p.m. Treatments in each group were shown in Table 1. On day 15 of lactation, mice were killed and total mammary glands, ovaries, uterus, adrenals and pituitary gland were removed and weighed.

Results

The results of experiment 1 were shown in Figures 1 and 2. There was no significant difference in the growth of the litter, the average body weight, daily food and water intakes of the mother between groups before injections. This shows that the lactational performance of the mother was essentially the same in each group.

During 5 days of treatments, the growth rate of the litter was depressed significantly in oxytocin injected groups with increasing doses of oxytocin. The average gains of litter weight in groups 2, 3 and 4 were 8.7 ± 1.4, 7.3 ± 1.1 and 6.0 ± 0.8 g (Mean ± S.E.), respectively and significantly different from 13.0 ± 0.4 g in controls (P<0.01 in each case). On the other hand, the average body weight of the mothers increased more in oxytocin injected groups while it increased gradually and almost linearly in controls, the average increase and % increases during this period being greater in oxytocin injected groups (3.2 ± 0.6 g and 7.5 ± 1.2 % (P<0.01), 3.5 g and 8.6 ± 2.5 % (P<0.01), and 4.5 ± 0.4 g (P<0.05) and 11.3 ± 1.4 % (P<0.01) in groups 2, 3 and 4, respectively) than in controls (1.5 ± 0.5 g and 3.6 ± 0.7 %).

Even with the withdrawal of oxytocin, the growth rate of the litters in oxytocin injected groups did not recover and several young in group 4 died of starvation, thus the average body weight of the litters in this group decreased. The body weight of the mothers which had been increased by oxytocin injections declined and reached to the control level toward day 12 of lactation.

The nursing behavior of the mother was not influenced by the treatments but a marked
engorgement of the mammary glands with milk was noticed in oxytocin injected groups.

The average daily food and water intake of the mother was shown in Figure 2. It increased gradually with slight fluctuations in controls throughout the experimental period, while in oxytocin injected groups it advanced slightly than in controls during the later part of the injection period and, in groups receiving higher doses, tended rather to decrease after oxytocin
The results of experiment 2 were shown in Table 1. The entire course of experiment was divided into 3 periods: the pre-injection period, 3 days from day 1, the injection period, 5 days from day 4, and the post-injection period, 6 days from day 9 of lactation. The effects on the daily food and water consumptions were quite similar to those in experiment 1, therefore excluded from the table.

During the pre- and post-injection periods, the results of litter growth and maternal body weight were similar to those in experiment 1. During the injection period, the litter growth was significantly inhibited in oxytocin injected groups irrespective of different preparations and routes of administration and the degree of inhibition was more pronounced with increasing doses of oxytocin. No direct comparison could be made between 2 different schedules of administration within experiment 2, but an inhibitory effect on litter growth tended to be more marked in a regimen of 9.00 a.m. ~ 2.00 p.m. than in a regimen of 9.00 a.m. ~ 6.00 p.m. when a group injected subcutaneously with 0.5 I.U. of Pitocin was compared with group 4 in experiment 1, and also when groups injected at 9.00 a.m. ~ 6.00 p.m. subcutaneously with Atonin were compared with groups injected at 9.00 a.m. ~ 2.00 p.m. subcutaneously with Atonin-0 or Pitocin (experiment 1) (vs Atonin-0 in 0.5 I.U., P<0.05; vs Pitocin in 0.25 or 0.5 I.U., P<0.05 in each case). No significant difference in response to oxytocin was observed between subcutaneous and intravenous injections at the same dose level. The body weight of the mother increased significantly more in oxytocin injected groups than in controls. An accumulation of milk in the mammary glands was noticed particularly in groups received higher doses.

When killed on day 15 of lactation, milk
Table 1. Effect of oxytocin injection on the average gains of litter weight and mother weight, and the average weights of mammary glands on day 15 of lactation in the mouse. Effect of different preparations, routes and schedules of administration

<table>
<thead>
<tr>
<th>Treatments(a)</th>
<th>No. of mice</th>
<th>Gain of litter weight (g)</th>
<th>Gain of mother weight (g)</th>
<th>Mammary gland weight on day 15 of lactation (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-injection period</td>
<td>Injection period</td>
<td>Post-injection period</td>
</tr>
<tr>
<td>Control, saline 0.05 ml/9.00 a.m. (6.00 p.m.)</td>
<td>14</td>
<td>5.5 ± 0.7</td>
<td>13.0 ± 0.5</td>
<td>12.5 ± 0.5</td>
</tr>
<tr>
<td>Atonin, s.c. 0.25 I.U. 9.00 a.m. 0.5 I.U. (6.00 p.m. 1.0 I.U.)</td>
<td>3</td>
<td>4.1 ± 0.9</td>
<td>9.8 ± 0.7**</td>
<td>8.6 ± 0.9**</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.5 ± 1.0</td>
<td>9.0 ± 1.1**</td>
<td>9.9 ± 1.5*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.8 ± 1.0</td>
<td>6.4 ± 0.8**</td>
<td>6.3 ± 2.3**</td>
</tr>
<tr>
<td>Atonin, i.v. 0.125 I.U. 9.00 a.m. 0.25 I.U. (6.00 p.m. 0.5 I.U.)</td>
<td>3</td>
<td>4.5 ± 1.2</td>
<td>9.5 ± 1.5**</td>
<td>10.1 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.6 ± 0.8</td>
<td>9.4 ± 0.5**</td>
<td>11.1 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.9 ± 0.2</td>
<td>8.2 ± 0.5**</td>
<td>9.0 ± 1.1**</td>
</tr>
<tr>
<td>Atonin-0, s.c. 0.125 I.U. 9.00 a.m. 0.25 I.U. (2.00 p.m. 0.5 I.U.)</td>
<td>6</td>
<td>5.1 ± 0.4</td>
<td>10.9 ± 0.7*</td>
<td>10.2 ± 0.8**</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5.5 ± 0.5</td>
<td>9.6 ± 1.1**</td>
<td>11.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4.5 ± 0.3</td>
<td>6.1 ± 0.7**</td>
<td>5.8 ± 1.3**</td>
</tr>
<tr>
<td>Pitocin, s.c. 0.5 I.U. 9.00 a.m. i.v. 0.5 I.U. (6.00 p.m.)</td>
<td>4</td>
<td>3.9 ± 0.9</td>
<td>7.9 ± 1.1**</td>
<td>9.0 ± 1.0**</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.9 ± 0.6</td>
<td>8.8 ± 1.6**</td>
<td>8.1 ± 1.8**</td>
</tr>
<tr>
<td>Atonin, s.c. 0.5 I.U. 9.00 a.m. (6.00 p.m.)</td>
<td>3</td>
<td>4.9 ± 0.8</td>
<td>8.7 ± 1.7**</td>
<td>8.4 ± 2.7**</td>
</tr>
</tbody>
</table>

(a) Treatments were given from day 4 to day 8 of lactation, twice daily.
(b) Litters were 6 young.
* ** Significantly different from control at P < 0.05 and P < 0.01, respectively.
accumulation was clearly observed in the mammary glands of some animals in oxytocin injected groups and the average weight of the mammary glands was greater in most of the oxytocin injected groups than in controls. There was no significant difference in the weights of other organs between oxytocin injected and control groups.

**Discussion**

The present findings that a graduated decrease in the body weight gain of litter occurred when increasing amounts of oxytocin were administered within doses used, irrespective of different preparations or routes of injection, show that exogenously administered oxytocin exerted an inhibitory effect on lactation in the mouse.

There are some evidences that oxytocin influences directly on the mammary secretory tissue. Oxytocin stimulated the glucose oxidation related to the protein synthesis of mammary tissue slices from lactating rats when added in vitro (Goodfriend and Topper, 1961; Cohen et al., 1962), and increased the contents of sodium, chloride and whey proteins and decreased the lactose content of the milk when injected intravenously into cows, presumably changing the permeability of the mammary epithelium (Wheelock et al., 1965). However, it is unknown whether these direct effects on mammary tissues, if any, were responsible for the inhibitory effect on litter growth observed in the present study.

It is most likely that oxytocin administration inhibited a milk ejection response resulting in inhibition of litter growth, as a coincidental increase of maternal body weight with accumulation of milk in the mammary glands was observed while the growth of their litter was inhibited despite that the litter vigorously mouthed the teats. Consequently, a possibility remains that oxytocin depressed the milk secretion indirectly through the primary inhibition of milk ejection, because accumulation of milk in the alveolar lumen followed by an increase of intramammary pressure retards and suppresses further synthesis and secretion of milk (Elliott, 1959; Folley, 1961).

It is not clear whether a slight decrease in daily consumption of food and water, which was observed in oxytocin treated groups, resulted in or from the inhibition of lactation. The strength of suckling stimulus, the secretory activity of the mammary gland and the dietary intake are interrelated each other. No data is available with reference to direct effects of oxytocin on dietary consumption. However, as shown in Figure 1 and 2, the inhibitory effect on litter growth seemed to precede to the influence on the dietary intake. Thus, it may be possible that the inhibition of milk ejection resulted in the accumulation of milk and the depression of litter growth, which in turn resulted in a decreased secretory activity and the weakening of the suckling stimulus, and therefore caused a resultant decrease of food intake. This supposition makes it difficult to conclude whether the result that the growth rate of litter did not recover and the milk accumulation was clearly noticed in the mammary glands in some animals even 6 days after oxytocin injections were withdrawn was due to the long-term inhibitory effect of oxytocin on the milk ejection process or was due to the weakened suckling stimulus of the young whose growth had been depressed during the injection period.

Considerable mechanisms for a possible inhibition of milk ejection by oxytocin are as follows; (a) the synthesis and release of endogenous oxytocin is suppressed by exogenous oxytocin, (b) the substance(s) which inactivates oxytocin is produced in the blood, and (c) the sensitivity of myoepithelial cells diminishes to be refractory to oxytocin released reflexly in response to suckling stimulus. Whichever of these mechanisms may be the case, a question arises why the depression of litter growth occurred even during a period of oxytocin injection, because it was reported that the contractility of the mammary gland engorged
with the milk following litter removal remained for 24 hr. to respond to intravenous injection of oxytocin (Silver, 1956; DeNuccio and Grosvenor, 1967). The following suggestion is offered that litters can remove the milk from the mother only within a short period after each oxytocin injection but they are unable to obtain the milk and lose their energy during other period where the milk ejection response is inhibited. This suggestion seems also applicable for the explanation of the results that a regimen of 9.00 a.m. ~ 2.00 p.m. of oxytocin administration exerted more marked inhibitory effect than a regimen of 9.00 a.m. ~ 6.00 p.m. at the same dose level, because it is possible that young might lose their energy more with increase in the length of fruitless suckling (starvation) period and because it has been demonstrated by several workers and believed in dairy husbandry practice that there is a progressive decline in the rate of milk secretion with increase in the length of milking interval (Ragsdale et al., 1924; Elliott, 1959; Schmidt, 1960; Wheelock et al., 1966), which would appear to fit more in the non-cisternal animals.

Another explanation may be considered that oxytocin which was transferred into young through the milk exerted some harmful effect on litter growth, from the reports that the preferential uptake of oxytocin by the mammary gland was demonstrated in lactating rats (Ginsburg and Smith, 1959) and that a small quantity of oxytocin was found in the milk of goats and cows after injection of large amounts of oxytocin (Noddle, 1962).

Referring to literatures, a case was reported in the cow in which repeated injections of oxytocin had brought about a refractoriness to the usual milk ejection stimuli in a normally responsive animal (Donker et al., 1954). More recently a similar inhibitory effect of oxytocin was observed in the cow (Carroll et al., 1968). It is of interest to study further on the mechanism by which inhibition of lactation is achieved by exogenous oxytocin, not only from the viewpoints of practical use of oxytocin in lactation studies but from endocrinological aspects.

References


