Exogenous Oxytocin and Lactation in the Mouse

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Abstract

Twenty-four primiparous mice were divided into four equal groups. The four groups were randomly assigned to injections of .5, .25, and .125 IU of synthetic oxytocin or oxytocin solvent. Average daily body weights of mothers and pups were compared over 15 days postpartum. Mammary glands were removed and processed for histological examination.

Average body weight gains of mothers were not significantly different among treatment groups. In general, there was no difference in average body weight gain of pups among treatment groups except that in the group nursing mothers injected with .5 IU oxytocin, average weights decreased slowly until by 12 days of age their weight gain adjusted for 4-day weights was less than the 25 and .125 oxytocin groups (P < .10). Microscopic examination of mammary glands revealed that involution occurred in 4 of 6 mice injected with .5 IU oxytocin. Also, the histological appearance of the .25 IU group suggested that this amount of oxytocin enhanced secretory activity of the cells.

Milk production response to exogenous oxytocin injection is dependent both on amount of oxytocin administered and on duration and intensity of the suckling stimulus applied.

Introduction

Studies of Mizuno and Schiiba (6) indicated that oxytocin injection of lactating mice decreased milk production such that an inverse relationship existed between dose of oxytocin and milk production. In a later paper Mizuno and Satoh (7) suggested that oxytocin injection reduced milk production by inhibiting the milk ejection response to normal suckling. The reduction in milk production contrasts with the results of Benson and Folley (1), Benson and Fitzpatrick (2), Johnson and Meites (4), and Thatcher and Tucker (8).

Our study was undertaken to repeat and expand the work of Mizuno and Schiiba (6).

Materials and Methods

Twenty-four primiparous mice of the HaM/ICR strain were divided into four groups and randomly assigned to one of four injection regimes. The control group received oxytocin solvent which contained NaAc, .1%; chlorobutanol, .5%; alcohol, .65%; acetic acid to pH 4. The other three groups received either .125, .25, or .5 IU of synthetic oxytocin (Syntocinon, Sandoz). All injections were given subcutaneously at a final volume of 50 μliters.

At Day 1 of lactation and thereafter, litter size was standardized to 8 pups. The daily protocol throughout the experiment was to separate pups from their mothers at 8:30 AM and at 1:30 PM. Mothers were weighed separately each day at 9 AM (on injection days immediately before ejection). On Days 4, 5, 6, 7, and 8 of lactation, mothers were injected twice daily at 9 AM and at 2 PM. Mothers were killed at 2:30 PM on the 15th day of lactation. Left and right abdominal inguinal mammary glands were removed separately and processed for histological examination (5).

Mice were maintained at 25 ± 2 C with a light:dark ratio of 14:10 hr. They were fed a standard laboratory ration (Purina Lab Chow) ad libitum and allowed free access to water. Weight gains were analyzed by covariance with weight at Day 4 the adjustment.

Results and Discussion

Average daily weights. Figure 1 presents the average daily mothers' weight over the 15 days of lactation. Control mothers were initially the lightest group and maintained this relative body weight essentially throughout the experiment. Moreover, average weight gains over the first 12 days of lactation agreed closely with Mizuno and Schiiba (6); the differences at Day 12 were 4 g and 3.5 g. Average weight of mothers was greater in our study being 36 g versus 33 g at Day 1 of lactation. Mizuno and
Schiiba (6) reported significant relative increases in body weight in the oxytocin-injected groups. The body weights of mothers began to increase on Day 5, peaked at Day 10, and decreased to control weights by Day 12. No such effect occurred in our study.

Figure 2 presents average daily litter weights from birth through 15 days of age. Clearly the control group weighed less (ca. 4 g) at 4 days of age than the oxytocin-injected groups. The covariance analysis adjusts for these pre-injection differences. A decrease in litter weight gain starting at Day 5 can be seen for those pups nursing mothers receiving the most oxytocin (the .5 IU group). By Day 12, the adjusted mean litter weight gains between the .5 IU oxytocin and the .25 IU and .125 IU oxytocin groups were significant at P < .10 level; by Day 14 this amounted to an average difference of 3.5 g. There were no other significant treatment differences. These results contrast with those of Mizuno and Schiiba (6) in which they showed a proportional decrease in litter weight gain starting in the injection period for each increase in amount of oxytocin injected. In fact by Day 12 their smallest difference in average litter weight was 7 g between controls and the .125 IU group, and their largest difference between controls and the .5 IU group was 15 g. Such large decreases in average litter weight were not at all evident in our study. Thatcher and Tucker (8) reported the injection of rats with 1 IU Synthocinon three times daily did not affect litter weight gain. Also Gachev et al. (3) reported that milk yield of rabbits injected with Synthocinon (.5 IU/kg body weight) was not reduced.

Mizuno and Schiiba (6) noted that the excised mammary glands were engorged with milk from those mice receiving oxytocin injections. Mizuno and Satoh (7) then suggested that failure of the litters to recover their lost weight during post-injection could indicate mammary gland involution. They reasoned that involution could result from incomplete milk ejection due to a relative lack of endogenous oxytocin caused by exogenous oxytocin administration.

Histological appearance. Histological examination of mammary gland preparations in
Fig. 2. Effect of oxytocin injections on average daily litter weights. Arrows indicate the five injection days.

The present study should clarify whether involution had occurred. Right and left glands showed similar characteristics to lend reasonable confidence to treatment comparisons. In general, appearance of mammary glands of control and .125 IU oxytocin injected group was that of ample secretion, columnar epithelial cells containing reasonably distinct nuclei, and

Fig. 3. Photomicrograph of mammary gland section from .25 IU oxytocin injected group. Photograph is 100 X.
distinct interalveolar connective tissue with occasional areas of alveolar disruption.

The group injected with .25 IU oxytocin showed fairly uniform epithelial hyperplasia, distinct columnar cells with round discrete nuclei, ample secretion, marked interalveolar connective tissue; all cells and secretion showed marked and uniform staining. Figure 3 is a photomicrograph of a fairly typical histological section from this group. The histological appearance of mammary tissue from the .25 IU oxytocin group suggests a definite enhancement in cellular integrity. Thatcher and Tucker (8) have shown that Syntocinon injections increased mammary gland RNA and DNA. Although the histological evidence of the present trial tends to support this, litter weight gains were not significantly increased for this treatment group.

The group injected with .5 IU oxytocin contained two mice with glands having a histological appearance similar to controls. Sections
from the remaining 4 mice showed definite though varying degrees of involution with reduced staining intensity. Figures 4 a and b are photomicrographs showing alveolar disruption. Some glands contained both columnar and cuboidal secretory cells, one showed areas of marked leucocytic infiltration, another contained cells and connective tissue with markedly reduced staining intensity and areas of interalveolar and secretory cell disruption with nuclei contained in the alveolar and ductular lumens. The histological appearance of this group is consistent with the finding that litter weight gains of this experimental group were reduced by the 12th day of age.

**Overall reflections.** A number of factors could contribute to the differences in results between our study and those of Mizuno and Schiiba (6). A discussion of these factors follows.

Different strains of mice were in the two studies. Strains of mice differ in their immunologic competence, milk producing ability, tolerance to stress, and a plethora of other factors. Therefore, strain of mice could influence results.

Tucker (9) has shown a correlation coefficient of .93 between daily litter weight gain and RNA content of mammary glands. This suggested that litter weight gains are a good measurement of milk production. All mice in our study had a greater average litter weight gain than those used by Mizuno and Schiiba (6); therefore, milk production was greater in our study. The gain in body weight of mothers noted by Mizuno and Schiiba (6) occurred concomitantly with a slight decrease in food and water intake for the oxytocin injected groups. From these results one would anticipate a decrease in body weight. However, they suggested this increased body weight reflected milk retained in the mammary gland. In further work, Mizuno and Satoh (7) suggested oxytocin injections caused involution. Involution suggests cessation (or reduction) of milk production, and, therefore, their explanation for increased body weight of mothers is inconsistent with their results.

An additional and perhaps more important difference between the studies was that our study used 8 pups per mother throughout whereas Mizuno and Schiiba (6) used 6 pups per mother at least initially. Tucker (9) has shown that when “suckling intensity” (as controlled by pups per gland) increases up to a ratio of one pup per gland, RNA and DNA content of mammary tissue increases. Therefore, to the extent that these nucleic acids reflect secretory activity, it appears that number of pups per gland influences the mammary glands’ secretory activity. “Suckling intensity” in the present trial was greater, being .8 pups/gland, than in the trials of Mizuno and Schiiba (6), .6 pups/gland. Moreover, this suckling intensity was maintained throughout the entire 15-day period in our study whereas Mizuno and Shiba noted that several pups died of starvation and others were in a weakened condition. There was a clue in our study of a depressive effect of oxytocin injection on milk production at the highest oxytocin. It is quite possible that milk production response to exogenous oxytocin injection is dependent both on amount of oxytocin administered and on duration and intensity of suckling stimulus applied.

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