Effect of Prolactin Upon Milk Secretion in the Spinal Cord-Sectioned Rat.* (30780)

CLARK E. GROSVENOR†
Department of Physiology and Biophysics, University of Tennessee Medical Units, Memphis

Ample evidence exists that suckling stimulates release of prolactin from the adenohypophysis(1-5). We reported recently that transection of the spinal cord of the lactating rat at T-13 resulted in a greatly diminished milk secretion in the 6 abdominal mammary glands (6,7). The level of milk secretion was not significantly increased by regular injections of oxytocin(6,7), or hydrocortisone(7), but was significantly improved following injections of 45-90 IU of prolactin per day(7). These data suggested that somatic sensory pathways from the mammary gland to the brain were involved in the suckling-induced release of prolactin. We were unaware, however, at the time of these studies that the first pair of abdominal mammary glands in the rat were reported by Denamur and Martinet(8) and by Linzell (J. L. Linzell, personal communication), to be innervated by T-13. Thus, the first pair of abdominal glands in our rats possibly were not completely void of somatic sensory connections to the central nervous system. To clarify the role of somatic sensory innervation in prolactin release, we have transected the spinal cord of lactating rats at T-11 and have tested the milk secretory response of the denervated glands with and without exogenous prolactin and under conditions of *ad lib* and restricted food intake.

Materials and methods. Lactating rats of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages at 76° ± 2° F, and were given a starch-casein-sucrose diet and tap water *ad libitum*. Each litter was reduced to 8 young on postpartum day two and to 6 young on postpartum day 4. Each litter and mother were weighed daily between 1:00 and 2:00 P.M. On postpartum day 2, the main galactophore of each of the 6 pectoral mammary glands of each lactating rat was exposed under light ether anaesthesia and ligated with nylon thread. After one or two days the litters nursed only the 6 abdominal mammary glands. The 6 abdominal mammary glands were denervated on postpartum day 10 or 11 by cutting the spinal cord at T-11 under ether anaesthesia then pithing and removing the cord by suction caudal to the transection. A sham cord-transection was performed in one group of rats in which the vertebral column at T-11 was cleanly exposed but the spinal cord was not damaged. One group of cord-transected rats received 3 units of oxytocin (Armour P.O.P.) per day to effect milk ejection, whereas 2 other groups received 3 units of oxytocin plus 51 units of prolactin (NIH Lot PS-5 assaying 17 IU/mg) per day. The injections were made subcutaneously in divided doses at approximately 8:00 A.M., 1:00 P.M. and 6:00 P.M. each day for 4 days commencing 4 to 6 hours after spinal cord transection. The sham operated rats were similarly injected with 0.2 ml saline 3 × daily. One or two days after the operation the hind feet became slightly edematous in all rats, which forced us to limit the period of the experiment to the 4 days following spinal cord transection.

Food and water intakes were determined daily after the postoperative period for the oxytocin group and for one group injected with oxytocin plus prolactin. Each rat in the other group injected with oxytocin plus prolactin was given an amount of food each day equal on a body weight basis to the average amount of food consumed on the corresponding day by the 11 rats receiving only oxytocin. Free access to water was permitted during the time of pair feeding. Rats which did not keep 6 young of approximately the same size during the postoperative period were excluded from the experiment. Differences between the groups were judged significant if the level of statistical probability was 5% or less.

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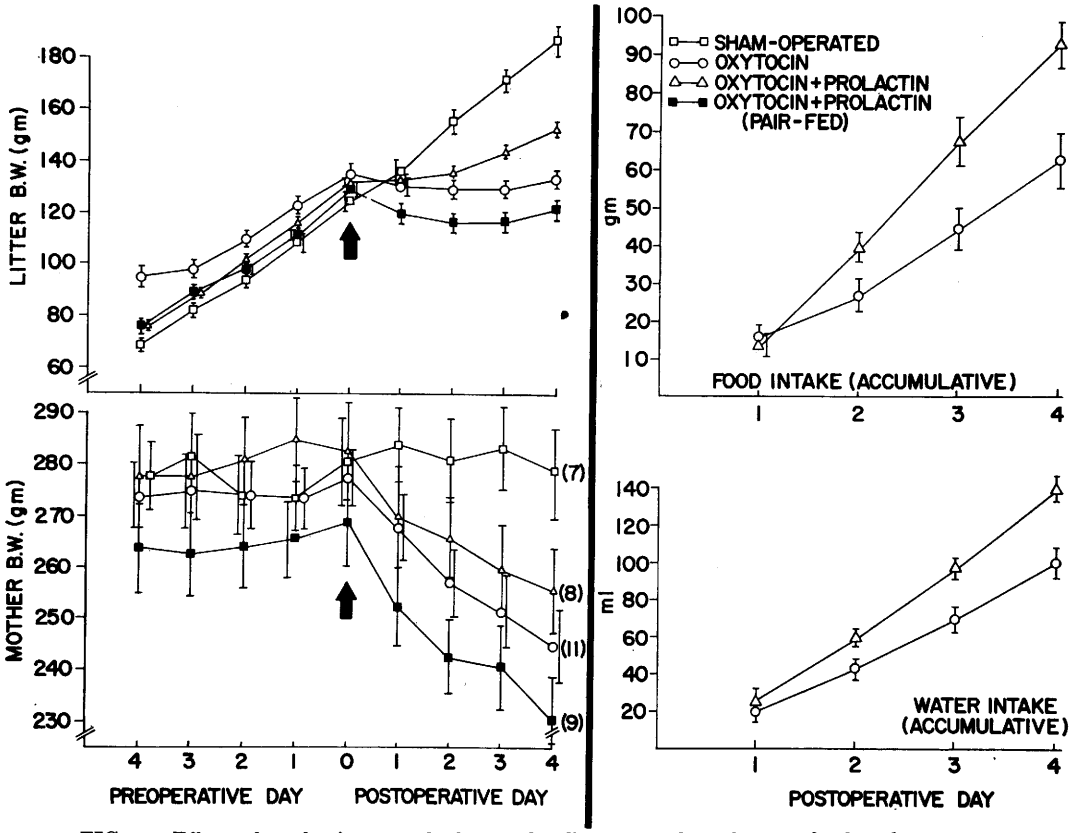

FIG. 1. Effect of prolactin upon body weight, litter growth and upon food and water consumption of lactating rats following spinal cord section at T-11. Galactophores of pectoral mammary glands ligated on postpartum day 2; abdominal glands denervated or sham operated on postpartum day 10 or 11 (arrow indicates day of operation). Oxytocin (3 IU) and prolactin (51 IU) injected 3 x daily in divided dosage after denervation and 0.2 ml saline injected 3 x daily to sham-operated rats. Numbers in parenthesis refer to numbers of rats. Values are means ± standard error of mean.

Results. There was complete paresis of the lumbar, sacral and caudal portions of the bodies of spinal cord-transected rats. The mothers resumed eating and drinking and exhibited normal behavior, *i.e.*, they retrieved their young and cleaned themselves and their young within a few hours following the operation. The young were always observed to be attached to the nipples of the mother even when the mother was eating or drinking. The young never were observed eating from the food cup of the mother.

The litters in each group gained steadily in weight during the preoperative period (Fig. 1). The litters of saline-injected, sham cord-transected rats continued to gain weight during the postoperative period. The weight of 9 of the 11 litters of oxytocin-injected rats declined the first day after transection of the spinal cord at T-11. Six of the 11 gained weight the second and third postoperative days and 10 of the 11 gained weight the last postoperative day. There was a net loss of 1.4 g for the 4-day period (Fig. 1). The litters of 5 of 8 *ad libitum*-fed rats injected with prolactin plus oxytocin lost weight the first postoperative day. Six of 8 gained weight the second day and 8 of 8 gained weight the third and fourth postoperative days. They were significantly heavier than those of oxytocin-injected controls by postpartum day 3 (Fig. 1). They had an average gain of 20 g during the 4-day period which represented a significantly greater weight gain than for litters of oxytocin-injected rats.

The mothers injected with prolactin and

oxytocin had a significantly greater accumulative food intake by the third postoperative day and a significantly greater accumulative water intake by the second postoperative day than did those injected only with oxytocin (Fig. 1). Each prolactin-injected mother ate an average of 91 g of food and drank 132 ml of water during the 4-day postoperative period which represented significant increases in comparison with the 64 g of food eaten and 92 ml of water drunk by oxytocin-injected mothers. The body weight of prolactin plus oxytocin injected mothers decreased daily after cord transection and to the same approximate extent as that of oxytocin-injected rats, whereas that of sham-operated mothers remained essentially constant (Fig. 1).

Seven of 9 litters of rats injected with prolactin and oxytocin and pair-fed to oxytocin-injected rats declined in weight the first day after cord-transection. Three of 9 gained weight the second day, 6 of 9 gained the third day and 8 of 9 gained the last postoperative day. There was, however, a net loss of 7.1 g for the 4-day postoperative period. The litter growth curve of this group ran approximately parallel to that of litters of oxytocin-injected rats (Fig. 1). The water consumption of the pair-fed mothers was not significantly altered from that of the *ad libitum*-fed oxytocin-injected group (data not shown). The maternal body weight of the pair-fed mothers decreased to a greater extent than that of the *ad libitum*-fed groups following spinal cord transection (Fig. 1). The difference, however, was not statistically significant.

The abdominal mammary glands of all rats at autopsy were large, healthy in appearance and well vascularized. The mammary tissue comprising the galactophore-ligated pectoral glands was yellowish-brown in color, small in area and relatively avascular.

Discussion. We have reported that litter growth is reduced to less than one-third of normal following transection of the spinal cord of lactating rats at T-13 and injections of oxytocin 3 times daily to better effect milk ejection(6,7). We observed in the present study that litter growth was reduced to a comparable extent following transection of the spinal cord at T-11. Spinal cord transection at T-11 undoubtedly totally deprives the abdominal mammary glands of somatic sensory connections with the brain, whereas the possibility exists that the upper pair of abdominal glands may be innervated in some rats following cord transection at T-13(8).

Undoubtedly, some of the reduction of litter growth which we obtained in this and in our previous studies(6,7) can be ascribed to the drastic nature of the experimental procedure and to interruption to the neurogenic stimulation of food intake elicited by suckling (9). However, the significant improvement of litter growth following prolactin administration to the T-11 cord-transected mothers suggests that the spinal cord normally serves as a pathway for sensory impulses from the mammary glands to the central nervous system which are fundamental in the suckling-induced release of prolactin(1-5). The dose of prolactin employed (51 IU per day) was within the dose range which maximally improved milk secretion in rats whose spinal cord was transected at T-13(7).

Mena and Beyer(10) reported that prolactin injections when given with oxytocin augmented milk secretion in the lactating rabbit with high spinal cord section. However, the rabbit apparently is affected relatively more from a deficit in oxytocin discharge than from a deficit in prolactin discharge following spinal cord section. Mena and Beyer(10) observed that oxytocin alone maintained an essentially normal level of milk secretion after spinal cord section, and after some days of treatment of prolactin, oxytocin administration by itself was able to maintain a good level of milk secretion in their rabbits.

The fact that limited milk secretion occurred following interruption of somatic sensory impulses from the mammary glands of our rats suggests that alternate pathways probably exist for release of prolactin and possibly from the release of other hormones from the adenohypophysis which are involved in milk secretion in this species. In addition to the release of prolactin elicited by stress(11), there is a strong likelihood that prolactin may

be secreted to some extent in the intact lactating rat in response to exteroceptive stimuli emanating from the young(12).

Lyons(13) and Cowie(14) reported that injections of prolactin to hypophysectomized lactating rats increased food intake and body weight of the lactating mother, as well as providing some maintenance of milk secretion. Benson(15) found that prolactin increased the daily food intake of adrenalectomized and intact lactating rats. We found that injections of prolactin significantly increased food and water intake in the spinal cord-transected mother but had no obvious influence upon maternal body weight. Our data suggest, therefore, that the additional food consumed as a result of prolactin administration was converted more readily into milk than into maternal tissue. The reduction in litter growth rate when the food intake of prolactin-treated rats was adjusted to that consumed by control oxytocin-injected rats suggests, moreover, that prolactin does not stimulate secretion of milk at the expense of maternal tissue; milk secretion is apparently increased by prolactin only when nutrients are available in amounts greater than needed to maintain the normal metabolic needs of the mother.

Summary. Milk secretion in the six abdominal mammary glands of lactating rats was assessed after spinal cord transection at T-11 and after prolactin replacement, to further define the role of somatic sensory pathways in the suckling-induced release of prolactin. The six pectoral mammary glands were rendered nonfunctional by ligation of the main milk ducts on postpartum day 2. Somatic sensory denervation of the 6 abdominal mammary glands was accomplished on day 10-11. Each mother nursed 6 young. The growth of litters of cord-transected lactating rats was greatly reduced in comparison to that of sham-operated rats. Prolactin (17 IU) plus oxytocin (1 IU) 3 × daily resulted in significant improvement in litter growth and in food and water intake of the mothers in comparison with oxytocin-injected control rats, but was without detectable effect upon the loss in maternal body weight which follows spinal cord transection. Litter growth following prolactin treatment was less than that of sham-operated rats. Prolactin did not increase litter growth when the mothers were pair-fed to oxytocin-injected mothers. These data indicate that the spinal cord serves as a pathway for sensory impulses from the mammary gland to the CNS which are fundamental in the reflex release of prolactin from the adenohypophysis. The data also suggest that prolactin stimulates food intake in some manner with the additional food converted more readily into milk than into maternal tissue.

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