Inhibition of Milk Ejection by Exogenous Oxytocin in Lactating Rats (35715)

R. P. Deis[1]
(Introduced by S. M. McCann)
Instituto de Investigacion Medica, Mercedes y Martin Ferreyra, Casilla de Correo 389, Cordoba, Argentina

It is well known that the release of oxytocin evoked by suckling in lactating rats is due to a reflex mechanism. Sensory stimuli originating in the mammary gland during suckling are transmitted to the CNS through the spinal cord and certain structures of the brain before passing to the hypothalamus. There is a considerable amount of evidence showing the role of the CNS in the regulation of oxytocin secretion in lactating animals (1). It seems that the CNS regulates both the release of oxytocin and the response of the mammary gland to the hormone in the lactating rat (2). Nevertheless, it is not known if the secretion of oxytocin is governed solely by the CNS or if it can be influenced by the level of the hormone in the blood. There is some evidence showing that exogenous oxytocin may interfere with the release of the hormone in the lactating cow (3) or modify the pituitary concentration of the hormone in the rat (4).

Several experiments performed in our laboratory have shown the existence of a central block in the release of oxytocin induced by suckling which is established 90 min after a normal suckling period of 30 min (Deis, R.P., unpublished). In this paper we have studied the effect of exogenous oxytocin on the endogenous release of the hormone evoked by suckling in lactating rats. Some of the results were communicated to the Sociedad de Biologia de Cordoba, Argentina (5).

Methods. White primiparous lactating rats (Institute strain) weighing 250–280 g were used. The experiments were performed as follows. Four days after delivery six offspring were left with the mother in a single plastic cage with a wire mesh cover. The experiments were started on the 10th day after birth. The litter was separated from the mother in the early morning for 9 hr. Before being returned to the mother, the young were weighed to the nearest 0.1 g. Then they were allowed to suckle for a period of 30 min. They were then reweighed and the weight gain of the young during the time of suckling was taken as an index of the amount of milk ejected by the mother and also as an indirect evidence of oxytocin secretion. The procedure was repeated during 2 consecutive days and the results taken as control. On the third day measurements were taken after applying the experimental conditions which were being studied. This was done because it has been observed that not infrequently the first time a litter is separated from the mother, the amount of milk subsequently obtained by the offspring is much smaller than the amount obtained on the second or third day of trial (6). In several experimental groups the same mother was used alternately as a control and as an experimental animal. In order to study the effect of neurohypophysial hormones on the release of oxytocin induced by suckling, synthetic oxytocin (Syntocinon, Sandoz) was injected in doses of 500, 250, or 100 m-u per animal to different groups of lactating rats, 180, 90, 60, or 30 min before the replacement of the litter. In another group, antidiuretic hormone (ADH) (Pitressin, Parke-Davis) in doses of 500 or 50 m-u was injected 90 or 30 min before the replacement of the young. The control group was injected with normal saline. In one group of mothers injected with 500 m-u of oxytocin 90

[1] Career Scientist of the Consejo Nacional de Investigaciones Cientificas y Tecnicas of Argentina.

min before the replacement of the litters a small dose of oxytocin (20 m-u/100 g body wt) was injected immediately before the period of suckling started. In two groups in which oxytocin, 500 m-u, was given 90 or 30 min before the period of nursing, sodium pentobarbital (2 mg/100 g body wt) was also injected intraperitoneally 30 min before the replacement of the litter. In the groups injected with oxytocin 90 min before suckling, two 30-min suckling periods were allowed and oxytocin (20 m-u/100 g body wt) was given immediately before the second period of suckling in order to induce milk ejection which was blocked in the first period by sodium pentobarbital.

In all groups oxytocin, ADH or normal saline was injected ip in a volume of 0.2 ml. The hormones were diluted in normal saline. Student's t test was used to assess the level of significance.

Results. Effect of 500 m-u of oxytocin injected ip into lactating rats, 30, 60, 90 or 180 min, before the replacement of the litters. In a group of nine rats the mean weight gain of litters after 30 min suckling of mothers which were injected with 500 m-u 90 min before the replacement of the young was significantly less than that of litters nursed by the control mothers injected with normal saline at the same time ($p<0.001$) (Fig. 1). In order to establish whether the oxytocin administered could modify the response of the myoepithelial cells to the hormone released by suckling instead of blocking its secretion, a small dose of oxytocin (20 m-u/100 g body wt) was given to seven mothers just before starting the suckling period. With this treatment the young obtained a normal amount of milk (6.05 ± 0.35 g) (mean ± SEM) which was not significantly different from that of the control group (5.35 ± 0.18 g) (Fig. 2). When 500 m-u of oxytocin was injected to the lactating rats 60 min before the suckling period, the amount of milk gained by the litter in 30 min of nursing was also significantly less than that obtained by the group nursed by the control mothers injected with normal saline ($p<0.001$) (Fig. 1). In a group of eight lactating rats injected with 250 m-u 60 min before the suckling period, the weight increase of the litter (4.80 ± 0.42 g) was not different from that observed in the control group (5.00 ± 0.36 g).

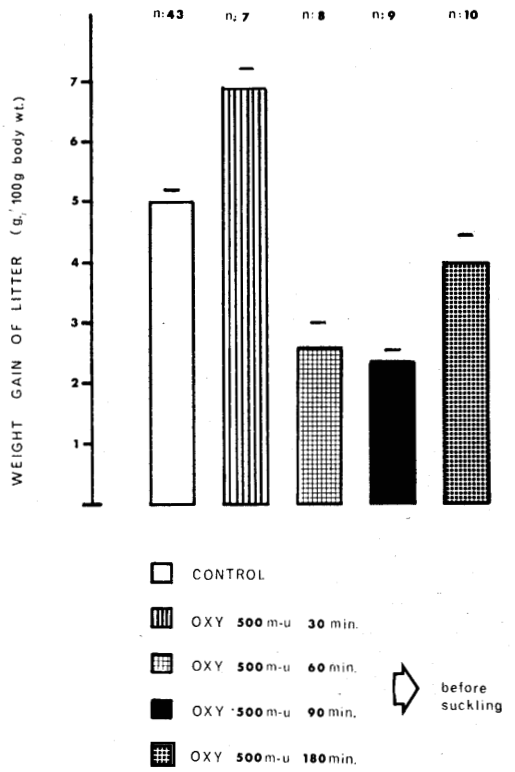

FIG. 1. Effect of 500 m-u of oxytocin injected at different times to lactating animals on the amount of milk obtained by the litter during 30 min of suckling. Each column represents the mean weight gain of the litter and the horizontal line the standard error. Open column: control rats injected with normal saline (0.2 ml). (The values represent the mean of controls of each experimental group). Other columns: groups receiving oxytocin 30, 60, 90, and 180 min before the replacement of the litter. Figures above each column show the numbers of rats tested.

Exogenous oxytocin (500 m-u) given 180 min before nursing did not cause as great a reduction in milk ejection. The amount of milk obtained by the litter in this group did not differ significantly from that of the control group. On the other hand, the injection of 500 m-u 30 min before suckling increased the amount of milk gained by the young compared with the amount obtained by the litters of control mothers injected with normal saline ($p<0.001$) (Fig. 1).

In order to establish whether or not the amount of milk obtained from rats injected with oxytocin was due to the effect of exogenous or endogenous oxytocin, sodium pentobarbital (2 mg/100 g body wt) was administered to two groups 30 min before the replacement of the litter to block the milk ejection reflex. In one group of six rats injected with 500 m-u of oxytocin 90 min before suckling, the amount of milk obtained by the young was 0.53 ± 0.24 g in the first 30 min suckling period and 4.57 ± 0.30 g in the 30 min period immediately following just prior to which the mothers had been injected with oxytocin (20 m-u/100 g body wt). In the other group of seven animals injected with sodium pentobarbital, the rats received 500 m-u of oxytocin 30 min before the suckling period. The weight gain of the offspring was 5.33 ± 0.49 g indicating that the amount of milk ejected was due to the effect of the exogenous oxytocin.

Since no difference was observed between the different control groups, the column of control rats in Figs. 1 and 2 represents the mean of the controls corresponding to the experimental groups in each figure. On the other hand, p values expressed in the text correspond to the difference between each experimental group and its corresponding control group.

Effect of different doses of oxytocin administered 90 min before the replacement of the litter. As Fig. 2 illustrates, the amount of milk gained by the offspring was significantly less than that of litters nursed by the control mothers ($p<0.001$) in the group of lactating rats injected with 250 m-u of oxytocin. At the same time this dose was significantly less effective than the 500 m-u dose in reducing the amount of milk obtained by the young ($p<0.05$). On the other hand, the weight gain of the offspring from the mothers injected with 100 m-u of oxytocin was not different from that obtained by the young from the control group.

Effect of ADH on the milk ejection reflex. In a group of six rats injected with 500 m-u of ADH 90 min before nursing, the mean weight gain of litters after 30 min suckling was significantly less than that of young nursed by control mothers injected with normal saline ($p<0.02$) (Fig. 3). On the other hand when 50 m-u of ADH was injected, the total amount of milk gained by the litter was not different from that obtained by the young from the control rats. If 500 m-u of ADH was injected to a lactating rat 30 min before nursing, there was no significant change in the amount of milk obtained by the young.

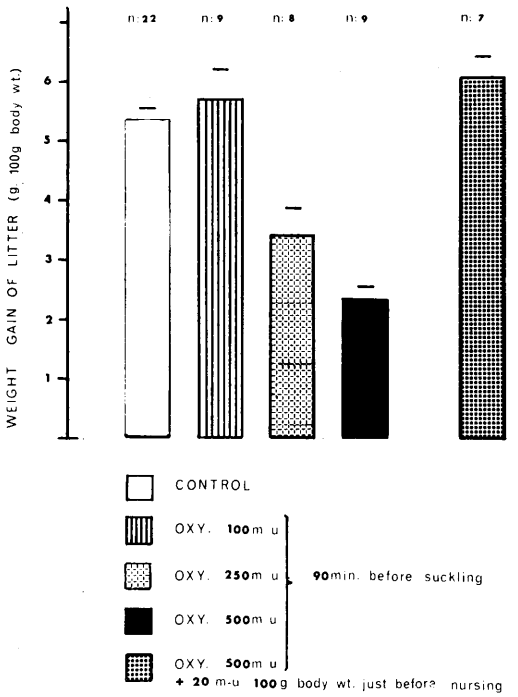

FIG. 2. Effect of different doses of oxytocin injected 90 min before the replacement of the litter. Each column represents the mean weight gain of the young after 30 min of suckling and the horizontal line the standard error. Open column: control rats injected with normal saline (0.2 ml). Other columns: groups of lactating rats injected with 100, 250, and 500 m-u of oxytocin, respectively. Last column: group of rats injected with 500 m-u of oxytocin 90 min before suckling and with 20 m-u/100 g body wt immediately before the replacement of the litter. Figures above each column show the numbers of rats tested.

Discussion. Two different doses of oxytocin, 500 and 250 m-u, when injected 90 min before replacement of the litters were capable of significantly reducing the amount of milk obtained by the young during 30 min of suckling. However, only the dose of 500 m-u was effective when injected 60 min before the suckling period. It seems that not only is a

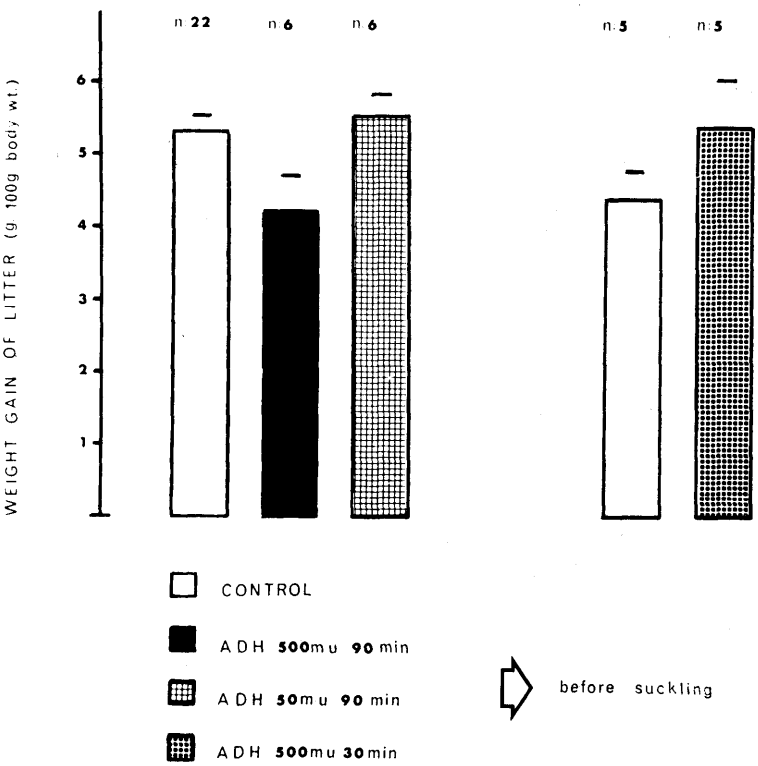

FIG. 3. Effect of ADH given by intraperitoneal injection to lactating rats. Comparison between control groups (open column), groups injected with 500 m-u, 90 and 30 min before the replacement of the litter (shaded column) and group injected with 50 m-u 90 min before suckling. Each column represents the mean weight gain of the litter and the horizontal line the standard error. Figures above each column show the numbers of rats injected.

critical period of time required for oxytocin to be effective, but also that the dose of the hormone is important. The effect of oxytocin on milk ejection when administered to the mother 90 min before the replacement of the young seems to be due to a partial block of the release of the hormone from the neurohypophysis induced by suckling and not to a peripheral effect on the mammary gland, since the administration of a small dose of oxytocin to the mother immediately before the suckling period induced a normal ejection of milk (Fig. 2). This would indicate that oxytocin probably acts at the CNS level. The results of these experiments agree with some observations made in our laboratory (Deis, R.P., unpublished) where it was found that after a nonsuckling interval of 90 min following a suckling period of 30 min, the litters were unable to obtain milk unless a dose of 20 m-u/100 g body wt of oxytocin was administered to the mothers immediately before the second replacement of the young. The litters were isolated from the mothers 9 hr before the first suckling period and then 90 min before the second. It is suggested, therefore, that under certain conditions, oxytocin is capable of blocking its own release.

It is difficult to explain why a dose of 500 m-u of oxytocin can affect milk injection when it is injected 90 or 60 min before nursing while 250 m-u is only effective when administered 90 min before the replacement of the offspring. These results indicate that the small dose requires a period of 90 min to trigger the stimulus which in turn will block the release of oxytocin from the posterior lobe, while the highest dose produces the same effect in 60 min. It seems probable that 500 m-u of oxytocin starts to be effective 60

min after being administered and its blocking effect persists for 1 hr since only a slight but not significant decrease in milk ejection is observed when this dose is injected 180 min before the suckling period. In order to clarify this point one could speculate about the existence of receptors at the CNS level. When a dose of 500 m-u is injected more receptors are stimulated and the response will take less time to be elaborated, *i.e.*, 60 min, while 250 m-u only partially stimulates the receptors and more time is required for the elaboration of the response, *i.e.*, 90 min.

From the results observed in the group of rats injected with 500 m-u of oxytocin 90 min before the suckling period and with sodium pentobarbital 30 min before the replacement of the young, it is possible to assume that oxytocin, when injected 90 or 60 min before suckling produces a partial block in oxytocin release. Thus, the amount of milk obtained by the litter is due to a small release of oxytocin induced by suckling and not to an effect of the oxytocin injected previously. On the other hand, in the rats injected with oxytocin (500 m-u) and sodium pentobarbital 30 min before the nursing period, the amount of milk obtained by the young was exclusively due to the injected oxytocin. This result indicates that oxytocin in the dose administered is capable of producing milk ejection in spite of being given half an hour before the suckling period actually starts, indicating a long lasting effect at the mammary gland level.

The fact that mothers injected with 500 m-u of oxytocin 30 min before the replacement of the litter are capable of ejecting more milk than the control mothers confirmed previous results and support the hypothesis that the CNS controls milk ejection by modifying the tonus of the duct system. When oxytocin is given before the young start to suckle, the milk removed from the alveoli to the ducts prevents the modification of the ductile tonus (2). Another explanation may be that the higher milk yield obtained would be due to an additive effect of the exogenous oxytocin plus the endogenous hormone released by suckling. This additive effect may demonstrate also that oxytocin (500 m-u) when injected 30 min before suckling does not produce a blocking effect on oxytocin release which could be obscured by the milk ejection induced by the exogenous oxytocin. If this were the case, the amount of milk obtained by the young should have been similar to that obtained by the litters of mothers treated with oxytocin 500 m-u and sodium pentobarbital 30 min before nursing since the dose of pentobarbital used completely blocks the milk ejection reflex (7).

The results obtained with ADH suggested a specific effect of oxytocin as opposed to ADH, since only a very high dose of ADH (500 m-u) elicited a significant decrease in the amount of milk obtained by the litter. This decrease was only 20% while 500 m-u of oxytocin produced a decrease of 70% when considering the amount of milk obtained by the young from control mothers as 100%. Furthermore it is more acceptable to compare 50 m-u of ADH with 500 m-u of oxytocin considering the ratio of ADH/oxytocin released by the suckling stimulus in most mammals (8, 9).

It is difficult to make a correlation between the amount of exogenous oxytocin necessary to induce a block in milk ejection with the quantities of hormone normally released by suckling. If an unknown area of the CNS receives oxytocin secreted from the posterior lobe, the hormone may reach this area via the blood system or through a shorter route, *i.e.*, a direct secretion into the cerebrospinal fluid (CSF). If we assume that oxytocin when released from the neurohypophysis may reach the CNS more directly than through the systemic circulation, when the hormone is injected intraperitoneally it may well be necessary to administer an apparently unphysiological dose in order to achieve a similar concentration to that obtained when oxytocin is secreted during suckling.

With the present results it is not possible to establish which type of process takes place in the CNS after the injection of oxytocin in the lactating rats. Further studies will be necessary to determine if the blocking effect is induced by a neural stimulus or by a short feedback mechanism. According to Knowles

(10), the CSF may form part of a feedback system linking the proximal and distal ends of the neurosecretory complex. In a recent paper Rodriguez (11) arrived at the same conclusion, postulating that in the toad the release of the neurohypophysial hormones is regulated by a feedback mechanism. Some morphological evidence is in favor of this hypothesis (11, 12). The possibility of an effect of oxytocin on nervous structures has received additional support from the work of Kawakami and Saito (13) who described various oxytocin-sensitive neurons at hypothalamic level in the cat. Another possibility to be considered is that a high dose of oxytocin could elicit at the mammary gland level afferent impulses which in turn will trigger the CNS. In cows a similar inhibitory effect of oxytocin on milk ejection has been described (3, 14).

The results of the present paper might be interpreted as suggesting that oxytocin plays a role in the regulation of its own release in the lactating rat (Fig. 4).

Summary. The effect of different doses of exogenous oxytocin ranging from 100 to 500 m-u on the milk ejection reflex was studied in lactating rats which were isolated for 9 hr from their litters. The litters were then returned to the injected mothers and allowed to nurse for 30 min. The ip injection of 250 or 500 m-u into lactating rats 90 min before the suckling period significantly blocked milk ejection. A small dose of oxytocin injected immediately before nursing to the latter group induced a normal milk ejection indicating that the blocking effect was not due to a lack of mammary gland response. A dose of 100 m-u did not block milk ejection. When oxytocin was injected 60 min before suckling, only 500 m-u decreased the amount of milk obtained by the young whereas the 250 m-u dose was ineffective. On the contrary when the 500 m-u of oxytocin was injected 30 min before nursing milk yield was significantly increased. This was caused by the milk ejecting activity of the exogenous oxytocin since the injection of sodium pentobarbital to block the milk ejection reflex did not prevent the pups from obtaining milk 30 min after oxytocin. Antidiuretic hormone at a dose of 50 m-u did not modify milk ejection, but a 500 m-u dose inhibited it. It is suggested that oxytocin may regulate its own release in lactating rats.

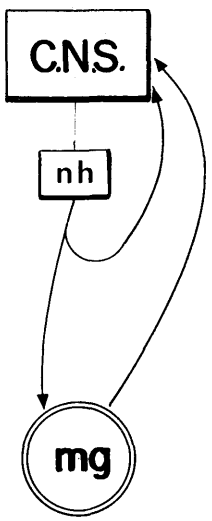

FIG. 4. Represents a possible feedback mechanism of oxytocin directly on the central nervous system (C.N.S.) or through the mammary gland (mg). nh: Neurohypophysis.

The author is most grateful to Mr. Luis Pucci and Miss Cándida Alvarez Tello for technical assistance. Oxytocin was kindly supplied by Sandoz Ltd., Argentina, and ADH by Parke-Davis of Argentina.

1. Tindal, J. S., *in* "Reproduction in the Female Mammal" (G. E. Lamming and E. C. Amoroso, eds.), p. 79. Butterworths, London (1967).

2. Deis, R. P., J. Physiol. London **197**, 37 (1968).

3. Donker, J. D., Koshi, J., and Petersen, W. E., Science **119**, 67 (1954).

4. Fendler, K., and Telegdy, G., Acta Physiol. Hung. **22**, 59 (1962).

5. Deis, R. P., Acta Physiol. Lat. Amer. **20**, 180 (1969).

6. Taleisnik, S., and Deis, R. P., Amer. J. Physiol. **207**, 1394 (1964).

7. Deis, R. P., and Orias, R., J. Physiol. London **197**, 47 (1968).

8. Dicker, S. E., and Tyler, C., J. Physiol. London **121**, 206 (1953).

9. Acher, R., and Fromageot, C., *in* "The Neurohypophysis" (H. Heller, ed.), p. 39. Butterworths, London (1957).

10. Knowles, F., *in* "Neurosecretion" (F. Stutinsky, ed.), p. 8. Springer-Verlag, Berlin/New York (1967).

11. Rodriguez, E. M., *in* "Neurosecretion" (W. Bargman, ed.). Springer, New York (in press).

12. Heller, H., Hasan, S. H., and Saifi, A. Q., J. Endocrinol. **41**, 273 (1968).

13. Kawakami, M., and Saito, H., Jap. J. Physiol. **17**, 466 (1967).

14. Carroll, E. J., Jacobson, M. S., Kassouny, M., Smith, N. E., and Armstrong, D. T., Endocrinology **82**, 179 (1968).

Received Feb. 19, 1971. P.S.E.B.M., 1971, Vol. 137.