Effects of Reserpine and Chlorpromazine on the Milk Ejection Reflex In Lactating Rats.* (30781)

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Effects of reserpine (RSP) on milk production and on secretion of pituitary prolactin have been reported by many workers (see 1 & 2 for review). Recent experiments of Kanematsu and Sawyer(3) and Kanematsu, Hilliard and Sawyer(4) indicate that the posterior median eminence-posterior tuberal region of the hypothalamus is the critical site of action of RSP in releasing prolactin from the rabbit adenohypophysis.

However, there have been only a few papers(5,6) dealing with the effects of RSP on milk ejection, which is another important phase of lactation. Milk ejection is known to be caused by oxytocin release stimulated by the suckling stimulus. Moon and Turner(5) reported blockade of the milk ejection response by reserpine, but Chaudhury(6) failed to confirm their results.

Both reserpine and chlorpromazine (CPZ) have been shown to block ovulation (see 7,8 for review), and CPZ has also been considered to stimulate the secretion of prolactin from the anterior pituitary gland(1,2). Chaudhury(6) reported that CPZ inhibited the milk ejection response in unanesthetized lactating rats.

Since electrophysiological studies have suggested that the site of action of CPZ is different from that of reserpine(9), different effects of milk ejection response might be expected. Therefore, the present experiments were designed to reexamine and compare the effects of these tranquilizers on the milk ejection response.

Materials and methods. Lactating rats of the Sprague-Dawley strain, weighing 280-390 g, were used. They were housed in an air conditioned, light controlled (14 hr light, 10 hr darkness) animal room and kept in individual cages. One or two days after delivery the litters were reduced to 8. The day following delivery was designated as the first day of lactation. Litters were removed from their mother in the evening after 6-8 days of lactation. They were kept in cages and covered with cotton wool to keep them warm. Around 9 A.M. of the following days, the mothers, deprived of their pups, were injected subcutaneously with reserpine (Serpasil, Ciba, N. J.) in the special Serpasil injectable solution or intramuscularly with chlorpromazine (Thorazine, Smith Kline & French Labs., Phila.). Untreated rats and animals receiving the special Serpasil injectable solution served as the intact and the injected controls, respectively. Doses of the drugs are given in Table I.

One to 1½ hours later, the 8 pups were placed with the mother and left for 2 hours to allow them to suckle. Before placing the litters with the mothers, the bladder of each pup was pressed to expel the accumulated urine. The 8 pups were weighed together to the nearest 0.1 g. The difference in the weight of pups during 2-hour suckling regimen was regarded as the milk yield of the mother rat. After the suckling regimen, the pups were returned to their own cages.

After 5 days of the suckling regimen (suckling period), the treatment with drugs and placebo was continued for another 4 days without the suckling (weaning period). Animals were killed 5 days after the final period of suckling. Ovaries, and abdominal and inguinal mammary glands were weighed. Ovaries and a piece of the mammary gland were fixed with Bouin's fluid and 10% formalin, respectively.

Results. Marked tranquility was observed by the start of the suckling regimen in the lactating rats injected with reserpine or chlorpromazine. However, the pups could readily suckle the teats of their mother while she was in the sedated condition. The daily sedation seemed to continue at least during the

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TABLE I. Effects of Reserpine and Chlorpromazine on Body and Organ Weights of Mother Rats.

Treatment	No. of animals	Change in body wt during treatment (g)	Body wt at autopsy (g)	Ovarian wt (mg/100 g B.W.)	Mamm. gland wt (g/100 g B.W.)	No. rats estrous in 5 days after weaning
Control, intact	5	+ 8.2 ± 3.4*	307.2 ± 5.5	34.38 ± 2.63	1.44 ± .07	4
Control, injected with special Serpasil vehicle	5	− 11.0 ± 3.6	331.6 ± 5.3	30.98 ± 4.94	1.10 ± .13	5
Chlorpromazine 5 mg/day	5	− 9.5 ± 2.6	295.0 ± 13.6	26.74 ± 1.10	1.62 ± .15†	4
Chlorpromazine 10 mg/day	5	− 26.3 ± 7.5	295.2 ± 7.9	24.08 ± .57	2.12 ± .04‡	2
Reserpine 100 μg/day	5	− 49.6 ± 2.6	218.0 ± 10.4	30.16 ± 2.45	1.82 ± .29§	0
Reserpine 200 μg/day	5	− 73.0 ± 5.3	229.6 ± 8.6	28.60 ± .73	2.28 ± .11‖	0

* Mean ± standard error.
†, ‡ $P < .05$, $P < .01$, respectively, compared with intact or injected controls.
§, ‖ $P < .05$, $P < .01$, respectively, compared with injected controls.

period of suckling. Daily gains in litter weight during suckling (representing milk yield) are seen in Fig. 1.

Pups could get a considerable amount of milk during the suckling period, although the amount was less than that in pretreatment period. Milk yield on the first day of the suckling period was similar to the pretreatment level in all groups except RSP-100 μg treated group. Usually only a small amount of milk could be obtained by pups on the last day of the suckling period. In the injected control group, milk yield decreased markedly from the second day of the treatment, and the average milk yield during the first 4 days of the regimen was only 62.5% of that during the pretreatment. Here the percentage of milk yield was appreciably lower than in the other groups: the percentages in the CPZ (high and low inclusive), RSP (high and low inclusive) treated groups and in the intact groups were 78, 73 and 78.5%, respectively.

During the suckling period all rats injected with drugs or the control solutions lost weight from 10 to 73 g, while intact animals gained weight (Table I). There was little weight change during the 4-day weaning period in the intact controls or CPZ-treated rats, but the injected controls showed some recovery; the reserpine animals continued to lose weight since they stopped eating. The initial body weight of the injected control group was higher than the other groups.

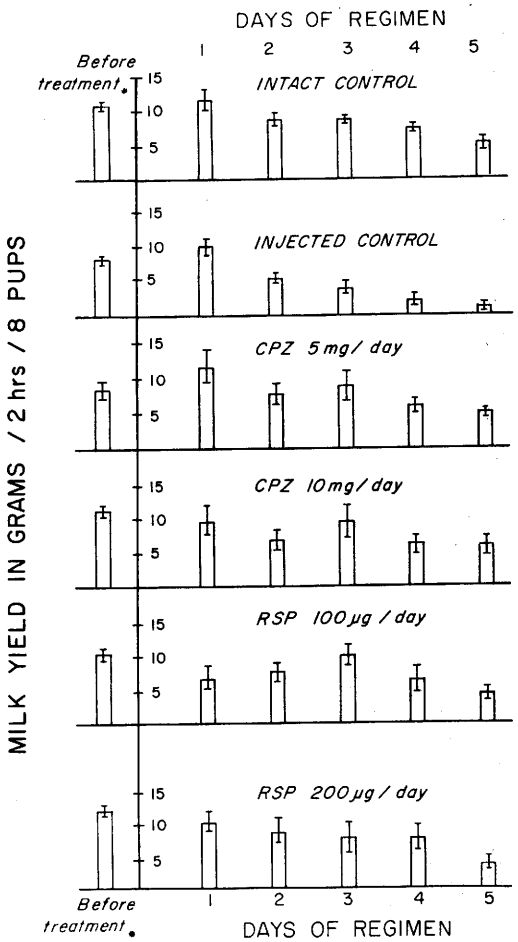

FIG. 1. Milk yield to suckling before, and 5 days after, treatment with chlorpromazine (CPZ) and reserpine (RSP).

Mammary gland weight of the injected control groups was the lowest encountered, although there was no significant difference in weight between the injected control and the intact control groups. In the CPZ- or RSP-treated groups, mammary gland weights were significantly higher than those of the injected control groups. The differences in weights of mammary glands of high and low dosage groups of either drug were not statistically significant.

Ovarian weights in the intact group were significantly higher than those in the CPZ-treated groups but not higher than in the RSP-treated groups.

Vaginal estrus was observed 3-4 days after the last day of the suckling regimen in the intact control, the injected control and the CPZ-treated groups. The number of animals showing vaginal estrus are included in Table I. No estrous smear appeared in the RSP-treated groups.

Histologically, the mammary glands of the intact and the injected control groups involuted nearly completely and the glands consisted only of ducts and cell clusters which were remnants of the lobulo-alveolar system. In some of the mammary glands end-bud-like structures with very small lumens were observed. Contrary to this observation, lobular-alveolar systems filled with milk-like substances remained in the mammary glands of animals treated with RSP. CPZ also retarded mammary gland involution, although it was less effective than reserpine in preventing the involution.

Discussion. In the present experiments neither RSP nor CPZ blocked the milk ejection response in unanesthetized lactating rats. Our results with RSP are thus compatible with the findings of Chaudhury(6), but fail to confirm the findings of Moon and Turner (5). The dosage used in the present experiments (100-200 μg/rat) and by Chaudhury (2.5 to 4.0 mg/kg) were higher than those used by Moon and Turner (10 μg/100 g)(5). It seems unlikely, however, that inhibition of milk ejection with reserpine would occur at low dosage and not at a higher dosage level; Tuchmann-Duplessis and Mercier-Parot (10) reported that the inhibiting effect of reserpine on gonadal activity was proportional to the dosage used. Furthermore, Moon and Turner noted blockade within 10 minutes of RSP injection, long before the maximal action of the drug is exerted, suggesting that blockade was exerted by factors other than reserpine.

Since in our experiments the suckling regimen began a sufficient time after RSP injection for the agent RSP to produce maximal sedation(11), and animals were especially quiescent at the beginning of the suckling regimen, it is impossible that the pups obtained milk before the RSP became effective.

In his chlorpromazine experiments Chaudhury(6) used a 25-minute suckling regimen, and he reported that while milk ejection was not blocked by CPZ in dosages of 1, 2, or 3 mg/kg it was inhibited by 5 mg/kg administered intraperitoneally immediately before suckling. Since the intramuscular doses used in the present experiments were approximately 3-6 times higher than Chaudhury's, the discrepancy between our results cannot be due to inadequate dosage on our part. However, since the maximum effectiveness of the drug, sedation-wise, appears within 15-30 minutes after injection, the possibility arises that our 2-hour suckling regimen was so prolonged that the effect of the drug had worn off before the end of the daily regimen. As a matter of fact, CPZ was injected 3-3.5 hours before the end of the suckling period. Nevertheless, the following observations tend to eliminate this possibility: animals still showed sedated behavior at the end of the regimen, and a preliminary experiment showed that almost all milk was obtained by the pups during the first hour of suckling in the intact and the CPZ injected animals.

In order to demonstrate further that the drug injected in the morning was still effective in the early afternoon, animals were weaned after the 5-day suckling regimen and checked 5 days for the appearance of vaginal estrus. In the intact and the injected control groups, 9 out of 10 rats showed vaginal estrus. However, all in the RSP-treated group and one out of 5 in the CPZ low dosage and 3 out of 5 in the CPZ high dosage group failed to show estrus. These findings

imply that reserpine is very effective in preventing ovulation even when injected 3 or 4 hours before the start of the critical period, (the critical period is 2-4 P.M., colony time). Although the blockade of ovulation by CPZ is not complete, as it is with RSP, the higher dosage of CPZ still showed a marked blocking effect on the recurrence of estrus and, presumably, on ovulation. Therefore, milk obtained by pups in the CPZ high dosage group is ejected from the mammary gland at a time when the effect of CPZ on the central nervous system is still marked.

The relatively low milk yield during the suckling regimen, especially on the fifth day, may be due rather to the condition of pups than to possible blockade of secretion of other lactation-maintaining hormones in the mother. Even though they were kept warm, the pups gradually became weaker during the suckling period.

The weight of the mammary gland in the RSP-treated groups was significantly higher than that in the injected control group. Histological studies clearly demonstrated that involution of the mammary gland was prevented by the treatment with drugs. This finding confirmed the observations of previous workers(12-15). In this connection it is interesting to recall the findings of Yokoyama and Ota(16) which showed that secretion of prolactin and of other lactation-maintaining hormones was continued in rats even after oxytocin secretion was blocked.

Summary. The effects of reserpine (RSP) and chlorpromazine (CPZ) on the milk ejection response in lactating rats were studied. Neither RSP nor CPZ blocked the milk ejection response. Pups suckling RSP- and CPZ-treated mothers could obtain as much milk as those in the intact control group. RSP injected each morning blocked the appearance of estrous smears completely for 5 days after weaning. The blockade by CPZ was not complete, but 3 out of 5 rats treated with high dosage of CPZ did not show estrous smears. Mammary glands of RSP- and CPZ-treated groups consisted of well maintained lobulo-alveolar system at 5 days after weaning, while in the injected and the intact control groups complete involution of the gland was observed. The results imply that RSP and CPZ blocked ovulation and activated or maintained prolactin secretion without blocking the milk ejection response.

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