Pup Survival and Prolactin Levels in Nicotine-Treated Lactating Rats[1] (37485)

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Nicotine exerts profound effects on several aspects of reproduction (1). In female rats it was shown that the number of sterile matings was much greater and the number of young born in fertile matings was decreased by long-term treatment with subconvulsive doses of nicotine administered to female rats (2, 3). Injection of nicotine during pregnancy prolonged gestation, decreased the birth weight of pups and caused mortality of one-third of the pups in litters within 48 hr after birth (4). Nicotine has also been reported to depress lactation in mice (5). In all of these earlier studies in which nicotine was shown to interfere with reproduction, there was no indication whether these effects were due to direct actions of nicotine on the central nervous system (6) or, if not, by what mechanism nicotine exerted its influence.

Recently, the neuroendocrine effects of nicotine on the rat hypothalamo–hypophysial axis have been critically investigated. The drug delays neurogenic activation of the proestrous surges of luteinizing hormone and prolactin (7, 8). The suckling-induced rise in prolactin after a period of separation from the pups (9) was also delayed following acute exposure to nicotine, but the milk ejection mechanisms were not blocked (10). This present study was undertaken to evaluate the effects of chronic administration of nicotine during pregnancy and lactation on the offspring and on the mother's milk production and ejection mechanisms. Nicotine dosages were low enough to permit normal gestation length and litter size.

Materials and Methods. Thirty-one mature female Sprague–Dawley (Simonsen) rats weighing 250 ± 40 g were maintained in a light- (14 hr light, 10 hr dark) and temperature-controlled room, and fed Purina laboratory chow and water *ad libitum*. The females were mated, and the morning on which sperm were observed in the vaginal smear (8-12 hr after suspected mating) was designated as Day 1 of pregnancy. On Day 5, the prospective mothers were divided into three groups. The first group received 0.5 mg nicotine tartrate (0.17 mg nicotine) in 0.2 ml 0.9% saline twice daily. The second group received 1 mg nicotine tartrate twice daily. The third control group received 0.2 ml 0.9% saline twice daily. Subcutaneous injections were given daily at 0900 and 1700 hr from Day 5 throughout pregnancy and during lactation for as long as the female had pups to nurse.

Pup weight and litter size were noted at parturition. Pups were counted and weighed and were examined qualitatively at these times for the presence of milk in their stomachs; in young pups, stomach milk can be observed as a white belt under the skin on the ventral surface. Observations were made to determine whether maternal behavior was normal (11).

Seven animals from the second group and 4 control rats were each catheterized with a chronic venous cannula inserted into the right atrium of the heart, via the external jugular vein (12), between Days 16–18 of pregnancy. The distal end of the cannula was connected to a swivel above the animal cage. This procedure permitted collection of blood

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samples without apparent disturbance to the animal. Blood samples (0.5 ml) were collected at 1300 hr daily starting on Day 20 of pregnancy and continuing until Day 5 of lactation, in those mothers having at least one surviving pup.

The blood samples were centrifuged and the plasma was collected for subsequent radioimmunoassay (RIA) of prolactin. RIA was performed with the *NIAMD Rat Prolactin* radioimmunoassay system (A. F. Parlow) obtained from the Rat Pituitary Hormone Program of the National Institute of Arthritis and Metabolic Diseases, NIH. The potency of the reference preparation *NIA MD-Rat Prolactin-RP-1* was 11 IU/mg. All samples were assayed in duplicate, and the two values were averaged.

An analysis of variance (SS-STP test) according to Sokal and Rohlf (13) was used to evaluate the success of females in each treatment in rearing their young. Student's *t* test was used to compare mean prolactin values.

Results. Effect of nicotine on pup survival. Neither duration of pregnancy nor litter size at birth was altered by either dosage of nicotine. However, the higher dose (2 mg) caused a significant decrease in mean pup weight at birth and in mean litter size at weaning (Table I). Mean litter size at weaning for the group receiving the high dose of nicotine was strikingly reduced; an average of less than 3 pups/litter reached that stage.

Although pups born to nicotine-treated females in the high dose group were lighter than pups in the other two groups, they appeared well formed and normal. Nevertheless, it was important to determine whether their low birth weight was a factor in their subsequent death. To test this possibility, pups from nicotine-treated mothers were fostered on untreated mothers that gave birth at the same time. Pups born to high dose nicotine mothers and fostered on normal mothers showed normal weight gain and survival.

The mortality of pups suckling nicotine-treated mothers was not due to inadequate maternal behavior, since all of the components of normal maternal behavior appeared in sequence (e.g., crouching, nest building, retrieving and licking). Death of the pups was due presumably to starvation because of lack of milk in the mammary glands.

To test for the presence of milk in the mammary glands, nicotine-treated females were lightly anesthetized with ether, and oxytocin (0.5 IU) was injected sc at two sites near nipples. The nipples were gently grasped with fine forceps and pulled upward; the base of the nipple and mammary gland was squeezed with two fingers. No milk was obtained by this procedure in any of 5 subjects; this procedure was effective in releasing milk in control animals. Recent work has shown that milk ejection itself is not blocked by nicotine (10).

Serum prolactin values. To test whether the lack of milk in the mammary glands might be due to a failure of pituitary prolactin secretion, plasma prolactin levels were measured. Inasmuch as only the high dose group had difficulty in maintaining their litters until weaning, plasma prolactin levels were compared between this group and saline controls.

Blood samples were taken beginning 2 days and 1 day prior to parturition (P-2, P-1, respectively). However, the number of sam-

TABLE I. Effect of Nicotine on Litter Size and Weight.

Nicotine tartrate (mg/day sc in 0.4 ml)	No. of litters	Duration of pregnancy	Litter at birth		Litter at weaning (no. of pups)
			No. of pups	Pup wt (g)	
0 (saline)	6	22.5 ± 0.34[a]	10.5 ± 0.80	6.63 ± 0.13	10.16 ± 0.79
1.0	8	22.6 ± 0.26	10.5 ± 0.37	6.90 ± 0.19	9.66 ± 0.33
2.0	17	22.7 ± 0.25	9.5 ± 0.87	5.74 ± 0.10[b]	2.42 ± 0.86[b]

[a] Mean ± SEM.

[b] $p < 0.01$ when compared with either of the other two groups.

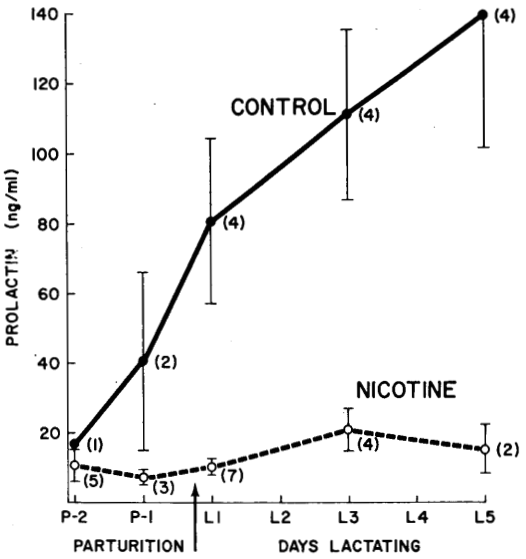

FIG. 1. Effect of nicotine tartrate (1 mg in 0.2 ml injected twice daily from Day 5 of pregnancy) on plasma prolactin levels during early lactation.

ples for these dates was small and, therefore they did not demonstrate significant differences. Nonetheless, a comparison of group means (Fig. 1) especially on P-1, indicates a trend toward lower prolactin levels in the nicotine-treated animals (7.56 ± 1.4 ng/ml compared to 40.5 ± 25.5 ng/ml in the saline controls). During the first 5 days of lactation (L1–L5) blood samples were taken from a larger number of subjects. On L1 plasma prolactin levels were strikingly higher in saline controls than in nicotine-treated animals (80.7 ± 23.3 ng/ml compared to 10.0 ± 2.8 ng/ml $p < 0.01$). This dramatic failure of prolactin release was also observed on the third day of lactation (13), when nicotine-treated females had a mean plasma content of 21.3 ± 6.5 ng/ml, compared with 113.2 ± 28.0 ng/ml in saline controls ($p < 0.05$). By L5 most of the pups in the nicotine group had died of starvation since they could not obtain milk. Pups survived in only 2 out of 7 litters previously sampled on L1; thus the sample size was reduced to 2 subjects. The mean prolactin level in these two animals was relatively lower compared with saline controls than on L3 (15.5 ± 7.5 ng/ml compared with 140 ± 38.2 ng/ml).

One female, not included in the data of Fig. 1, that received the high dose of nicotine was able to rear 9 of her 11 pups to weaning. Prolactin levels in this female were high, similar to those of saline controls. Her mean plasma prolactin level for L1, L3 and L5 was 121.0 ng/ml compared to 111.3 for the saline controls, and 15.6 ng/ml for the remainder of the females receiving the high dose of nicotine and which had lost most of their pups.

Discussion. Our findings reveal a chronic inhibitory influence of nicotine on plasma prolactin levels in lactating rats. This does not rule out the possibility of simultaneous inhibitory influences on secretion of other hormones such as ACTH and somatotropin, which also contribute to the lactogenic response. However, we have unpublished data indicating a rapid large stimulatory effect of nicotine on secretion of adrenal corticosterone in acute experiments. In the present study we noted that in the one mother which was resistant to nicotine to the extent that she lactated normally, plasma prolactin levels remained as high as in controls. Therefore the extremely low levels of prolactin commonly observed following treatment with nicotine may well account for the failure of milk secretion in lactating mothers, resulting in starvation of the majority of pups.

In short-term experiments with acute injections of nicotine, in which the highest daily dosage of the current experiments was administered within 5 min, the drug delayed the suckling-induced release of prolactin for only 1–2 hr (10). Similar acute injections delayed the proestrous surges of prolactin (8) and LH (7) and to completely block the proestrous surges repeated injections were necessary over a 6-hr period. Rather than resulting in tolerance, the long-term effects of twice daily injections of nicotine appear to be additive and by the end of pregnancy, 24-hr inhibition of release of prolactin has apparently been achieved by the two daily injections 8 hr apart.

Our experiments were not designed to determine the precise mechanism by which nicotine interferes with prolactin release. It is

known that nicotine exerts pharmacological effects on a number of brain neurohumors, which may include a mediator (or mediators) influencing hypophysiotropic hormones. Nicotine releases acetylcholine (14), catecholamines (15–17), vasopressin (18) and serotonin (19). However, recent evidence indicates that serotonin is facilitory rather than inhibitory to prolactin release mechanisms (20).

The precise means whereby the brain itself affects the rate of secretion of various hypophysiotropic substances is still an open question. However, there is evidence indicating that catecholamines (CA) in the brain may be involved (21–24). It has been shown that epinephrine inhibits the release of prolactin by rat pituitaries *in vitro*, and that CA *in vitro* suppress the synthesis of rat pituitary prolactin (23). Turning to *in vivo* studies, Barraclough and Sawyer (25) showed that depletion of brain CA by reserpine and chlorpromazine induced sustained release of prolactin in the rat. Khan and Bernstorf (26) confirmed these results and also noted the appearance of lactation. Initiation of milk secretion was also observed in the rabbit after reserpine implants were placed in the hypothalamus (27). Recently, Kamberi, Mical and Porter (22) demonstrated a decrease in plasma prolactin concentration, determined by radioimmunoassay, after injection of dopamine, epinephrine or norepinephrine into the third ventricle. MacLeod and Lehmeyer (23) have even suggested that the prolactin-inhibiting factor (PIF) may be itself a CA but this has been questioned by the *in vitro* experiments of Koch, Lu and Meites (28) and the intrapituitary infusions of Kamberi, Mical and Porter (22).

Administration of nicotine has been shown to cause a significant release of CA (15–17, 29), and it has been suggested that the alkaloid might produce some of its actions in the CNS by stimulating release of endogenous CA (15). Changes in turnover rate provide a more sensitive indication of neurohumoral metabolism than do changes in tissue concentrations of an amine (30, 31) and Bhagat (32) showed that chronic administration of nicotine resulted in an increased turnover rate and utilization of exogenous CA in the rat brain. Thus it is tempting to postulate that the mechanism by which nicotine decreases plasma prolactin levels may involve its stimulatory influence on CA in the brain.

Summary. Administration of 0.5 and 1 mg nicotine tartrate twice daily to female rats through pregnancy and lactation affected neither duration of pregnancy nor litter size at birth. However, pups born to females given the higher dose of nicotine were lighter in weight at birth than pups in the two other groups, and most of them died of starvation before weaning because the mother's mammary glands contained so little milk. Blood was collected via a chronic intra-atrial cannula, and radioimmunoassay of prolactin was performed. Plasma prolactin levels of the group receiving the high dose of nicotine were much lower than in the low dose nicotine and saline groups. It is suggested that failure of prolactin release following chronic nicotine administration was responsible for low milk production and starvation of the pups. The mechanism by which nicotine affects prolactin levels may involve effects on brain catecholamines.

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