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Evidence for Adrenergic and Cholinergic Components in Milk Let-Down Reflex in Lactating Rat.* (23342)

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A cholinergic component apparently is involved in activation of the hypothalamo-hypophyseal mechanism for release of antidiuretic and luteinizing hormones and, in addition, an adrenergic link has been proposed for luteinizing hormone and ACTH release(1). However, very little appears to be known about the release of oxytocin from the posterior pituitary gland other than acetylcholine, when injected into the dog, results in increased uterine activity apparently due to liberation of oxytocin(2). Since oxytocin is released as a result of nursing or milking stimuli in lactating animals thereby resulting in milk let-down(3), it seemed of interest to investigate the possibility of cholinergic and adrenergic components in this phenomenon. In the present study, the effects of various blocking agents on let-down in lactating rats have been studied and results compared with normal values previously obtained(4).

Materials and methods. Adult primiparous lactating rats weighing 200-300 g were assayed for milk let-down according to the method of Grosvenor and Turner(4). These were divided into 3 groups: *Ergot Group.* Each mother received a single subcutaneous injection of one of the following ergot drugs‡ 10 minutes prior to nursing: methylergonovine tartrate (Methergine), 1-2 mg/kg; an equi-proportional mixture of methanesulfonate salts of dihydroergocornine, dihydroergokryptine and dihydroergocristine (Hydergine), 2-4 mg/kg; dihydroergotamine methanesulfonate (DHE 45) 2-4 mg/kg. In addition, some mothers received .2 USP/kg oxytocin ("P.O.P." Armour) subcutaneously 8 minutes after Methergine or 10 minutes after Hydergine and DHE 45, *i.e.*, immediately before nursing. *Dibenamine Group.* Each mother received a single subcutaneous injection of Dibenamine HCl§ which was administered to one group in dose of 40-60 mg/kg 15-60 minutes prior to nursing and 60-80 mg/kg 120 minutes prior to nursing to a second group. A third group was injected with 80 mg/kg 120 minutes before nursing followed by .2 USP/kg oxytocin subcutaneously immediately before the animals commenced to nurse. *Atropine Group.* Each animal in this group received a single subcutaneous injection of atropine sulfate in dose of 700 mg/kg either 15-60 minutes or 150-220 minutes prior to nursing. Eighteen of these were anaesthetized with Nembutal (45 mg/kg) and oxytocin administered intravenously while the young were actively sucking. Gross observations were made on effects of each drug on behavior of the animals.

Results. Ergot Group. All ergot drugs, in dosage employed in the present study, sig-

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TABLE I. Milk Removal by Nursling Rats on 14th Day Postpartum.

Treatment	No. of rats	Time of treatment prior to nursing (min.)	Avg wt of litters (g)	Avg wt of milk (g)	Avg % Milk wt / Litter wt	SE
Control†	40		144.0	5.5	3.8	.19
Methergine, 1-2 mg/kg	10	10	132.8	1.7	1.3	.31*
Idem, then Oxytocin, .2 USP/kg	5	10 2	162.8	6.8	4.2	.19
Hydergine, 2-4 mg/kg	13	10	150.0	2.6	1.6	.35*
Idem, then Oxytocin, .2 USP/kg	5	10 —	177.6	8.7	4.9	.28
DHE 45, 2-4 mg/kg	10	10	151.0	3.3	2.2	.38*
Idem, then Oxytocin, .2 USP/kg	5	10 —	155.2	7.9	5.1	.46
Dibenamine, 40-60 mg/kg	11	15–60	187.0	7.6	4.1	.39
" , 60-80 "	10	120	174.5	4.2	2.3	.29*
Idem, then Oxytocin, .2 USP/kg	5	120 —	177.6	9.0	5.1	.37
Atropine, 700 mg/kg	8	15– 60	165.1	5.0	3.4	.47
Idem	10	150–220	159.0	1.2	.8	.11*
Atropine, 700 mg/kg, then Oxytocin, .05 USP/kg i.v. into Nembutal anaesthetized mothers	9	150–220 —	166.6	6.1	3.7	.40
Atropine, 700 mg/kg, then Oxytocin, .1 USP/kg i.v. into Nembutal anaesthetized mothers	9	150–220 —	173.9	6.1	3.6	.23

* $P < .01$. † Data from Grosvenor and Turner(4).

nificantly inhibited milk let-down in lactating rats when compared with control values previously obtained(4). Methergine was the most potent inhibitor, followed by Hydergine and DHE 45, in that order (Table I). All treated animals showed normal behavioral patterns in gathering their young preparatory to nurse and during the nursing period and only those treated with Methergine showed any visible sign of possible central nervous system depression evidenced primarily by lethargy. No animal under any treatment showed signs of central excitation.

Dibenamine Group. It was noted all rats of this group reacted to treatment with Dibenamine by being highly restless and irritable for approximately 30-45 minutes post-injection after which they gradually became calm and assumed normal behavior. Such excitation did not, however, affect nursing behavior or affect let-down response in those animals permitted to nurse during the period of maximum excitation (Table I). Milk let-down was significantly inhibited when 60-80 mg/kg Dibenamine was administered 120 minutes prior to nursing. There was no apparent difference in inhibitory effects of the 2 doses.

Atropine Group. The response of lactating rats to atropine was one of extreme excitability commencing 30-60 minutes post-injection and lasting for several hours. Milk let-down was significantly inhibited in those rats injected 150-220 minutes before nursing but was not inhibited in those treated 15-60 minutes before nursing.

Inhibition following ergot or Dibenamine administration was reversed when .2 USP/kg oxytocin was injected subcutaneously just prior to nursing resulting in normal or above normal yields of milk. In atropinized anaesthetized animals normal yields also were obtained when .05 or .1 USP oxytocin was injected intravenously.

Discussion. Neural pathways involved in release of oxytocin from posterior pituitary

gland following nursing stimuli are obscure though the brain, hypothalamus and supraopticohypophyseal tract apparently are involved(1). It has been shown that adrenalin prevents release of antidiuretic hormone(5,6) and may perhaps inhibit milk let-down by preventing discharge of oxytocic hormone(7). However, in the present study sympatholytic agents, like some ergot alkaloids and Dibenamine, inhibited milk let-down indicating the possibility of an adrenergic component involved in this reflex. That the inhibition is not peripheral is evidenced by observations that exogenously administered oxytocin was capable of eliciting normal milk yields when injected after any sympatholytic tested. However, all ergot drugs have complex central nervous system effects at various dose levels which are not well understood so the possibility exists ergot alkaloids may inhibit milk let-down in the rat primarily by central depression such as is obtained with Nembutal (8). Inhibition obtained with Methergine, which has no demonstrable sympatholytic activity(9) supports this concept, for it was noted Methergine-treated animals which showed symptoms of possible central depression were inhibited more in their let-down response than were those treated with either Hydergine or DHE 45. Similar symptoms were noted previously(10) with ergotamine-treated lactating rats. On the other hand, neither Hydergine nor DHE 45 showed visible signs of possible central depression of excitation, yet milk let-down was significantly inhibited. Dibenamine has a direct excitant action on the central nervous system which develops soon after administration but which appears to be independent of its adrenergic blocking action(11). This was apparent in the present study for Dibenamine elicited visible symptoms of central excitation which persisted for 30-45 minutes though milk let-down was not inhibited in any animal who nursed during this period. Let-down, however, was significantly inhibited when the drug was injected 2 hours prior to nursing after which time adrenergic blockade is maximum, or very nearly so(11). It is interesting to note high concentrations of sympathin are present in hypothalamus(12) and therefore possibly the locus of adrenergic blockade of milk let-down in lactating rats.

Analysis of data obtained from atropine-treated lactating rats points to the possible presence of a cholinergic component in the let-down reflex. However, it might be argued that inhibition of let-down in atropinized animals might be the result of unopposed adrenergic impulses which might evoke such intense constriction of mammary gland blood vessels that circulating oxytocin would be prohibited from reaching the effector myoepithelial cells. It has been shown adrenalin in appropriate doses can block milk let-down response to both endogenous and injected oxytocin(7,13-15). We feel some vasoconstriction of the blood supply of the mammary gland does occur since .1 USP/kg oxytocin injected intravenously failed to elicit greater milk removal than .05 USP/kg for in a previous study the former dose resulted in a 55% greater milk yield(8). However, the observation that .05 USP/kg oxytocin resulted in a normal let-down response would seem to indicate that vasoconstriction is not severe enough to prevent normal let-down, and it is therefore postulated the principal site of blockade is primarily central rather than peripheral. It was apparent that 1-2 hours must elapse from time of injection until cholinergic paralysis of milk let-down is maximum since atropine, when administered 15-60 minutes before nursing, failed to block the response. It is postulated from the results of this study that adrenergic and cholinergic nerve links are present in the neurohumoral arc responsible for discharge of oxytocin from the posterior pituitary gland.

Summary. The amount of milk obtained by litters of lactating rats treated with various ergot alkaloids, Dibenamine or atropine, was compared with control values previously obtained. All ergot drugs tested significantly blocked the let-down response. Methylergonovine (Methergine) was the most potent inhibitor, followed by the dihydroergotoxine complex (Hydergine) and dihydroergotamine (DHE 45), in that order. Results indicate action on central nervous system is probably responsible for some, or perhaps all, milk let-down inhibitory action of ergot drugs. This

observation was based primarily on results with Methergine, a non-sympatholytic compound. Dibenamine evoked symptoms of central excitation which persisted for 30-45 minutes after injection though milk let-down was not inhibited in those animals who nursed during this period. Milk let-down, however, was significantly inhibited when the drug was injected 2 hours prior to nursing, after which time adrenergic blockade has occurred. Atropine induced a highly significant blockade of the let-down response when injected 150-220 minutes before nursing, but failed to block the response when administered 15-60 minutes before nursing. Oxytocin administered after any of these drugs restored normal milk let-down, indicating blockade was central rather than peripheral. Since Dibenamine, Hydergine and DHE 45 are powerful adrenergic blocking agents and atropine a cholinergic blocking agent, it is postulated both adrenergic and cholinergic links are present in the neurohumoral arc responsible for discharge of oxytocin from the posterior pituitary gland.

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Digestive Action of Gastric Juice Following Various Types of Gastric Resection for Duodenal Ulcer.* (23343)

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The present report evaluates operations designed for treatment of duodenal ulcer by comparison of the digestive action of the gastric juice following such procedures. For this purpose the erosive effect of gastric juice on the cat's esophagus has been measured and pepsin and acidity have been determined. These data have been compared with similar data from a study of control non-ulcer patients and another group of patients with untreated duodenal ulcer. Previous experiments (1) have shown gastric juice from 72% of untreated duodenal ulcer patients produced grade 3 to 5 digestion of the cat's esophagus during a 2 hour perfusion period. Only 9% of gastric specimens from control non-ulcer patients produced such severe degrees of esophageal erosion. Acidity and pepsin concentrations in the gastric juice of the majority of duodenal ulcer patients were found to be greater than in controls(1). A correlation of the data reported below, together with the observed incidence of ulcer recurrence following the several operative procedures studied

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