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Ergotamine, Oxytocin and Milk Let-Down in Lactating Rat.* (22789)

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It has been demonstrated(1-4) that adrenalin in appropriate doses can block the milk let-down response to both endogenous and injected oxytocin. Adrenalin presumably acts by constricting mammary blood vessels thus blocking passage of oxytocin to mammary myoepithelium(5-6). Ergotamine, also a peripheral vasoconstrictor(7), has recently been shown to inhibit milk let-down in lactating rats(8). The present paper is concerned with effects of exogenous oxytocin on ergotamine-inhibition of milk let-down in lactating rats. Evidence is presented to indicate that ergotamine exerts its inhibition by means other than through vasoconstrictive effects.

Materials and methods. Docile lactating albino rats, each with its first litter and weighing 200-260 g, were housed in individual cages and given free access to food and water. Each litter was reduced to 8 young shortly after birth and, whenever possible, these were equally distributed as to sex. Rats with less than 8 young were discarded. Each of 42 litters when 14 days old was isolated from their mother for 10 hours. During this time they were kept warm to prevent any undesirable effects from exposure. Each litter was then divided equally. One half was weighed as a group, killed and their stomachs examined for the presence of milk. The other half was replaced with the mother and allowed to suck for exactly 20 minutes. Length of time before each mother began nursing was recorded. Young were then removed, weighed as a group and immediately killed by decapitation. Their stomachs were opened and semisolid curds removed and weighed (Table I). The mothers received single subcutaneous injections as follows: (a) 17 received 1 mg/kg ergotamine tartrate.‡ Of these 10 were injected 10 minutes, the rest, 2 minutes before replacement of litters. (b) 10 received 1 mg/kg ergotamine 10 minutes before replacement of litters followed by oxytocin ('Pitocin', Parke Davis & Co.) 8 minutes later. Oxytocin was given to 5 rats in dose of .4 I.U./kg and to remaining 5 in dose of .2 I.U./kg. (c) 5 were treated with .2 I.U./kg oxytocin 10 minutes before replacement of litters followed by 1 mg/kg ergotamine 8 minutes later. (d) 10 served as controls of which 5 were uninjected. The remaining received comparable injections of distilled water 10 minutes prior to replacement of litters. Topical effects of saline solutions of 1:2000 ergotamine and .2 I.U./ml oxytocin alone, and in combination, were studied in 5 lactating rats. Each mother was separated 4-6 hours from its litter on the 14th day postpartum. Nembutal anaesthesia (3 mg/

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TABLE I. Average Quantity of Milk, Expressed as % Litter Body Weight, Secured in 20 Min. by Litters, Each with 4 Young, on the 14th Day Postpartum.

Treatment	No. of rats	Time of treatment prior to nursing (min.)	Avg wt of litters (g)	Avg wt of milk (g)	% milk / Litter wt	S.E.
Control	10	10	89.2	3.3	3.7	.43
Ergotamine, 1 mg/kg	10	10	86.8	.6	.7	.21
Idem	7	2	94.0	2.0	2.1	.68
Ergotamine, 1 mg/kg, then oxytocin, .2-.4 IU/kg	10	10 2	96.9	4.2	4.3	.31
Oxytocin, .2 IU/kg, then ergotamine, 1 mg/kg	5	10 2	97.7	4.2	4.3	.51

100 g) was injected intraperitoneally, an abdominal mammary gland exposed and observed with a stereoscopic dissecting microscope (x16-x32). The exposed tissue was kept moist with physiological saline solution and covered with a thin sheet of polyethylene. Drugs were administered as drops at the tip of a 24 gauge needle and gently placed on mammary tissue without touching it with the needle.

Results. At the end of 10 hours isolation, stomachs of half of each litter contained no milk. It was assumed, therefore, that stomachs of other half of each litter permitted to suck following isolation, were also empty prior to sucking. In each case upon placing the litter back with their mother after isolation, she was observed to hastily gather her young and to commence nursing within 3 minutes.

Amount of milk obtained by litters of lactating rats injected with ergotamine 10 minutes prior to litter replacement was very small in comparison with that obtained by control offspring ($P = .001$), or with that obtained by young of mothers treated with ergotamine 2 minutes before litter replacement ($P = .03$). This latter group obtained an average of only 57% as much as control offspring though the quantity difference was not significant ($P = .06$). When oxytocin was injected before or after ergotamine insignificantly larger ($P = .4$) quantities of milk were obtained by offspring in comparison with control group. The amount was, however, significantly greater than that obtained by litters of rats treated with ergotamine either 10 or 2 minutes prior to replacement of young. ($P = .001$ and .005 respectively). There was little difference in amount of milk obtained by offspring of uninjected and distilled water-injected controls.

Topical administration of ergotamine to living rat mammary tissue resulted in constriction of arterioles but not of venules or of alveolar capillaries. Constriction occurred within 1-10 minutes with relaxation taking place 3-15 minutes later, depending upon vessel diameter. Effect on mammary alveoli and ducts varied from no response, in most instances, to slight transient contraction of a few alveoli. Oxytocin administered alone caused almost immediate (2-4 sec.) contraction of alveoli which resulted in ducts filling with milk but there was no apparent effect on the vascular bed. If the glands were not engorged, milk did not return to the alveoli, which remained contracted. If alveoli were greatly distended, milk squeezed into ducts gradually ran back into alveoli again. Such relaxation occurred within 1-5 minutes. It was possible, therefore, in some cases to apply ergotamine and/or oxytocin to same area of tissue and obtain another contractile response as soon as relaxation of alveoli and arterioles occurred.

Ergotamine and oxytocin mixed together and applied topically, evoked both alveolar contraction and arteriole constriction. Moreover, oxytocin applied at height of ergotamine-induced arteriole constriction, elicited its usual alveolar contraction with no apparent delay in contraction time. Drops of saline applied directly to mammary tissue had no apparent effects.

Discussion. These observations would

seem to rule out ergotamine inhibiting milk let-down in lactating rats by vasoconstriction of mammary vessels. There remain at least two other possibilities to explain this inhibition. It is generally accepted(9,10) that mammary myoepithelial cells are the contractile elements responsible for squeezing milk from alveoli and ducts. There may then be a competition between oxytocin and ergotamine at the myoepithelium level for results from injecting ergotamine 2 minutes prior to nursing, suggests a certain level of the drug must be present in general circulation before full inhibition of milk let-down is assured. The other possibility is ergotamine through sympatholytic action and central nervous system depression blocks release of oxytocin from posterior pituitary gland. As neural pathways involved in the milk let-down reflex are obscure, it is perhaps premature to speculate on the site of neural blockage by ergotamine. It has been shown, however, that electrical stimulation of anterior hypothalamus(11) and vagi(12) in lactating sheep and goats, and supraoptico-hypophyseal tract(2,13,14) in lactating rabbits resulted in let-down in milk. It is possible that ergotamine inhibits milk let-down in lactating rats through blockage of one or more of these areas.

Summary. 1. The quantity of milk secured by litters of lactating rats treated with ergotamine 10 minutes before nursing was significantly less than that obtained by control offspring. Oxytocin administered before or after ergotamine restored normal milk let-down. 2. Topical application of ergotamine to living rat mammary tissue resulted in arteriole constriction but did not interfere with normal myoepithelial contraction induced by oxytocin. 3. Results indicate ergotamine does not inhibit milk let-down through vasoconstriction of mammary blood vessels thus prohibiting access of oxytocin to effector myoepithelium. Rather, a competition between ergotamine and oxytocin for the effector tissue or a neural block inhibiting release of oxytocin by posterior pituitary gland seem more probable.

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Fixation of Carbon Dioxide by *Actinomyces* and *Lactobacillus bifidus*. (22790)

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In recent studies on fermentation of glucose by *Actinomyces bovis* and *Lactobacillus bifidus*(1) a net fixation of CO_2 was definitely observed in some fermentations and suggested in others. The overall stoichiometry of 2 fermentations by *Actinomyces* indicated that CO_2 was being fixed in a manner consistent with the operation of the Wood-Werkman reaction(2) with the formation of succinic acid. The data suggested that this same reaction was functioning to a minor extent in one strain of *L. bifidus;* but that the major path-