Failure of Oxytocin to Initiate Mammary Secretion in Rabbits or Rats.* (26143)

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Benson and Folley(1) demonstrated that oxytocin inhibited mammary involution in postpartum rats after litter removal, and this has been confirmed(2). Since it was previously shown that prolactin could similarly inhibit mammary involution(3), the British workers hypothesized that the effects of oxytocin were mediated through anterior pituitary release of prolactin. We have questioned this hypothesis for several reasons, including the observation that oxytocin did not initiate lactation in animals with developed mammary glands(4-5). Recently however, Haun(6) reported successful induction of lactation in mature New Zealand White female rabbits with multiple injections of oxytocin for 2 days, after initially priming them with estrogen for 21 days. We have attempted to duplicate his procedure, and in addition have given oxytocin to pseudo-pregnant rabbits and estrogen-primed rats. Oxytocin failed to initiate mammary secretion in these animals.

Methods. Five groups of mature virgin New Zealand White female rabbits, weighing 3.5-4.5 kg, were injected subcutaneously with 0.1 mg estradiol in corn oil on alternate days for 20 days. On the 21st and 22nd days, a control group was injected intraperitoneally with physiological saline 4 times daily; and 3 groups, with .3, .6 and 1.2 IU oxytocin‡ respectively, given intraperitoneally 4 times daily. Mammary glands were inspected for lactation *via* mid-ventral incision on the 25th day. In another experiment, pseudopregnancy was induced in mature virgin rabbits of the same strain and weight by a single intravenous injection of 100 IU chorionic gonadotropin. This results in excellent mammary lobule-alveolar development by the 12th day. On days 12-16 of pseudopregnancy, one group received no further treatment and served as controls; another group was injected subcutaneously with 10 mg prolactin§ once daily; and 3 groups received .3, .6 and 1.2 IU oxytocin respectively, given intraperitoneally 4 times daily. On the 17th day mammary glands were examined for lactation *via* mid-ventral incision.

For the rat experiment, mature virgin females of the CFN Carworth strain, weighing 180-220 g, were injected subcutaneously with 10 μg estradiol in corn oil once daily for 10 days to develop a lobule-alveolar system. For the next 5 days, separate groups were injected with saline, oxytocin, vasopressin, prolactin, ACTH,‖ and prolactin and ACTH, respectively. On the 16th day, the rats were killed and the right inguinal mammary gland was removed, fixed and stained by standard histological technics(2), and examined for secretion microscopically.

Results. In the estrogen-primed rabbits, 2 of 6 control rabbits (Group 1) had a slight amount of milk in the ducts and there was little or no lobule-alveolar development. Only 2 of 18 oxytocin-injected rabbits (Groups 2-4) had a small amount of milk in the ducts, and the mammary glands were no better developed than in controls. In the pseudopregnant rabbits, 2 of 6 controls (Group 5) showed a slight amount of serous secretion in the ducts, whereas mammary glands of all 6 prolactin-injected animals (Group 7) were distended with milk which could easily be expressed from the nipples. Oxytocin (Groups 6-9) did not initiate milk secretion at any

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‡ Oxytocin was supplied through courtesy of the late Dr. D. A. McGinty, Parke, Davis and Co., Detroit.

§ Prolactin (1 mg = 15 IU) was donated by Endocrinology Study Section, N.I.H.

‖ ACTH gel (40 USP units/cc) was supplied through courtesy of Dr. R. O. Stafford, Upjohn Co., Kalamazoo, Mich.

TABLE I. Effects of Oxytocin* and Prolactin† on Initiation of Lactation in Estradiol-Primed‡ and Pseudopregnant§ Rabbits.

Group and No. of rabbits	Treatment	No. with milk	Remarks
	Estradiol-primed rabbits (examined on 25th day)		
1 (6)	Controls, saline 4× daily on days 21-22	2	Slight duct secretion
2 (6)	.3 IU oxytocin *idem*	1	*Idem*
3 (6)	.6 IU oxytocin "	1	"
4 (6)	1.2 IU oxytocin "	0	No secretion
	Pseudopregnant rabbits (examined on 17th day)		
5 (6)	Controls, 17th day pseudopregnancy	0	Serous secretion in 2
6 (6)	10 mg prolactin daily on days 12-16	6	Copious milk throughout
7 (3)	.3 IU oxytocin 4× daily on days 12-16	0	Serous secretion in 1
8 (3)	.6 IU oxytocin *idem*	0	*Idem*
9 (3)	1.2 IU oxytocin "	0	No secretion

* Oxytocin (Parke-Davis) was inj. intraper.
† Prolactin (NIH, 15 IU/mg) was inj. subcut.
‡ .1 mg estradiol inj. subcut. on alternate days for 20 days.
§ Pseudopreg. induced by single intrav. inj. of HGG.

dose level, although 2 animals showed slight serous secretion in the ducts (Table I).

In the rats (Table II), mammary glands of controls (Group 1) regressed to a bare duct system, as did mammary glands of oxytocin (Groups 2-5) and vasopressin (Groups 6-7) rats. No secretory activity was observed in these rats. Neither prolactin (Groups 8-9) nor ACTH (Groups 10-11) alone induced secretion, although mammary lobule-alveolar structure was well maintained by prolactin.

TABLE II. Effects of Oxytocin,* Vasopressin,† Prolactin,‡ and ACTH§ on Initiation of Lactation in Estradiol-Primed Rats.

Group and No. of rats	Treatment for 5 days after estradiol	No. with secretion
1 (10)	Controls, saline 2× daily	0
2 (5)	1 IU oxytocin 2× daily	0
3 (5)	1 " " 4× "	0
4 (5)	2 " " 2× "	0
5 (5)	10 " " " "	0
6 (5)	1 " vasopressin 2× daily	0
7 (5)	5 " " " "	0
8 (5)	1 mg prolactin 2× daily	0‖
9 (5)	2 " " " "	0¶
10 (5)	1 IU ACTH " "	0
11 (5)	2 " " " "	0
12 (5)	1 mg prolactin + 1 IU ACTH 2× daily	4

* † Oxytocin and vasopressin (Parke-Davis) were inj. subcut.
‡ Prolactin (NIH, 15 IU/mg) was inj. subcut.
§ Depot-ACTH gel (The Upjohn Co.) was inj. subcut.
‖ ¶ Mammary lobulo-alveolar structure was maintained.

When both prolactin and ACTH were administered (Group 12), mammary maintenance and secretion were observed in 4 of 5 rats. This shows that both prolactin and ACTH are necessary to initiate mammary secretion in estrogen-primed rats, as reported previously(7).

Discussion. Oxytocin failed to initiate mammary secretion in estrogen-primed or pseudopregnant rabbits in contrast to the report by Haun(6), although his procedure in respect to strain and size of rabbit, hormone dosage and length of treatment was duplicated as closely as possible. A private communication from Folley¶ also states that "a recent attempt to repeat Haun's results on New Zealand White rabbits by Benson has failed completely." It is possible that the limited mammary secretion noted by Haun was due to estrogen rather than oxytocin stimulation. Neither oxytocin nor vasopressin inhibited mammary involution or initiated secretion in estrogen-primed rats. By contrast, injections of prolactin alone inhibited mammary involution, and injections of both prolactin and ACTH initiated mammary secretion in estrogen-primed rats. Thus oxytocin did not induce prolactin release in either rabbits or rats.

Other work also indicates that prolactin release from the pituitary is independent of

¶ Letter dated March 30, 1960.

oxytocin secretion. Thus (a) hypothalamic lesions in the rat inhibit oxytocin release and prevent litters from obtaining milk, although milk production and hence prolactin secretion continue in a normal fashion(8); (b) single pituitaries transplanted to the kidney of rats secrete greater than normal amounts of prolactin and initiate mammary secretion, despite absence of obvious oxytocin stimulation(9); (c) whereas suckling evokes an immediate decrease in pituitary prolactin content, indicating discharge of this hormone, injections of oxytocin are ineffective in this respect(4). Benson and Folley's(1) demonstration that oxytocin inhibits mammary involution in postpartum rats can be explained by its direct action on the mammary gland(5,10).

Summary. Oxytocin failed to initiate lactation when injected 4 times daily into 45 mature female New Zealand White rabbits initially primed with estradiol or made pseudopregnant to develop their mammary glands. Prolactin injections initiated copious milk production in pseudopregnant rabbits. 2. Neither oxytocin nor vasopressin inhibited mammary involution or induced secretion in estrogen-primed rats, whereas injections of prolactin alone retarded mammary involution and addition of ACTH initiated mammary secretion. 3. It is concluded that oxytocin does not induce prolactin release in rabbits or rats.

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Effect of Diazoalkane-yielding Compounds on Strains of *Escherichia coli* Resistant to 1-Methyl-3-nitro-1-nitrosoguanidine.* (26144)

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1-Methyl-3-nitro-1-nitrosoguanidine (NG) has anticancer activity in mice(1,2,3) and antibacterial activity *in vitro*(4,5). Two mutants of *Escherichia coli* strain S which are resistant to NG have been selected. The first-step resistant mutant S/Ng 1 was selected from the wild type, strain S. The second-step mutant S/Ng 2 was selected from S/Ng 1(6). It was believed that some insight into the mechanism of action of NG could be gained by testing the strains of *E. coli* resistant to NG against structurally similar compounds.

Skinner *et al.*(3) have synthesized a series of nitrosoguanidines differing from NG in the substituent on N-1. In addition, 2 commercially available compounds, like NG, yield diazomethane under alkaline conditions. These are N-methyl-N-nitrosourea (MNU) and N - methyl - N - nitroso - *p* - toluenesulfonamide (MNTS). Another compound, S - methyl-N-nitropseudothiourea (MNPT) (7) will, like NG(8,9) nitroguanisate amino acids.

Methods. Strain S (obtained from Dr. A.

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