Effects of Oxytocin and Blocking Agents upon Pituitary Lactogen Discharge in Lactating Rats.* (23775)

CLARK E. GROSVENOR† AND CHARLES W. TURNER
Department of Dairy Husbandry, University of Missouri, Columbia

It is known that nursing stimuli are necessary for maintenance of milk secretion and initiation of milk "let-down" by causing reflex discharge of lactogenic hormone from adenohypophysis and oxytocin from neurohypophysis(1). Because of the integration and coordination of these hormones in maintaining lactation it has been postulated that oxytocin might be the humoral link for release of lactogenic hormone(2,3). Basis for such postulation is mainly the ability of injected oxytocin to retard mammary gland involution in the rat. We thought it necessary to test the validity of this concept by determining if oxytocin could induce discharge of pituitary lactogen in nursing rats whose reflex release of lactogen and oxytocin have been blocked and to see if cholinergic and adrenergic components were involved in reflex discharge of lactogen as has been found previously in regard to release of oxytocin in response to nursing stimuli in lactating rats(4).

Materials and methods. Ninety-five adult primiparous lactating rats of the Sprague Dawley-Rolfsmeyer strain, weighing 270-320 g, were housed in individual cages and fed Purina Lab Chow and water *ad libitum*. Each litter was adjusted to 6 young shortly after birth and when 14 days old separated from their mother for 10 hours, then replaced and allowed to suck for 30 minutes. The mothers were treated as follows: Fifteen received single subcutaneous injections of 60 mg/kg Dibenamine-Cl‡ 2 hours before nursing. Twenty-five others received single subcutaneous injections of 700 mg/kg atropine-SO_4 either 20 minutes or 2½ hours before nursing. Remaining animals were anaesthetized with Nembutal (45 mg/kg *i.p.*) 10-20 minutes prior to end of isolation period. When completely anaesthetized each mother was laid on her side and her young replaced. Forty of these received oxytocin§ in doses of .05-.1, 3 or 6 USP/kg in a volume not exceeding .06 ml into the superior epigastric vein a few minutes after the young were replaced and while they were actively sucking. Fifteen similarly received distilled water. After nursing, each mother was killed, her pituitary gland removed rapidly, weighed and frozen until assayed for lactogenic hormone. Five glands were pooled for each assay. These were crushed in an agate mortar, suspended in a measured amount of distilled water and assayed in common adult pigeons by the Reece-Turner intradermal method(5).

Results. Data previously obtained(6) indicated a pituitary lactogen level of 2.44 Reece-Turner (R-T) units/100 g following isolation of mother and litter for 10 hours on 14th day postpartum (prenursing level). Following 30 minutes nursing, the level had dropped to 1.66 R-T units/100 g (postnursing level). In the present investigation Dibenamine and Nembutal effectively blocked lactogen release from pituitary gland since nursing evoked no decline from control prenursing level (2.39 and 2.46 R-T units/100 g, respectively). Atropine injected 2½ hours before nursing also totally blocked lactogen release in response to nursing stimuli (2.35 R-T units/100 g) but only partial blockade occurred when the drug was administered 20 minutes prior to nursing (2.17 R-T units/100 g). Oxytocin injected i.v. into Nembutal-anaesthetized nursing rats in dose of .05-.1 USP/kg, which dose previously had been determined as the amount of hormone released as a result of nursing stimuli in 14-

* Contribution from Mo. Agr. Exp. Sta., Journal Series No. 1813. Approved by Director.

† Postdoctoral Fellow of N.I.H. This investigation was supported in part by research grants from Public Health Service.

‡ Kindly supplied by Smith, Kline and French Laboratories, Philadelphia.

§ Kindly supplied by Armour Laboratories, Chicago.

TABLE I. Lactogen Content of 14-Day Postpartum Lactating Rat Pituitary Gland. Data obtained after 30 min. nursing following 10 hr isolation of mother and litter of 6 young.

Treatment	Time of treatment prior to nursing (min.)	No. of rats	Avg wt of mothers (g)	Avg pituitary wt of mothers (mg)	Avg Reece-Turner lactogen	
					Units/pituitary	Units/100 g
Control, prenursing*	—	15	289.9	10.5	7.08	2.44
Control, postnursing*	—	15	292.5	9.3	4.85	1.66
Dibenamine, 60 mg/kg subcut.	120	15	293.7	9.9	7.03	2.39
Atropine, 700 mg/kg subcut.	150	15	289.2	10.8	6.79	2.35
Idem	20	10	277.1	9.6	6.00	2.17
Nembutal, 45 mg/kg intrap.	10–20	15	306.1	10.8	7.52	2.46
Idem + .05-.1 USP/kg oxytocin intrav.	10–20 —	10	289.7	10.6	7.22	2.49
Idem + 3 USP/kg oxytocin intrav.	10–20 —	15	312.0	11.3	6.88	2.22
Idem + 6 USP/kg oxytocin intrav.	10–20 —	15	286.8	11.4	6.13	2.14

* Data from Grosvenor and Turner(6).

day lactating rats(7), failed to alter pituitary lactogen from the control prenursing level (2.49 R-T units/100 g). Amounts of oxytocin 30x and 60x the upper physiological dose of .1 USP/kg produced but a slight diminution from control prenursing level (2.22 and 2.14 R-T units/100 g, respectively).

Discussion. We have shown(6) nursing stimuli evoke very rapid discharge of lactogenic hormone from the pituitary gland of lactating rats. In the present study, we have been able to prevent totally such discharge with Dibenamine and atropine injected in doses and at times these drugs previously have been shown to block oxytocin release in the lactating rat(4). The evidence in regard to blockade of oxytocin release was interpreted as indicating cholinergic and adrenergic links were involved somewhere in the neural pathway from mammary gland to pituitary gland. We believe, from results of the present study, a similar interpretation applies to lactogen discharge in response to nursing stimuli. Since it is well known such stimuli evoke reflex release of oxytocin and lactogen(1), it is possible there exists a common sensory pathway from mammary gland to hypothalamus. We suggest, therefore, adrenergic and cholinergic components are involved, perhaps in the hypothalamic area, in simultaneous discharge of these hormones.

The role of oxytocin acting as a humoral agent responsible for lactogen discharge has not been substantiated by our present experiments. Nembutal has been shown(7) to block oxytocin release in response to nursing stimuli in lactating rats and, in the present investigation, lactogen discharge as well. Physiological doses of oxytocin failed to effect lactogen release in anaesthetized lactating rats though it may be argued only a small portion of the quantity administered i.v. actually reached the anterior pituitary gland. However, sufficient oxytocin certainly gained access to the pituitary when injected i.v. in doses 30 and 60 x the physiological level, yet insignificant discharge of lactogen occurred. If oxytocin were capable of evoking release of pituitary lactogen we should have obtained a decline from the prenursing level of 2.44 R-T units/100 g to a level approaching that resulting from 30 minutes nursing (1.66 R-T units/100 g). Meites (personal communication) and Meites and Turner(8) also have been unable to demonstrate any change in pituitary lactogen following administration of

oxytocin or vasopressin in the rat, guinea pig or rabbit.

The observations of Benson and Folley(2, 3) that oxytocin maintains involuting mammary gland of rats for a period of time in a state of active secretion following daily oxytocin administration must be explained in terms other than their suggestion that oxytocin causes discharge of lactogenic hormone from the anterior pituitary gland, attractive as it may appear.

Summary. 1) The effect of various compounds upon release of lactogenic hormone from the hypophysis in response to nursing stimuli has been studied in 14-day postpartum lactating rats. 2) Atropine, Dibenamine and Nembutal effectively blocked pituitary lactogen discharge since 30 minutes nursing evoked no decline (2.35, 2.39 and 2.46 Reece-Turner units/100 g, respectively) from previously obtained control prenursing level (2.44 R-T units/100 g). The normal level following 30 minutes nursing was previously determined as 1.66 R-T units/100 g. Since Dibenamine is a potent adrenergic blocking agent and atropine a cholinergic blocking agent, it is suggested cholinergic and adrenergic links are involved in the reflex arc responsible for lactogen discharge from rat pituitary gland. 3) Oxytocin injected i.v. into Nembutal-anaesthetized lactating rats in physiological doses failed to alter pituitary lactogen (2.49 R-T units/100 g) from the control prenursing level. Amounts of oxytocin 30 and 60 x this dose produced but a slight discharge. These results suggest oxytocin has no stimulatory effect upon pituitary lactogen release in the lactating rat, and are contrary to the recent hypothesis that oxytocin is a humoral link involved in lactogen discharge.

1. Folley, S. J., *The Physiology and Biochemistry of Lactation,* Edinburgh, London, Oliver & Boyd, 1956.

2. Benson, G. K., Folley, S. J., *Nature* (London), 1956, v177, 700.

3. ———, *J. Endo.,* 1957, v14, xl.

4. Grosvenor, C. E., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 719.

5. Reece, R. P., Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.,* 1937, 266.

6. Grosvenor, C. E., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 723.

7. ———, *ibid.,* 1957, v95, 131.

8. Meites, J., Turner, C. W., *Endocrinology,* 1942, v30, 726.

Received November 25, 1957. P.S.E.B.M., 1958, v97.

Serotype Composition of the Adenovirus Group. (23776)

WALLACE P. ROWE, JANET W. HARTLEY, AND ROBERT J. HUEBNER
U. S. Department of Health, Education, and Welfare, P.H.S., N.I.H., Nat. Inst. of Allergy and Infectious Diseases, Bethesda, Md.

A previous report(1) described 14 immunologically distinct viruses possessing the attributes of the adenovirus group(2). This communication describes 9 additional serotypes of human and monkey origin, as well as a subtype of type 7, designated 7a.

Materials and methods. Criteria for including a new virus in the adenovirus group have been described previously(1,3). All types reported here demonstrated characteristic cytopathogenicity for HeLa cell and monkey kidney tissue cultures, ether resistance, lack of pathogenicity for 1-day-old and adult mice and adult rabbits, and ability to demonstrate complement fixing antibody responses in paired serums of at least 4 humans infected with other adenovirus types. The strains designated as prototypes represent, of necessity, the first strain of the type received and tested in this laboratory. Neutralization tests were performed in tube cultures of trypsin-dispersed rhesus monkey kidney cells,* maintained in medium 199. Adenovirus neutralization procedure 2(3) was employed throughout, using the same criteria for neutralization with

* Received from Microbiological Associates, Bethesda, Md.