Lactogenic Activity of Human Chorionic Somatomammotropin in Rhesus Monkeys[1] (36422)

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Although human chorionic somatomammotropin (HCS)[2] has been shown to induce the synthesis of casein-like phosphoproteins in pregnant mouse mammary gland explants (1) and to induce milk formation following its acute injection into the mammary ducts of rabbits sensitized with chorionic gonadotropin (2, 3) nothing is known about the effect of HCS on mammary gland function in primates. Because HCS and monkey chorionic somatomammotropin are immunologically similar (4), the present study was undertaken to determine whether HCS had any effect on mammary glands in nonpregnant Rhesus monkeys, and to determine whether HCS could alter milk production in female Rhesus monkeys with well-established lactation.

Methods. To determine the effect of HCS on nonpregnant monkey mammary gland tissue *in vivo*, HCS, 17β-estradiol and progesterone were administered in the following combinations to female Rhesus monkeys weighing between 3.5 and 4.5 kg: 1) HCS, 25 mg twice daily for 7 days; 2) progesterone,[3] 20 mg/day, and 17β-estradiol,[3] 5 mg/day for 4 weeks; 3) HCS plus progesterone plus 17β-estradiol in the same doses for 2 weeks. There were four monkeys in each treatment group. Breast biopsies were obtained by the method of Speert (5) before each treatment and at the end of each therapy period. The tissue was fixed immediately in Zenkersformalin solution and was subsequently sectioned and stained with hematoxylin–eosinazure[4]. For purposes of comparison, breast biopsies were taken from one pregnant Rhesus monkey and from three Rhesus monkeys who were breast-feeding their 3–6-week-old infants.

The dosages of 17β-estradiol and progesterone employed were equivalent to those previously shown by Speert (5) to produce mammary gland enlargement and development in nonpregnant Rhesus monkeys. These dosages of the sex steroids undoubtedly exceed the physiologic range. Hopper and Tullner (6) have recently reported that the mean total estrogens excreted daily by pregnant Rhesus monkeys is 30 μg/day. Previous work in our laboratory indicates that daily administration of 20 mg progesterone to nonpregnant Rhesus monkeys produces mean plasma progesterone concentrations of 17 ng/ml (7) whereas Neill, Johansson, and Knobil (8) have reported that the mean plasma progesterone concentration of pregnant Rhesus monkeys is 4 ng/ml. The HCS administered was Lederle Purified Placenta Protein (Lot #716049)[5] which, in our hands, was about 75% as potent as the most highly purified Lederle Preparation (Lot #4142-C-190-1).[5] With the doses administered serum HCS concentrations were obtained which are comparable to those observed during the third trimester of human pregnancy (9).

To determine whether well-established lactation could be altered in Rhesus monkeys, HCS (1.25 mg/kg of mother's body wt) was

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[2] Alternately known as Human Placental Lactogen (HPL), Chorionic Growth Hormone-Prolactin (CGP), or Purified Placenta Protein (PPP).

[3] Mann Research Laboratories, Inc., New York, NY.

[4] Kindly performed by Dr. Matthew Block.

[5] Supplied by Dr. Ira Ringler, Lederle Laboratories; Pearl River, NY.

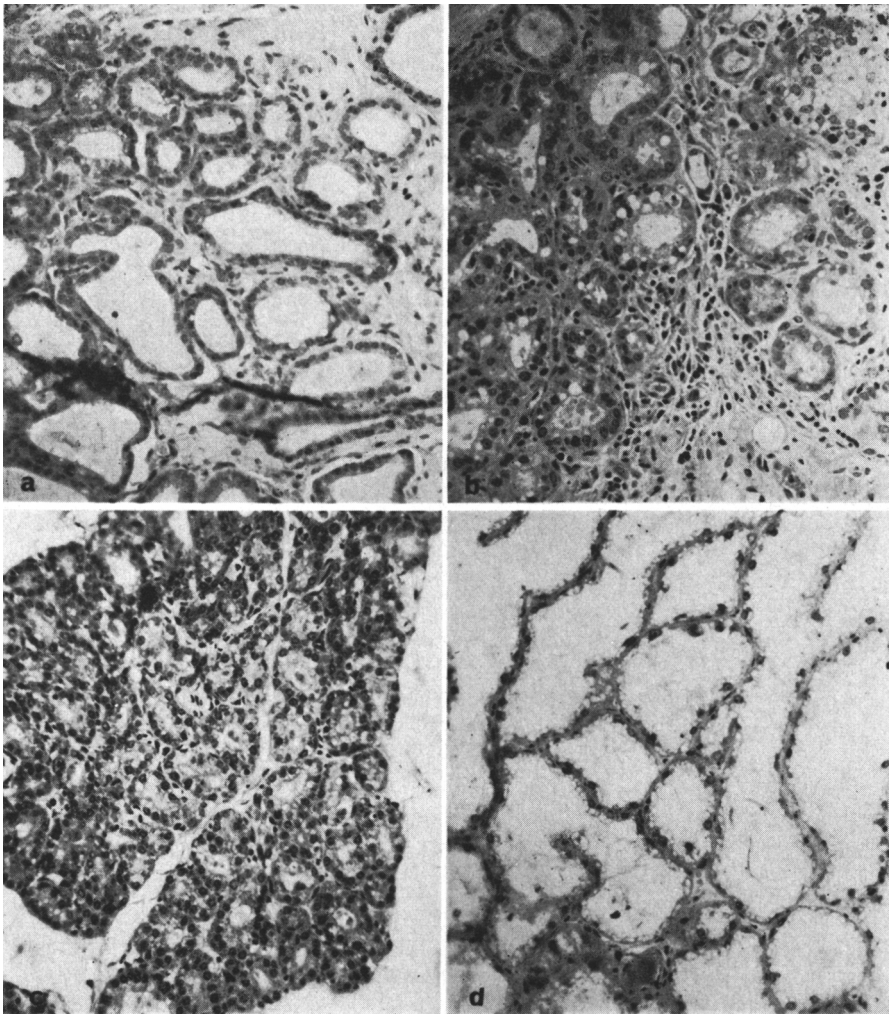

FIG. 1. (a). Nonpregnant monkey mammary tissues after progesterone + 17β-estradiol for 2 weeks. (b). Nonpregnant monkey mammary tissue after progesterone + 17β-estradiol two weeks followed by HCS + progesterone + 17β-estradiol for two weeks. (c). Midpregnancy monkey mammary tissue. (d). Mammary gland from a lactating monkey. All illustrations magnified ×160, and stained with hematoxylin–eosin–azure.

administered daily to three lactating Rhesus monkeys weighing between 4.8 and 6.2 kg for two weeks. Breast biopsies were obtained before and at the completion of the HCS treatment. The suckling monkey infants were weighed every second or third day for 3 weeks preceding HCS treatment, during HCS treatment and for 2 weeks after completing HCS treatment. The newborn monkeys were 3–6-weeks-old before we started HCS administration to the mother.

Results. The administration of HCS alone to nonpregnant nonlactating Rhesus monkeys produced no change in the basic mammary gland morphology. Breast biopsies obtained before and after only HCS treatment contained scant amounts of alveoli and ducts. However, as shown in representative biopsies (Fig. 1), the administration of 17β-estradiol plus progesterone daily for 4 weeks produced the expected increase in amount of mammary gland mass and alveolar duct differentiation (Fig. 1a) (5). When HCS treatment was added to the 17β-estradiol plus progesterone

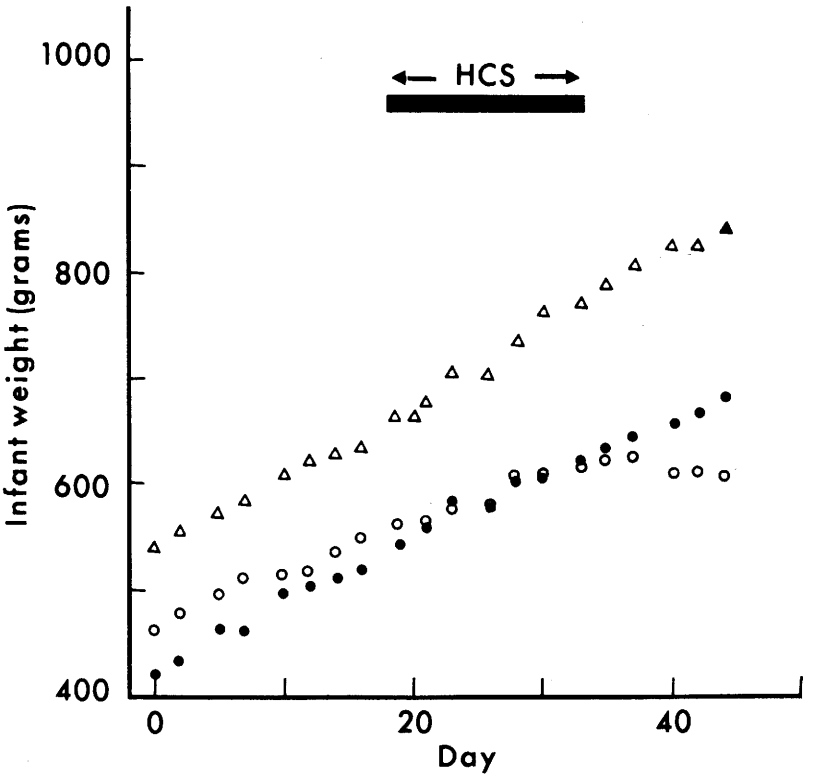

FIG. 2. Weight gain of three suckling infant monkeys before, during, and after treatment of their mothers with human chorionic somatomammotropin (HCS) 1.25 mg/kg/day.

treatment (Fig. 1b), the mammary glands developed additional morphologic changes which more nearly resembled the breast tissue obtained from the pregnant Rhesus monkey during the last trimester of gestation (Fig. 1c). The combination of HCS plus 17β-estradiol plus progesterone produced enlargement of the lumens of the ducts and alveoli in the mammary tissue with deposition in the ducts of large amounts of eosinophilic material. Also, the cytoplasm of the cells surrounding the lumens frequently contained vacuoles and the nuclei were larger and basally located within the cell. However, the pregnancy mammary gland contained alveoli in greater numbers and density than the HCS + progesterone + 17β-estradiol treated glands. Nevertheless, the lumina after hormone therapy as well as during gestation, were much less distended with secretory material than those mammary gland tissues obtained from lactating monkeys (Fig. 1d).

As shown in Fig. 2, HCS had little discernible effect on apparent milk production in monkeys with well-established lactation. There was no consistent change in the rate of weight gain of the suckling monkeys when their mothers were treated with HCS as compared with their pre- or posttreatment rates of weight gain. The mean $\pm$ SEM rate of weight gain during HCS treatment (5.78 $\pm$ 0.85 g/day) was not significantly different from the pre- or posttreatment rates of growth 5.48 $\pm$ 0.36 and 3.15 $\pm$ 1.79 g/day, respectively). Moreover, examination of the breast biopsies obtained from these lactating monkeys before and after HCS treatment revealed no discernible changes in mammary gland morphology.

Discussion. The results of this study indicate that mammary glands from nonpregnant primates and rodents respond similarly to the lactogenic activity of human chorionic somatomammotropin (HCS). It should be

noted, however, that this expression of lactogenic activity in mammary tissue does not occur unless the HCS is administered to animals that have been previously stimulated with estrogenic and/or progestational hormones. Josimovich (2) and Friesen (3) demonstrated the lactogenic activity of HCS in rabbits previously pretreated with chorionic gonadotropin, presumably stimulating endogenous progesterone production. Turkington and Topper (1) confirmed the similarity of HCS to ovine prolactin in mammary gland explants obtained from pregnant mice. In the present study, not until the nonpregnant monkeys were pretreated with 17β-estradiol and progesterone did HCS produce morphologic changes in the mammary tissue which were similar to those of pregnant monkeys. Nevertheless, the lactogenic activity of HCS + progesterone + 17β-estradiol in monkeys, as assessed by mammary gland morphology, was considerably less than that observed during puerperal lactation (Fig. 1). Also, HCS administration produced no discernible effect on milk production in monkeys after lactation was established (Fig. 2).

Unfortunately, the present study does little to elucidate the mechanisms which are responsible for the initiation of lactation in primates in the immediate postpartum period. Very little data are available with regard to the hormonal factors which affect the initiation of lactation in primates, and studies in other mammals reveal considerable variation from species to species with regard to hormonal requirements (10). The rapid decrease of circulating hormonal products of the conceptus postpartum has been postulated to take part in this process. In women, it has long been known that synthetic estrogen administration in the immediate postpartum period may be useful in suppressing lactation (11). On the other hand, Meites and Sgouris (12) found that both estrone and progesterone were necessary to inhibit the milk production stimulated by prolactin in rabbit mammary glands. By contrast, Kuhn has reported that progesterone inhibits the formation of lactose in lactating rats and that prolactin administration to pregnant rats is incapable of stimulating lactose production in rat mammary tissue (13). More recently, Turkington and Hill demonstrated that progesterone inhibited lactose synthetase activity in mouse mammary gland explants by repressing prolactin-stimulated formation of one of the two protein components of lactose synthetase (α-lactalbumin) (14). At present, there are no data concerning the effect of progesterone withdrawal on the initiation of puerperal lactation in primates. In view of the failure of HCS administration to reduce milk production in monkeys with well-established lactation in the present study, it seems unlikely that the rapid decrease of HCS in the postpartum period plays (4, 15) any significant role in the initiation of puerperal lactation in primates.

Summary. The lactogenic activity of human chorionic somatomammotropin (HCS)[2] has been assessed in mature, nonpregnant female Rhesus monkeys. When HCS (25 mg twice daily) was administered alone no changes in mammary gland morphology were observed. However, when HCS was administered for 2 weeks to animals previously treated for 2 weeks with 17β-estradiol (5 mg/day) plus progesterone (20 mg/day), histologic changes in mammary tissue suggestive of milk production were observed in the mammary glands. In addition, HCS alone neither altered mammary gland morphology in monkeys with well-established lactation nor affected the rate of weight gain in their suckling monkeys. Thus, in primates, HCS secretion during pregnancy appears to facilitate progesterone and/or estrogens in preparing mammary tissue for lactation.

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