caloric intake. This conclusion is consonant with another in which prolactin was shown to stimulate fattening in unfed fish[13]. The possibility of a shift from fat synthesis to increased protein synthesis in bromocriptine-treated animals deserves further study.

Because insulin has many other vital activities, reduction of insulin itself is not a practical way to reduce fat stores. However, reduction of prolactin secretion has been practiced extensively in obstetrics and gynecology with few deleterious side-effects. The low dose of bromocriptine used in this study is equivalent on a weight basis to that used in humans for such purposes[14]. Thus a selective suppression of insulin's lipogenic activities by reducing prolactin secretion may offer a practical means for treating obesity.

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Inhibition by SMS 201-995 of normal mammary gland growth in mice

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Summary. Twice daily s.c. injection of 5 ng or 50 ng of SMS 201-995 between 25 and 55 days of age induced a significant retardation of normal mammary gland growth in C3H/He virgin mice, associated with the reduced plasma GH level. Meanwhile, plasma prolactin level and the pattern of estrous cycle were affected little by SMS treatments. The results indicate an involvement of GH in normal mammary gland growth in mice.

Key words. GH; mammary gland; mice; prolactin; somatostatin.

Somatostatin was initially isolated from the hypothalamus[2] and subsequently found in the gastrointestinal tracts and pancreatic islets[3]. Somatostatin and its analogs have widespread physiological roles including the suppression of the secretion of hormones from gut and pancreas, gastric acid secretion and pancreatic exocrine secretion[3–6]. However, the most representative action of somatostatin is the inhibition of pituitary growth hormone (GH) secretion[2, 7, 8] and most analogs are more potent and longer acting than the native molecule[9, 10].

Despite the accumulation of a considerable amount of data, the question of the role of GH in mammary gland growth is still far from being conclusively answered. Exogenous administration of GH from different species, as used in previous studies, is likely to be one of the factors preventing reliable interpretation of the results. In this paper, we studied the effects of chronic administration of SMS 201-995, a somatostatin analog, on mammary gland growth and the circulating levels of GH and prolactin in virgin mice.

Materials and methods. Animals and treatments. A highly inbred strain of C3H/He mice maintained in our laboratory were used. At 25 days of age, virgin mice were divided into 3 groups. The 1st, 2nd and 3rd groups received twice daily (08.00 and 17.00) s.c. injections of 0.05 ml physiological saline, 5 ng SMS 201–995 (Sandoz Ltd., Basel, Switzerland) and 50 ng SMS, respectively,

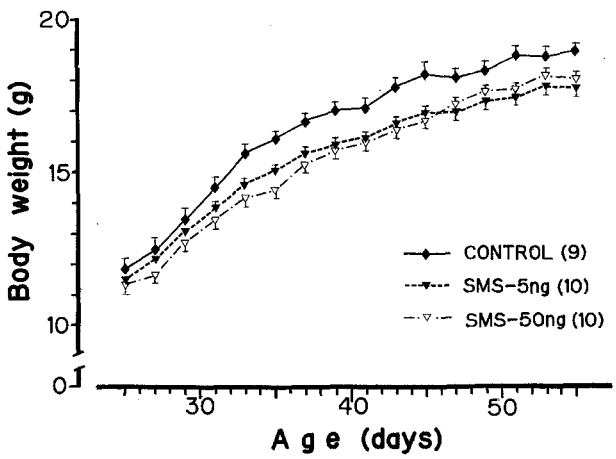

Figure 1. Body weight change in each group (Mean ± SEM). Each dose of SMS, dissolved in 0.05 ml physiological saline, was injected subcutaneously twice daily between 25 and 54 days of age and once on the morning of day 55. Control received vehicle only. Number of mice weighed is indicated in the parentheses.

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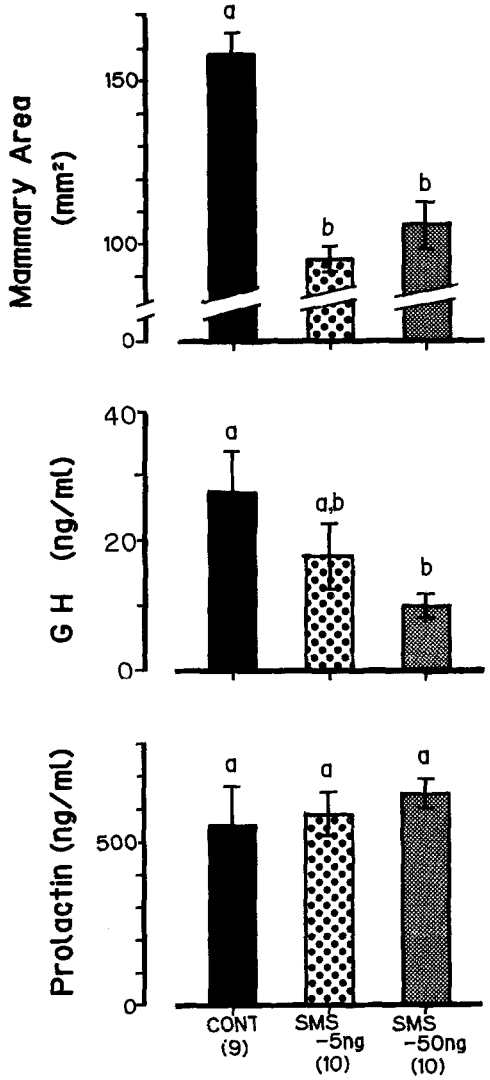

Figure 2. Mammary area and plasma levels of GH and prolactin in each group (Mean ± SEM). See fig. 1 for details of treatments. Number of samples is indicated in the parentheses. Values with different superscripts are significantly different at $p < 0.05$ or 0.01.

for 30 days and once on the next morning, and all mice were killed by decapitation under light ether anesthesia 2–3 h after the last injection. Each dose of SMS was dissolved in 0.05 ml physiological saline, divided into the small aliquots necessary for each injection and kept at −20 °C. Throughout the experiment, mice were kept in plastic cages (16 × 28 × 13 cm), 4–5 each, maintained in an animal room air-conditioned (21–23 °C and 60–70% relative humidity) and artificially illuminated (14 h of light from 05.00 to 19.00), and provided with a commercial diet (Lab MR Breeder, Nihon Nosan Kogyo KK, Yokohama, Japan) and tap water ad libitum.

Body weight: Body weight was measured every other day between 25 and 55 days of age. *Estrous cycle:* Vaginal smears were checked every morning (08.30–09.30) during the last 12–15 days

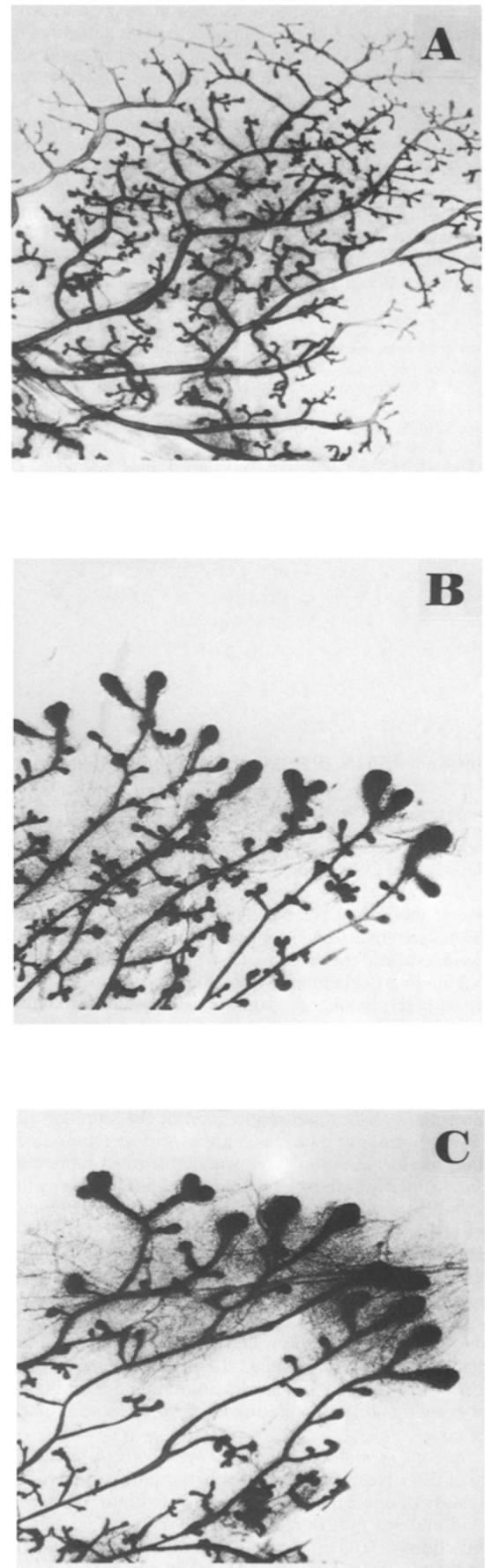

Figure 3. Representative whole-mount preparation of the third thoracic mammary gland in each group (× 12). See fig. 1 for the detail of treatments. *A* Control; *B* SMS 5 ng group; *C* SMS 50 ng group.

of injection. *Plasma levels of GH and prolactin:* Just before killing, mice were bled by orbital puncture under light ether anesthesia. Plasma was kept at −20 °C until the assay of hormones by homologous radioimmunoassay using the kits donated by Dr Parlow, Torrance, CA, USA. *Mammary gland growth:* At autopsy, bilateral third thoracic mammary glands were prepared for whole-mount evaluation. As an index of mammary gland growth, mammary area, the area bounded by a line joining the periphery of the ducts[11], was measured and the mean for the bilateral glands represented the value for the individual. *Endocrine organ weights:* At autopsy, anterior pituitary, adrenals and ovaries were also removed and weighed.

Results. Body weight change (fig. 1): Body weight was apparently smaller in mice treated with both doses of SMS than in the control at any age examined, although the difference was not always statistically significant. There was little difference in weight between two experimental groups at all ages. *Mammary gland growth* (figs 2 and 3): Mammary area was significantly smaller in both experimental groups than in the control (fig. 2). Furthermore, the glands of the former were morphologically more premature than that of the latter; the tops of the mammary ducts were still swollen in mice given SMS and the number of duct branchings seen in these mice was much less than that of the control (fig. 3). No difference was seen between the two experimental groups in the degree of mammary gland growth. *Plasma levels of GH and prolactin* (fig. 2): GH level was apparently decreased by SMS, while the differences between the control and 5 ng groups, and between the two experimental groups, were not statistically significant owing to large variations in the values for the former two groups. There was little difference among groups in plasma prolactin level. *Estrous cycle and endocrine organ weights:* Only a slight difference was observed among groups in either the pattern of the estrous cycle or endocrine organ weights (data not shown).

Discussion. This study shows that the chronic suppression of pituitary GH secretion by SMS resulted in a marked retardation of normal mammary gland growth in virgin mice. We previously found that neonatal treatment with monosodium glutamate (MSG) also induced the inhibition of normal mammary gland growth in mice at advanced ages associated with the reduced GH in the circulation[12]. Furthermore, there was a significant correlation between plasma GH level and mammary gland DNA content in mice[13]. All observations have demonstrated the involvement of GH in mammary gland growth in mice.

While common actions of GH and prolactin are often observed in several species[14], Markoff and Talamantes[15] pointed out that mouse GH showed only about 10% of the lactogenic activity of prolactin. Studies in our laboratory also suggest the minor participation of GH in mammary gland growth compared with prolactin[13, 16]. In this study, either the circulating prolactin level or the pattern of the estrous cycle as an index of ovarian hormone secretory activity was affected little by SMS treatment, which was in good agreement with the previous results of neonatal MSG treatment[12]. GH is shown to be effective on mammary glands only in intact animals[17]. Thus, the manifestation of GH effects on mammary gland may be dependent upon the presence of other mammotropic hormones.

Apparent retardation of body growth in SMS treated mice reflects the chronic decline of GH secretion due to SMS.

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