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Suppression of Normal and Preneoplastic Mammary Growth and Uterine Adenomyosis with Reduced Growth Hormone Level in SHN Mice Given Monosodium Glutamate Neonatally*

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Abstract—*As a step in the evaluation of the role of growth hormone (GH) in mammary tumorigenesis, the effects of neonatal treatment with monosodium glutamate (MSG) on normal and neoplastic mammary growth, plasma levels of GH and prolactin (PRL) and the pattern of estrous cycles were determined in SHN virgin mice. The development of uterine adenomyosis, which is under similar hormonal control, was also examined in these mice. The formation of precancerous mammary hyperplastic alveolar nodules (HAN) was markedly inhibited and the number of 'ghosts', the remnants of regressed HAN, was increased by the single injection of* 4 mg *MSG on the day of birth, although spontaneous mammary tumorigenesis was not inhibited. The incidence of adenomyosis was suppressed by MSG treatment. Plasma GH level was chronically lower in the MSG-treated mice than in the controls. In contrast, plasma PRL levels and the pattern of estrous cycles were little affected by the treatment. These results indicate an involvement of GH in the development of HAN and uterine adenomyosis in mice.*

INTRODUCTION

DESPITE the accumulation of a considerable amount of data, the role of GH in mammary tumor development is still far from a definitive conclusion [1]. The pharmacological dose of bovine or ovine GH used in mice or rats in previous studies may be a major factor preventing reliable interpretation of the results.

We have previously found that a single injection of MSG in neonatal mice resulted in a marked suppression of the formation of preneoplastic HAN [2] and pregnancy-dependent mammary tumors [3]. The same treatment also caused a decline in the pituitary content of both GH and PRL [2].

The primary objective of this study was to examine the consequence of neonatal treatment of mice with MSG on normal, preneoplastic and neoplastic development of the mammary gland and on the circulating levels of GH and other mammotropic hormones. MSG-mediated alterations of endogenous hormone levels was considered to be a useful approach for evaluating the role of GH in mammary tumorigenesis.

The incidence of uterine adenomyosis [4–6] was also studied in this experimental model since the role of GH in this pathogenesis in the uterus is quite obscure, although the hormones involved appear to be similar to those in pathological changes in the mammary glands [4, 6, 7].

MATERIALS AND METHODS

Animals and treatments

The inbred strain of SHN virgin mice were used. On day 0 (day of birth) half of the female pups of each litter received a single subcutaneous injection of 4 mg MSG (WAKO Pure Chemical Inc., Osaka, Japan) dissolved in 0.01 ml of 0.9% saline (MSG-treated group). The remaining female littermates were given the vehicle only (control). Each litter was weaned on day 20, kept in a Teflon cage (30 × 16 × 15 cm), maintained in an animal room air-conditioned (22 ± 1°C and 55–70% relative humidity) and artificially illuminated (14 hr of light from 5:00 a.m. to 7:00 p.m.), and provided with a commercial diet (CE–2: CLEA Japan Inc., Tokyo, Japan) and tap water *ad libitum*.

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Body weight change

The body weights of a number of mice in each group were measured every 10 days after weaning until 7 months of age.

Estrous cycle

In order to estimate the pattern of ovarian hormone secretion, vaginal smears were checked every morning for 42 and 50 days beginning 1.5–2 months and 5–6 months of age, respectively.

Plasma levels of GH and PRL

Blood was collected by orbital puncture from a number of mice in each group at 1, 2, 5, 6, 7 and 9 months of age. After centrifugation at 1000 ***g*** for 20 min, plasma was frozen and stored at −20°C. GH and PRL levels were determined by homologous radioimmunoassay.

Spontaneous mammary tumorigenesis

All mice were checked for palpable mammary tumors every 7 days throughout their life spans, beginning at 3 months of age. Mammary tumor-bearing mice were killed 3 weeks after the appearance of the first tumor and the age at onset, and the number and the size of tumors were recorded.

Normal and preneoplastic mammary gland growth

At autopsy, the bilateral third thoracic mammary glands with no tumors were removed and used for wholemount preparations, which were checked under 10-fold magnification. The degree of mammary end-bud formation was rated from 1 to 7 in increments of 1. The numbers of HAN and the ghosts, the remnants of regressed HAN, were counted.

Uterine adenomyosis and endocrine organ weights

Uteri were removed, fixed in Bouin's solution, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin–eosin for histological observation of the development of adenomyosis.

Pituitary and adrenal glands and ovaries were also removed and weighed.

Statistics

The statistical significances of differences between groups were evaluated by the Mann–Whitney U test, χ^2 test and Student's t test for plasma GH levels, mammary tumor incidence and the other parameters respectively.

RESULTS

Body weight change

While the body weight increased in both the MSG-treated and the control mice, the extent of the increase was much more pronounced in the MSG-treated group. The difference in the body weight between the two groups became statistically significant after 90 days of age ($P < 0.01$) (data not shown). The increased body weight of the MSG-treated mice is consistent with the well-known obesity caused by neonatal MSG treatment [8].

Estrous cycle (Table 1)

There was little difference in the pattern of estrous cycle between the control and the MSG-treated mice, whereas estrous and metestrous stages became shorter with longer diestrous stages in the older mice than in the younger mice of both groups.

General features of mammary tumorigenesis (Table 2)

No significant difference was seen between the groups in incidence, age at onset, progression rate and number of mammary tumors per tumor-bearing mouse.

Normal and preneoplastic mammary gland growth (Fig. 1)

Both mammary rating and the number of HAN at autopsy of tumor-bearing mice were significantly lower in the MSG-treated group than in the control group ($P < 0.01$). In contrast, the number of ghosts was significantly higher in the MSG-treated mice (2.4 ± 0.4, mean ± S.E.M.) than in the controls (0.3 ± 0.1) ($P < 0.01$).

There was no difference between groups in the size of HAN expressed in terms of the mathematical mean of the major two diameters (data not shown).

Plasma levels of GH and PRL (Fig. 1)

Plasma GH level was significantly lower in the MSG-treated mice than in the control at any month examined ($P < 0.01$).

PRL levels, however, remained unaltered by MSG treatment except at 6 months of age, when the control level was significantly reduced ($P < 0.01$).

Incidence of uterine adenomyosis and endocrine organ weights (Table 3)

Incidences of both adenomyosis and subserosal nodules, an advanced state of adenomyosis, were significantly inhibited in the MSG-treated mice as compared to the control mice ($P < 0.05$).

Pituitary weights of MSG-treated mice were also reduced ($P < 0.01$), while there was little difference in the weights of the adrenals and the ovaries between groups.

DISCUSSION

Neonatal treatment with MSG was found to decrease the pituitary content [9] and serum levels [10] of GH around 40 days of age in rats and

Table 1. Percentage of each stage of estrous cycle in each group (mean ± S.E.M.)

Age at the start of examination (months)	Group and treatment*	Period examined (days)	Percentage against total period examined			
			Proestrus	Estrus	Metestrus	Diestrus
1.5–2	control (12)†	42	6.6±1.4	16.3±0.8	37.3±8.3	39.9±6.6
	MSG (12)	42	5.8±1.2	13.7±1.5	51.8±5.5	28.8±3.9
5–6	control (12)	50	5.2±0.9	4.3±0.8	17.3±5.1	73.2±4.9
	MSG (12)	50	5.3±1.2	4.0±0.8	26.6±8.8	64.0±8.2

*On day 0 (day of birth) half of the female pups of each litter of SHN mice received a single subcutaneous injection of 4 mg MSG dissolved in 0.01 ml physiologic saline (MSG). The remaining female littermates were given the vehicle (control). Each litter was nursed normally until 20 days of age.
†No. of mice examined.

Table 2. General features of mammary tumorigenesis in each group.

Group and treatment*	Incidence (%)	Onset age (months)	No. of tumors†	Progression of the first tumor			Age at death of mice with no tumors
				Initial‡ (mm)	Final‡ (mm)	% change	
Control	97.5 (39/40)§	6.9 ± 0.2‖ (38)¶	1.7 ± 0.1 (38)	4.5 ± 0.3 (38)	14.5 ± 1.7 (38)	242 ± 35 (38)	15.4 (1)**
MSG	95.7 (44/46)	7.6 ± 0.4 (42)	1.7 ± 0.2 (36)	5.8 ± 0.4 (33)	17.1 ± 1.6 (33)	185 ± 21 (33)	6.4, 12.2 (2)

*See Table 1 for detail of treatments.
†No. of mammary tumors per tumor-bearing mouse which appeared during 3 weeks after the first tumor appearance.
‡Mammary tumor sizes expressed in terms of the geometric means of the major two diameters at appearance (initial) and after 3 weeks (final).
§No. of mice with mammary tumors/initial no. of mice examined.
‖Mean ± S.E.M.
¶No. of mice or mammary tumors examined.
**No. of mice which died without mammary tumors.

Table 3. Incidence of uterine adenomyosis and endocrine organ weights in each group

Group and treatment*	Incidence of adenomyosis (%)	Incidence of adenomyosis with subserosal nodules (%)	Endocrine organ weights (mg)			
			No. of samples	Pituitary glands	Adrenal glands	Ovaries
Control	57.5 (23/40)‡	15.0 (6/40)	39	3.22 ± 0.10†	12.4 ± 0.3	22.2 ± 0.7
MSG	34.8 (16/46)	2.2 (1/46)	39	2.80 ± 0.10	12.0 ± 0.4	21.8 ± 0.8
P	<0.05	<0.05		<0.01	>0.05	>0.05

*See Table 1 for detail of treatments.
†Mean ± S.E.M.
‡No. of mice with adenomyosis/total no. of mice examined.

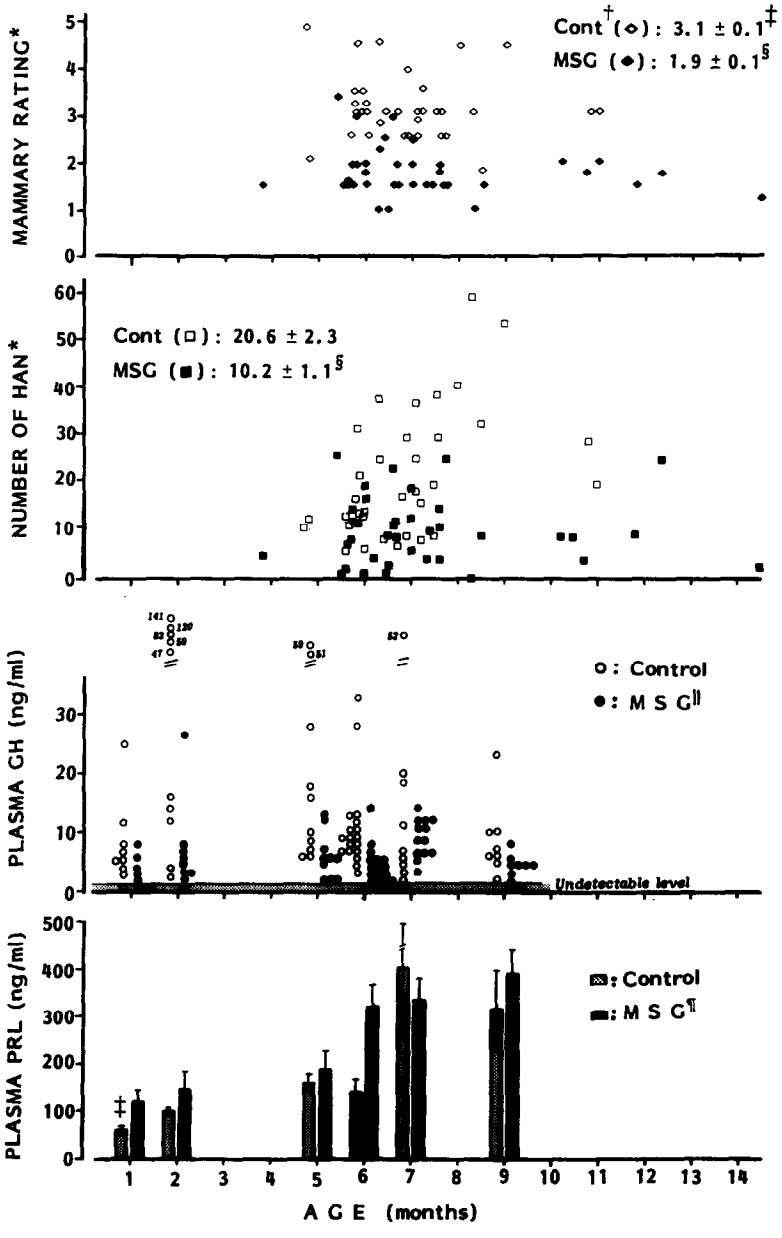

*Fig. 1. Changes with age in mammary rating, the number of HAN and plasma levels of GH and PRL in each group. *Mean and sum of the values of the bilateral third thoracic mammary glands in mammary rating and the number of HAN, respectively. †See Table 1 for detail of treatments. ‡Mean ± S.E.M. §Significantly lower than the control at $P < 0.01$. ‖Significantly lower than the control at all months at $P < 0.01$. ¶Not significantly different from the control except at 6 months.*

pituitary contents at 60, 150 and 420 days in mice [2]. In the present study plasma GH levels were significantly lower in mice given MSG neonatally than in the controls during 1–9 months of age, while there was little difference between groups in either plasma PRL levels or the pattern of estrous cycle as an index of ovarian hormone secretory activity. Associated with the reduced GH level, the formation of HAN as well as normal end-buds was markedly inhibited by MSG treatment. These results thus indicate the involvement of GH in the growth of preneoplastic mammary tissue. The manifestation of a GH effect on the mammary glands apparently may occur in the presence of other mammotropic hormones, because the present results show that the secretion of PRL, estrogen and progesterone remains unaltered in the MSG-treated mice, and because it has been shown that, if GH is effective on mammary glands, it is in intact animals [1].

It is not clear at present whether the effect of GH on the mammary glands is direct or indirect

through its activity on the general metabolic pathway, or both. However, the direct role of GH has been reported recently. Mammary gland DNA synthesis in SHN virgin mice declines significantly when cultured in the medium deficient of GH [11]. Itturi and Welsch [12] also found that GH stimulated DNA synthesis in DMBA-induced rat mammary tumors *in vitro*.

Although a significant inhibition of HAN formation was caused by neonatal MSG treatment, mammary tumorigenesis was virtually unaffected by the treatment. While GH may participate in mammary DNA synthesis, which is a limiting factor for mammary tumorigenesis [13], the effect of PRL is more marked than that of GH [11]. Furthermore, PRL was found to play a key role in the initial progression of mammary malignant foci [14]. Only GH secretion was suppressed, and not PRL secretion, by neonatal MSG treatment. Thus, that the possible inhibition of mammary tumorigenesis by reduced GH was masked by a PRL effect would partly account for the lack of inhibition of mammary tumorigenesis despite the decreased HAN in the MSG-treated mice observed in this study.

The present results also show that the decline of the development of uterine adenomyosis by neonatal MSG treatment corresponds with the decreased plasma GH levels, even though there is no change in plasma PRL and the pattern of estrous cycle. The results thus suggest that, as in the mammary glands, GH plays a role in the development of adenomyosis. A mechanism of the action of GH on the uterus is unclear at present.

Finally, this study is the first report showing that chronic modulation of endogenous GH may affect biological events in the mammary gland and in the uterus.

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