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Depression of delayed-type hypersensitivity in mice with hypothalamic lesion induced by monosodium glutamate: involvement of neuroendocrine system in immunomodulation

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SUMMARY

Delayed-type hypersensitivity (DTH) was depressed in mice that had been treated with monosodium glutamate (MSG) in their suckling period. Analysis of the DTH depression by use of the macrophage migration inhibition assay showed dysfunction of DTH effector T cells. The neuronal loss of nuclei in the hypothalamus, which elaborates the corticotropin-releasing factor and the hypersection of adrenocorticotrophic hormone, was observed in the MSG-treated mice. Therefore, DTH response may be modulated by the neuroendocrine system.

INTRODUCTION

Many investigators have studied the effect of the central nervous system on the immune response. They have found, for example, that hypothalamic lesions afford significant protection against lethal anaphylaxis in rats or guinea-pigs (Luparello, Stein & Park, 1964; Szentivanyi & Filipp, 1958), suppress the humoral and cell-mediated immune response in guinea-pigs or rats (Macris *et al.*, 1970; Schiavi *et al.*, 1975; Tyrey & Nalbandov, 1972) and inhibit lymphocyte stimulation in whole blood cultures by antigens or mitogens in guinea-pigs (Keller *et al.*, 1980). In addition, it has been shown that the immune response elicits a decrease in noradrenaline synthesis in the hypothalamus (Besedovsky *et al.*, 1983). These reports strongly suggested that the hypothalamus is involved in the immune response. On the other hand, the hypothalamus also governs the release of the hormones from the anterior pituitary gland by their releasing factor in the hypothalamus, which travel via the pituitary-stalk portal vessels to the anterior pituitary gland (Schally *et al.*, 1968). Besedovsky & Sorkin (1977) proposed the immune-neuroendocrine network system which is based on existence of afferent-efferent pathways betwenn immune and endocrine systems.

Olney (1969) reported that monosodium glutamate (MSG) selectively destroys the preoptic and arcuate nuclei of the hypothalamus in mice. In the present study, we examined the effect of hypothalamic lesions induced by MSG on the delayed-type hypersensitivity (DTH) response in mice. The results showed that DTH response was depressed in MSG-treated mice and that their plasma levels of adrenocorticotrophic (ACTH) were higher than those of their control mice. We also showed that the low DTH response in MSG-treated mice was due to dysfunction of DTH effector T cells and that DTH responses might be regulated by the neuroendocrine system in the hypothalamic-pituitary-adrenal axis.

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MATERIALS AND METHODS

Mice and MSG treatment

Female and male C3H/HeSlc (C3H) and C5BL/6 Slc (B6) mice were obtained from the Shizuoka Agricultural Experimental Animal Co-operation, Hamamatsu, Shizuoka, Japan, and they were bred in our animal facility from breeding pairs. Litters of the mice were divided into two groups: one was treated with MSG dissolved in saline, the other, which served as a control, was given only vehicle. Two mg of MSG (Nakarai Chemicals, Kyoto, Japan) per gram of body weight were injected subcutaneously (s.c.) from the second to the seventh day of their life. When weaned at 21–25 days, mice of three to four litters were grouped according to sex and MSG treatment. Age difference between the oldest and the youngest mice of each group was less than 5 days. They were maintained in an air-conditioned room with controlled lighting (12 hr light, 12 hr dark) and temperature ($24 \pm 1°$). A maximum of 10 mice was kept per cage. They were provided with a commercial diet and water *ad libitum*. Mouse body weight and naso-anal length were measured for the calculation of Lee index as an indicator of obesity (Bernardis & Patterson, 1968).

Antigen and immunization

Bacillus Calmette–Guérin (BCG) cell wall (CW) and purified protein derivatives (PPD) were kindly donated by Dr Brehmer, Robert Koch Institute, Berlin, BCG CW vaccine was prepared

by the method of Brehmer, Anacker & Ribi (1968). The typical procedure used in this study was described in our previous paper (Yamamoto & Kakinuma, 1978). The vaccine (0·2 ml) containing 300 μg of BCG CW was injected s.c. into mice to induce the DTH response. For the induction of DTH to antigens other than BCG, 10^3 colony-forming units (CFU) of live *Listeria monocytogenes* (*Listeria*), strain EGD, were injected intravenously (i.v.) into mice. *Listeria*-soluble protein (LSP), which was used as a test antigen of foot pad tests, was prepared according to the method of Youdim, Stutman & Good (1973). In order to measure DTH responses, the footpad test was performed 4 weeks or 10 days after the immunization with BCG CW or live *Listeria*, respectively. The procedure was detailed in an earlier report (Yamamoto & Kakinuma, 1978).

Bone marrow cell transfer to irradiated mice

Mice were irradiated in a plastic box with 900 rads X-rays from a Toshiba KXC-2-18-2 X-ray source equipped with 0·5 mm Cu and 0·5 mm Al filter and then injected i.v. with 1×10^7 syngeneic bone marrow cells.

Measurement of plasma ACTH

In order to measure the plasma ACTH in mice, the CIS-Sorin ACTH radioimmunoassay kit (Commissariat A l'Energie Atomique, Gif-sur-Yvette, France) was used. As the kit has the advantages of being a direct assay and requiring only 0·1 ml of plasma, plasma ACTH was assayed without extraction for ACTH. For measurement of plasma ACTH, mice were decapitated in the morning between 08·30 hr and 10·30 hr. According to the methods of Greer & Rockie (1968), mice were anaesthetized for ACTH release by placing them in a large closed jar with paper towels saturated with ether in the bottom. The mice were dacapitated to collect the plasma 10 min after exposure to ether.

Hormone

Synthetic ACTH was purchased from N.V. Organon (BH OSS, The Netherlands).

Preparation of non-adherent cells

The procedure was described previously (Kato & Yamamoto, 1982). Briefly, after erythrocytes were removed with 0·83% NH_4Cl-Tris buffer, spleen cells were washed three times with Hanks' balanced salt solution (HBSS) and resuspended in RPMI-1640 medium (Nissui Pharmaceuticals Co., Tokyo, Japan). One ml of peritoneal exudate cell (PEC) suspension containing 5×10^7 cells was applied to a 5-ml syringe column containing Sephadex G-10 followed by incubation for 45 min at 37°. The non-adherent cells were eluted with RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) (Gibco Laboratories, Grand Island, NY).

Macrophage migration inhibition (MI) test

The MI test was performed as described in our previous paper (Kato *et al.*, 1981). Briefly, peritoneal exudate cells (PEC) used in the MI test were collected in HBSS on the Day 3 after intraperitoneal (i.p.) injection of 2 ml of 12% casein sodium (Nakarai Chemicals Ltd, Kyoto, Japan) saline. The cells were washed three times with HBSS and then resuspended in RPMI-1640 media, to which was added 100 U of penicillin, 100 μg of streptomycin and 15% FCS per ml. The PEC was centrifuged in capillaries at 200 *g*, and the portion of capillary containing packed cells was then attached with silicone to the bottom cover slip of Sykes–Moore tissue culture chambers. The cells in quadruplicate capillary tubes were assayed, and the number of cells in a capillary tube was 3×10^7 cells. The chambers were filled with the above-mentioned RPMI-1640 medium with or without 50 μg of PPD/ml and incubated at 37° for 24 hr. The areas of a cell migration from the open end of capillary tubes were traced on papers after projection and enlargement and then measured with a planimeter. MI activity was expressed with the percentage migration inhibition.

$$\text{MI activity} = \frac{\text{migration area of the PEC with 50 } \mu\text{g/ml of PPD}}{\text{migration area of the PEC without PPD}} \times 100.$$

The significance of differences between the migration area of PEC with PPD and without PPD was determined by the two-tailed Student's *t*-test.

RESULTS

Obesity indexes and lesions of brains in MSG-treated mice

We calculated the obesity index of C3H and B6 mice treated with MSG at 4·5 months after birth. Table 1 shows that the obesity index of MSG-treated mice of both sexes was significantly higher than that of their controls. We also examined histologically the brains of MSG-treated mice. Figure 1 shows the section of hypothalamus of C3H mice 4·5 months after MSG injection, indicating the neuronal loss of hypothalamic nuclei.

BCG CW- and live *Listeria*-induced DTH in MSG-treated C3H mice

We examined DTH responses in MSG-treated C3H mice. Each group of six female or male MSG-treated mice and their control mice was immunized with 300 μg of BCG or with 10^3 CFU of live *Listeria*. They were tested for footpad swelling with corresponding antigens, as described in the Materials and Methods. Figure 2 shows that lower footpad swellings against both antigens were observed in MSG-treated mice and not in the controls.

Table 1. Obesity index in MSG-treated mice at 4·5 months after birth*

Strain	Sex	No. of mice	Obesity index† MSG treatment	Obesity index† Controls
C3H	Female	6	0·395±0·021	0·335±0·009 ($P<0·001$)
	Male	6	0·370±0·010	0·313±0·009 ($P<0·001$)
B6	Female	5	0·372±0·001	0·327±0·009 ($P<0·001$)
	Male	5	0·369±0·012	0·328±0·005 ($P<0·001$)

* Two mg of MSG per gram of body weight of neonatals were injected s.c. from the second to the seventh day of their lives.

† Obesity index = $\frac{\sqrt[3]{\text{body weight}}}{\text{naso-anal length}}$ (mean ± standard deviation).

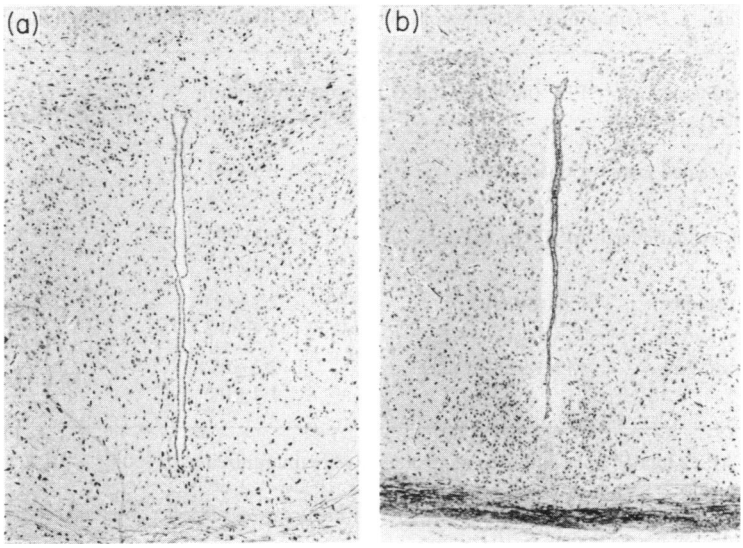

Figure 1. Section through hypothalamus of (a) a C3H mouse treated with MSG, and (b) a control mouse 4·5 months after birth. The neuronal loss of hypothalamic nuclei in the MSG-treated mouse was shown. Magnification × 52.

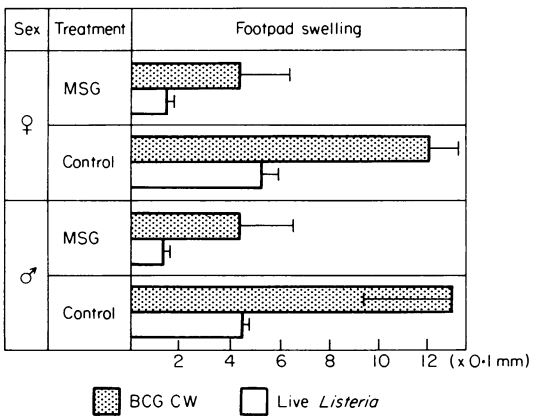

Figure 2. Each group of six female and male MSG-treated C3H mice and their controls were immunized with 300 μg of BCG CW or 10^3 CFU of live *Listeria*. Footpad tests were performed 4 weeks or 10 days after BCG CW or live *Listeria* immunization, respectively.

MI activity of PEC from MSG- and BCG CW-treated C3H mice

We next examined the MI activity of PEC from MSG-treated mice with depressed footpad responses. Each group of six female MSG-treated mice and their control mice was immunized s.c. with BCG CW. Footpad testing was performed 4 weeks after BCG CW immunization. The PEC from each group were collected 4 days after footpad testing and used for the MI test. A strong footpad swelling and a positive MI activity of PEC were observed in control mice not in MSG-treated mice (Table 2).

MI activity of the mixture of non-adherent cells of PEC from MSG- and BCG CW-treated mice and PEC from MSG-treated or control mice

In the preceding experiment, there was no MI activity of PEC from MSG- and BCG CW-treated mice (Table 2). In order to examine the cause of this phenomenon, non-adherent cells of PEC from MSG- and BCG CW-treated mice (MSG-effector

Table 2. MI activity of PEC from MSG-treated mice immunized with BCG CW

Freatment	Footpad swelling (mm)	Presence of PPD (50 μg/ml)	Migration area of PEC (cm^2)*	MI activity (%)
MSG (6)	0·08 ± 0·04†	−	3·1 ± 0·1	98
		+	3·0 ± 0·4	
Control (6)	0·55 ± 0·16†	−	3·8 ± 0·1	72 ($P < 0·001$)
		+	2·7 ± 0·3	

* Mean ± SD.

† Significant difference between MSG treatment and control ($P < 0·001$).

Table 3. Dysfunction of MI-effector cells from MSG- and BCG CW-treated mice*

Non-adherent (effector) cells from:	PEC (indicator cells) from:	Presence of PPD	Migration area (cm^2)	MI activity§ (%)
MSG-treated mice	Control mice	−	2·6±0·1	107
		+	2·8±0·8	
MSG-treated mice	MSG-treated mice	−	2·2±0·2	90
		+	2·0±0·2	
Control mice	Control mice	−	2·2±0·3	64 ($P<0·01$)
		+	1·4±0·3	
Control mice	MSG-treated mice	−	2·2±0·2	64 ($P<0·01$)
		+	1·4±0·3	

* MSG-treated mice and their control mice were immunized with 300 μg of BCG CW 4 weeks before.

† Non-adherent cells from BCG CW effector PEC of these mice were obtained by passing them through a Sephadex G-10 column.

‡ PEC from control or MSG-treated mice were collected and used as indicator cells of the MI test.

§ 1×10^6 non-adherent cells were mixed with 2×10^7 indicator cells and their MI activities were measured.

Table 4. Plasma ACTH level of MSG-treated mice

Anaesthetization by ether	Sex	Treatment	No. of mice	Obesity index*		Plasma ACTH† (pg/ml)	
−	Female	MSG	6	0·379±0·013	($P<0·01$)	246±56·1	($P<0·01$)
		Control	6	0·323±0·005		125±51·2	
	Male	MSG	4	0·381±0·011	($P<0·01$)	301±78·5	(NS)‡
		Control	4	0·334±0·023		289±201·0	
+	Female	MSG	5	0·372±0·010	($P<0·001$)	884±44·6	($P<0·001$)
		Control	4	0·327±0·009		232±104·1	
	Male	MSG	5	0·370±0·012	($P<0·001$)	916±185·9	($P<0·001$)
		Control	5	0·328±0·005		442±76·6	

* Obesity index: see footnote in Table 1.

† Mean±SD.

‡ Not significant.

cells) were mixed with PEC from MSG-treated or control mice (MSG-PEC or control-PEC) at a ratio of 1:20. In addition, as their controls, non-adherent cells of PEC from BCG CW-immunized mice (control-effector cells) were mixed with MSG-PEC or control PEC in the same way. Then, MI activity of these mixtures was determined. Table 3 shows that MI activity was observed in a mixture of control-effector cells and control-PEC or MSG-PEC, but not in the mixtures of MSG-effector cells and control-PEC or MSG-PEC.

Plasma ACTH level of MSG-treated mice

We measured plasma ACTH level of MSG-treated mice. Table 4 shows that in a non-anaesthetized group of plasma ACTH level of female MSG-treated mice was higher than that of their controls. However, there is no significance between male MSG-treated mice and their control mice. A high plasma ACTH level was observed in some male mice and may have been induced by stress due to fighting. In the anaesthetized group the plasma ACTH level of both sexes of MSG-treated mice was much higher than their controls.

Development of DTH response in MSG-treated mice by transfer with normal bone marrow cells

Since the depression of the DTH response in MSG-treated mice seemed to be related to a high level of their plasma ACTH, we thought that a high level of plasma ACTH might impair the immune-competent cells derived from bone marrow involved in DTH development. Thus, we examined whether the induction of BCG CW-induced DTH was observed by transfer of normal bone marrow cells to MSG-treated mice. Twelve female MSG-

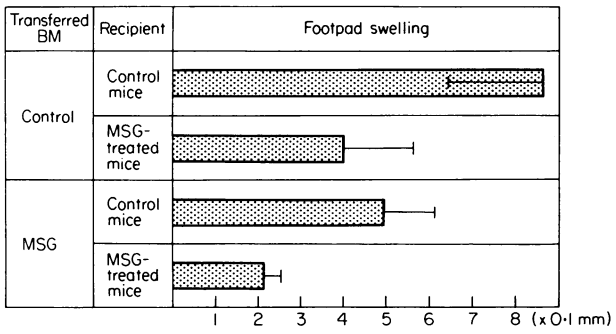

Figure 3. Ten million bone marrow cells from female MSG-treated mice or controls were inoculated into the 900 rads X-ray-irradiated female MSG-treated and control mice. All of these mice were immunized s.c. with 300 μg of BCG CW immediately after bone marrow cell inoculation and examined for footpad DTH response 4 weeks after the immunization.

treated mice were irradiated with 900 rads X-rays and divided into two groups: one group was transfused with 10^7 bone marrow cells from MSG-treated mice (MSG-MSG-mice), and the other group was transfused with 10^7 bone marrow cells from control mice (MSG-control-mice). In addition, twelve control mice were also irradiated with 900 rads-X-rays and divided into two groups: one group was transfused with 10^7 bone marrow cells from MSG-treated mice (control-MSG-mice), and the other group was transfused with 10^7 bone marrow from control mice (control-control-mice). All of these mice were immunized s.c. with 300 μg of BCG CW immediately after the transfer and examined for footpad DTH response with 10 μg of PPD 4 weeks after the immunization. DTH response in the control-MSG-mice was inhibited in comparison with that in the control-control-mice, while DTH response in MSG-control-mice was enhanced in comparison with that in MSG-MSG-mice (Fig. 3).

BCG CW-induced DTH response in ACTH-treated mice

In addition, we examined the effect of ACTH on the DTH response. Seven female mice were injected i.p. with 15 μg/kg of synthetic ACTH every 3 days for 5 weeks. In addition, seven mice untreated with the hormone were used as a control group. All mice were immunized s.c. with 300 μg of BCG CW 1 week after the beginning of hormone treatment and examined for footpad DTH response with 10 μg of PPD 4 weeks after immunization. Footpad reaction was inhibited in the hormone-treated mice ($0{\cdot}48 \pm 0{\cdot}33$ mm) in comparison with that in the control mice ($0{\cdot}83 \pm 0{\cdot}18$ mm) ($P < 0{\cdot}05$).

DISCUSSION

We have shown that both female and male C3H and B6 mice that had produced hypothalamic lesions by treatment with MSG in their suckling period had a significantly increased Lee index, an indicator of obesity, as has been described by several investigators (Nagasawa, Yanai & Kikuyama, 1974; Olney, 1969; Redding *et al.*, 1971; Tanaka *et al.*, 1978). Furthermore, these MSG-treated C3H mice were found to be markedly suppressed in their DTH responses induced by BCG CW or *Listeria* immunization. In addition to the DTH suppression in these MSG-treated C3H mice, BCG CW-induced lung granuloma response, which has been regarded as a cell-mediated immune response in B6 mice (Yamamoto *et al.*, 1982), and IgE antibody response in C3H mice were reduced by the treatment of MSG in their suckling period (data not shown). These findings suggested that hypothalamic lesions in MSG-treated mice were closely associated with the reduction of immune responses. Many reports have indicated that hypothalamic lesions modify various immune responses, as described previously.

We examined the mechanisms of DTH depression in MSG-treated mice using MI assay, an *in vitro* correlate of DTH response. The results showed that no MI activity of PEC from MSG- and BCG CW-treated mice was observed despite the presence of 8–12% of θ antigen-positive cells (T cells). Thus, in order to explain the depression of MI activity of PEC from MSG-treated mice, we proposed two possibilities. First, the elaboration of MI factor (MIF) in T cells of PEC from MSG-treated mice may be inhibited. Second, macrophages in the PEC from the mice may be unresponsive to MIF even though T cells produced MIF. To clarify these possibilities, MI activity of mixture of MSG-effector cells with MSG-PEC or control-PEC was measured. As a control, we used control-effector cells instead of MSG-effector cells. The results showed that the MI activity of the mixture of control-effector cells with MSG-PEC was observed, but not of the mixture of MSG-effector cells with control PEC, suggesting that macrophages in PEC from MSG-treated mice were sensitive to MIF and that elaboration of MIF in T cells of PEC from the mice was inhibited. Thus, we believe that the DTH depression in MSG-treated mice may be due to the dysfunction of DTH effector T cells. On the other hand, Roszman *et al.* (1982) reported that suppressor macrophages, which inhibited the lymphocyte function, were induced in rats with electrolytic preoptic anterior hypothalamic lesions. The character of the suppressor macrophages seems to be similar to that of live BCG-induced suppressor macrophages in PEC, which suppressed the MI activity of effector PEC from BCG CW-immunized mice (Kato *et al.*, 1981). On the other hand, in the present study, MI activity of a mixture of non-adherent (effector) cells and PEC from MSG-treated mice was observed (Table 3), suggesting that no suppressor macrophages against MI activity were induced in PEC from MSG-treated mice. Thus, the mechanism of immune suppression in the MSG-treated mice may be different from that of animals with electrolytic anterior hypothalamic lesions.

We have shown histologically the neuronal loss of hypothalamic nuclei in MSG-treated mice (Fig. 1). Olney (1969) and Olney & Sharpe (1969) reported originally that MSG selectively destroyed the preoptic and arcuate nuclei of the hypothalamus in mice and induced the acute degeneration of hypothalamic neurons in Rhesus monkeys.

On the other hand, it is generally accepted that specific neurohormones secreted by the hypothalamus are carried to the anterior pituitary gland via the hypothyseal portal vessels where they exert stimulatory or inhibitory effects on the synthesis and release of the trophic hormones (Schally *et al.*, 1968). Chemical or physical damage to the hypothalamus may thus result in changes in the content and release of anterior pituitary hormones. Redding *et al.* (1971) reported that the anterior pituitary levels of growth hormone (GH) and luteinizing hormone were markedly reduced in female and male MSG-treated rats. In the

present study, MSG treatments resulted in a decrease of approximately 10–12% in naso-anal length at 4·5 months after birth. Growth retardation in MSG-treated mice probably resulted from the impairment of GH secretion and/or synthesis by the anterior pituitary. Moreover, the hypothalamus is known to modify ACTH secretion from the anterior pituitary through the corticotropin-releasing factor (CRF) (Mangili, Motta & Martini, 1966; Schally *et al.*, 1968). We have shown that plasma ACTH levels in female and male MSG-treated mice with hypothalamic lesions were higher than those of control mice (Table 4). These findings were consistent with those of Greer & Rockie (1968) and Halász, Slusher & Gorski (1967), indicating that ACTH secretion, estimated indirectly by measuring plasma corticosterone content, was higher in rats that received the deafferentation of basal hypothalamus than that in control rats. We have shown by bone marrow transfer (Fig. 3) that DTH effector T-cell function is apparently inhibited in MSG-treated mouse recipients with the hypersecretion of ACTH. We have also shown that the DTH response in control mice receiving MSG mouse bone marrow was enhanced in comparison with that in MSG-mice receiving mouse bone marrow (Fig. 3). This suggests that DTH effector T-cell dysfunction in MSG-treated mouse bone marrow is partially eliminated in control mouse recipients with a physiological level of plasma ACTH. The mechanisms of inhibition of DTH effector T-cell function in MSG-treated mice remain unresolved. It has been established that in the hormone regulation of the hypothalamic-pituitary-adrenal pathway the hypothalamic lesion produces CRP, which in turn triggers the secretion of ACTH by the pituitary. The ACTH then acts to stimulate secretion by the adrenal gland of the corticosteroid, hormone having an immune suppressive effect. In the present study, we have also shown that footpad response was depressed in mice treated with synthetic ACTH. In this context, Wahl, Altman & Rosenstreich (1975) reported that glucocorticosteroid inhibited the macrophage migration inhibition factor (MIF) production or blocked the action of MIF on macrophages. Therefore, although we could not measure plasma adrenocortical hormones in mice, DTH effector T-cell function is probably inhibited by excessive adrenocortical hormone, such as glucocorticosteroid, which is secreted from adrenal cortex by excessive plasma ACTH stimulation in MSG-treated mice. Glucocorticoids have various immunomodulatory effects: a high concentration of corticosteroids induces thymic involution (Bellamy, Janssens & Leonad, 1966; Ishidate & Metcalf, 1963), reduces mitotic activity in T cells (Ishidate & Metcalf, 1963) and inhibits phagocytic activity of human leucocytes (Christie, Kjosen & Solberg, 1977). On the other hand, a lower concentration of glucocorticoids enhances thymocyte differentiation (Ritter, 1977), increases mitotic activity (Whitfield, MacManus & Rixon, 1970) and stimulates antibody *in vitro* (Ambrose, 1964; Smith, Sherman & Middleton, 1972). In contrast to the effects of exogenous glucocorticoids, we showed in the present study that immune responses might be modulated by endogenous hormone interaction among CRF, ACTH and adrenocortical hormone. It is endocrinologically accepted that the hypothalamus is responsive to the feedback effect of the circulating adrenocortical hormone, and that the effects, including negative regulatory influence, determine the hypothalamic activity to produce the CRF (Yates & Maran, 1974). In addition, circulating adrenocortical hormone also acts at the pituitary level to determine pituitary sensitivity to CRF (Yates & Maran, 1974). Moreover, Besedovsky *et al.* (1975) reported that serum corticosterone levels increased several fold during the primary immune response of rats and mice. Therefore, from these findings, it is conceivable that a hormone regulatory circuit in the hypothalamus-pituitary-adrenal axis could modulate the immune response, whereas the immune system could be a potential influence on the hormone regulatory circuit.

Finally, although we could not measure the plasma GH in this study, GH in MSG-treated mice may reduce in a manner similar to that in MSG-treated rats reported by Redding *et al.* (1971). GH may be involved in immune responses since GH has been shown in dwarf mice to influence the development of the lymphoid system and T-cell dependent immune response (Duguesnoy, 1972; Fabris, Pierpaoli & Sorkin, 1971). A study on the effect of GH on DTH response is in prospect.

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