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Alterations in Immunoreactive Somatostatin Levels in Hypothalamic and Gastroenteropancreatic Tissue as a Consequence of Neonatal Treatment with Monosodium Glutamate[1] (42026)

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Abstract. The present study was conducted to evaluate the effects of neonatal treatment of male rats with monosodium-L-glutamate (MSG) on levels of immunoreactive somatostatin (IRS) in specific regions of the gastroenteropancreatic (GEP) system, in discrete hypothalamic areas, and in peripheral blood. In two identical experiments, IRS concentrations measured in the arcuate nucleus and median eminence of adult, MSG-treated rats were significantly reduced in comparison to IRS levels measured in control littermates. Levels of IRS in the preoptic–periventricular nucleus were significantly reduced only in one experiment. In contrast, neonatal MSG treatment resulted in a twofold increase of IRS levels in the pancreas and antral region of the stomach. Peripheral plasma IRS concentrations were significantly elevated in MSG-treated rats only in one experiment. Since MSG-treated rats have a deficiency in growth hormone (GH) secretion, an additional experiment was performed to determine if GH replacement therapy could reverse some or all of the changes in IRS concentrations induced by MSG treatment. With the exception of a further increase in antral IRS levels, replacement with rGH failed to restore IRS levels in other tissues or plasma of MSG rats to levels measured in control rats. These results show that neonatal MSG treatment not only affects IRS levels in the hypothalamus and blood as previously reported, but in parts of the GEP system as well. Further, effects on hypothalamic IRS levels are opposite to those on GEP IRS levels. The significance of these findings may relate to the altered metabolic state of these animals as a consequence of perturbations in the secretion of other hormones from the hypothalamus, anterior pituitary gland, and possibly the GEP system.

Following the initial observations by Olney in mice (1), a significant number of reports have documented the long-term deleterious effects of neonatal treatment with monosodium-L-glutamate (MSG) on behavior, metabolism, and endocrine function in other rodent species and nonhuman primates (2–8). At least some of the disturbances, particularly those associated with the reproductive system, may be attributed to the destruction of neurons in the inner layer of the retina and arcuate nucleus (ARC) as a direct result of MSG treatment (1, 2, 4, 6, 9–12). Recently, Millard *et al.* (12) reported that the reduction in pulsatile growth hormone (GH) secretion observed in MSG-treated male rats is due primarily to a deficit in the secretion of a putative GH-releasing factor (GRF). Indeed, after the publication of the aforementioned report (12), several groups isolated, sequenced, and synthesized a GRF from human pancreatic tumors (13, 14) and subsequently from the rat hypothalamus (15). Using an antiserum raised against human pancreatic GRF which cross reacts with rat GRF, Merchenthaler *et al.* (16) demonstrated the existence of GRF-containing cell bodies in the ARC thus lending credence to the hypothesis put forth by Millard *et al.* (12) to explain depressed pulsatile GH secretion in MSG-treated rats.

In addition to the probable deficiency in GRF secretion in MSG-treated rats, these rats exhibit elevations in circulating levels of immunoreactive somatostatin (IRS) (17). This would suggest another possible cause for depressed GH secretion in MSG-treated rats, however, administration of an antisomatostatin sera to MSG-treated rats produced

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only a modest increase in mean plasma GH levels while failing to restore high amplitude GH pulses (12). Moreover, levels of IRS in the medial basal hypothalamus (MBH) of MSG-treated rats are lower than IRS levels measured in the MBH of control rats (5, 7). Although measurements of steady-state IRS levels in the MBH do not provide information concerning the effects of neonatal MSG treatment on synthesis/release mechanisms in somatostatinergic neurons, the observation that GH increases the secretion of IRS from hypothalamic fragments *in vitro* (18, 19) suggests, albeit indirectly, that IRS release from median eminence (ME) peptidergic nerve terminals may be decreased in GH-deficient, MSG-treated rats. Therefore, in light of the localization of IRS in the D cells of the pancreas and gastrointestinal tract (20, 21) and its possible role in regulating nutrient homeostasis (22), the following study was performed to determine whether levels of IRS in gastroenteropancreatic (GEP) organs are altered as a consequence of neonatal MSG treatment. For comparison with previously published reports, measurements of IRS levels in discrete hypothalamic areas and peripheral plasma were performed.

Materials and Methods. *Animals.* Adult male (300–350 g) and female (225–275 g) rats (Charles River Labs, Wilmington, Mass.) were allowed to mate in clear plastic cages in a light-controlled room (lights on from 0500–1900 hr) maintained at 22–24°C. Pregnant female rats were individually placed in cages. Beginning 2 days after parturition, male pups (five to eight per litter) were injected ip with either 4 mg/g BW MSG (Sigma Chemical Co., St. Louis, Mo.) dissolved in double distilled water or an isosmotic control consisting of 10% NaCl on alternate days through Day 10 of age. The offspring were weaned at 21 days of age and were used for experimentation either at 3 (Experiments 1 and 3) or 5 (Experiment 2) months of age. All rats were provided with food and water *ad libitum*.

Experimental protocols. Three separate experiments were conducted to ascertain the effects of neonatal MSG treatment on IRS levels in discrete areas of the hypothalamus, in parts of the GEP system, and in peripheral plasma. Experiments 1 and 2 were essentially identical.

Experiments 1 and 2. On the day of sacrifice, body weights (Experiments 1 and 2) and nasal–anal lengths (Experiment 2) were recorded from all rats. The Lee index ($\sqrt[3]{\text{body wt.}}/\text{nasal–anal length}$), a measure of obesity, was calculated in Experiment 2. For each experiment, one group each of control and MSG-treated rats was decapitated between 1000 and 1200 hr and trunk blood was collected into heparinized glass tubes on ice. The brain was placed under a stereomicroscope and the ME was rapidly dissected as described previously (23). The ME fragments containing somatostatin nerve terminals then were homogenized in 100 μl ice-cold 2 *N* acetic acid, boiled for 5 min to inactivate degradative enzymes, and the homogenate was stored frozen at −20°C until assayed for IRS content. The remainder of the brain was placed on dry ice, transferred to snap vials, and stored frozen (−20°C). At a later time (within 1 week of sacrifice), the brains were mounted and sliced in a freezing microtome–cryostat. The preoptic component of the periventricular nucleus (PVN) and ARC (containing somatostatin fiber tracts en passage to the ME) were microdissected as described in a previous report (24). The preoptic PVN was chosen for analysis since somatostatin perikarya located in this area seem to be involved in regulating GH secretion while somatostatin cell bodies of the PVN located caudal to the preoptic division appear to be important in modulating thyroid-stimulating hormone secretion (25). Samples of the PVN and ARC were homogenized in 50 μl 2 *N* acetic acid on ice and treated as described above for the ME.

Concurrently with microdissection of the ME, portions of the pancreas (body and tail, 150–250 mg), stomach (antrum, 150–250 mg), duodenum (proximal part, 125–175 mg), jejunum (15–20 cm from pylorus, 100–125 mg), and descending colon (100–125 mg) were quickly removed, washed in ice-cold 0.9% NaCl, blotted, and weighed. Tissue fragments were homogenized in 750 μl 2 *N* ice-cold acetic acid, sonicated, placed in a boiling water bath for 5 min, and stored frozen at −20°C.

Trunk blood was centrifuged in a refrigerated centrifuge at 1000*g* for 10 min. The plasma was extracted according to the pro-

cedure of Patel *et al.* (26) using acid–ethanol (95 parts absolute ethanol and 5 parts 1 *N* HCl) in a ratio of 1 part plasma and 2 parts acid–ethanol. The mixture was vortexed, centrifuged at 1000*g* for 20 min, aspirated into another chilled glass tube, lyophilized, and stored at −20°C until assay of IRS levels.

Experiment 3. After observing consistent alterations in IRS concentrations in some tissues of MSG-treated rats (see Results), the following experiment was performed to examine whether GH replacement therapy could reverse these changes. For this study, MSG-treated rats were divided into two groups. One group of rats was injected subcutaneously with 40 μg rGH (NIAMDD rGH B-7) every 6 hr (160 μg/day) for 10 days while another group of MSG-treated rats was injected with 200 μl of vehicle (0.01 N NaOH in 1% bovine serum albumin–phosphate-buffered saline). This dosage regime was based on a reported secretory rate of GH in male rats of 140 μg/rat/24 hr (27) and the observation that administration of bovine GH for 12 days to hypophysectomized rats restored depressed IRS levels in the hypothalamus (28). A separate group of adult male rats treated with 10% NaCl during the first 10 days of life was injected with vehicle for 10 days. Body weights, nasal–anal lengths, and Lee indexes were recorded before and after rGH or vehicle treatment. One hour after the last injection (1000 hr), all rats were sacrificed and tissue and blood samples were obtained as stated under Experiments 1 and 2.

Assay of IRS content. Immunoreactive somatostatin content in various tissues and plasma was determined by a radioimmunoassay (RIA) procedure reported by Arimura and co-workers (29) using antiserum R101 produced by immunizing rabbits with cyclic somatostatin conjugated to human serum γ-globulin (kindly provided by Dr. A. Arimura). [Tyr^1]-Somatostatin for radiolabeling and native somatostatin for use in the standard curve was purchased from Peninsula Labs (Belmont, Calif.). The sensitivity of the assay was 5–7 pg/tube (90% of total binding) with 50% of total binding of labeled hormone to antibody occurring at 35–40 pg/tube.

On the day of the assay, tissue samples were thawed, centrifuged, and the supernatants were appropriately diluted with assay buffer for detectability of IRS levels in the RIA. Lyophilized plasma was solubilized with 500 μl assay buffer and assayed directly. Inter- and intraassay variability determined on tissue supernatants of ME fragments averaged 14.3 and 8.8%, respectively. Samples obtained in a given experiment were run in the same assay. Levels of IRS reported in tissue and plasma were not corrected for procedural losses as determined by adding synthetic somatostatin to samples prior to extraction. Losses in the various tissue and plasma samples varied between 0 and 13%.

Protein assay. Measurements of the protein content of PVN, ARC, and ME samples were performed by a micromodification of the method of Lowry *et al.* (30).

Statistical analysis. All data obtained in Experiments 1 and 2 were analyzed by Student's unpaired *t* test. For Experiment 3, differences in body weight, nasal–anal length, and Lee index prior to and after GH or vehicle replacement were analyzed by Student's paired *t* test. Analysis of variance (one-way) followed by the Student–Newman–Keul's multiple comparison test was used to evaluate significant changes in IRS levels in tissue and plasma in Experiment 3.

Results. *Experiments 1 and 2 (Table I).* Unless otherwise stated, results refer to both experiments. As previously reported by a number of laboratories, MSG-treated rats weighed significantly less ($P < 0.01$), were significantly smaller ($P < 0.01$), and were more obese ($P < 0.01$, higher Lee index) than their control littermates (data not shown).

Neonatal MSG treatment resulted in significant reductions ($P < 0.01$) in IRS concentrations in the ARC and ME (Experiments 1 and 2) and in the PVN (Experiment 1 only). In most instances, IRS concentrations were reduced by approximately 50%. In contrast, IRS concentrations in the pancreas and antrum of MSG-treated rats were doubled in comparison to pancreatic and antral IRS levels in control rats. Concentrations of IRS in the duodenum, jejunum, and colon of MSG-treated rats were not significantly different from levels of IRS measured in these tissues from control rats. Immunoreactive somatostatin concentrations in the peripheral plasma of MSG-treated rats were significantly elevated ($P < 0.01$) in comparison to plasma

TABLE I. EFFECTS OF MSG ON IRS LEVELS IN VARIOUS TISSUES AND IN PERIPHERAL PLASMA

	Experiment 1		Experiment 2	
	Control	MSG	Control	MSG
Hypothalamus (ng IRS/ μg protein)				
PVN	0.056 ± 0.004 (9)	0.032 ± 0.005 (9)**	0.103 ± 0.012 (7)	0.107 ± 0.006 (15)
ARC	0.256 ± 0.031 (9)	0.069 ± 0.008 (8)**	0.423 ± 0.060 (6)	0.201 ± 0.017 (16)**
ME	2.933 ± 0.318 (9)	1.667 ± 0.118 (9)**	2.368 ± 0.348 (8)	1.420 ± 0.088 (18)**
GEP tissue (ng IRS/mg wet wt)				
Pancreas	0.336 ± 0.031 (9)	0.637 ± 0.070 (10)**	0.521 ± 0.029 (6)	1.119 ± 0.095 (16)**
Antrum	1.810 ± 0.127 (7)	3.243 ± 0.451 (7)*	1.550 ± 0.199 (6)	2.498 ± 0.205 (16)*
Duodenum	0.523 ± 0.019 (7)	0.643 ± 0.095 (7)	0.549 ± 0.023 (6)	0.641 ± 0.032 (15)
Jejunum	0.330 ± 0.044 (8)	0.393 ± 0.061 (9)	0.407 ± 0.021 (7)	0.467 ± 0.033 (17)
Colon	0.253 ± 0.041 (8)	0.304 ± 0.033 (10)	0.189 ± 0.016 (6)	0.210 ± 0.014 (15)
Peripheral plasma (pg IRS/ml)	55.5 ± 3.8 (9)	76.2 ± 3.6 (8)**	30.5 ± 4.6 (6)	39.9 ± 2.8 (15)

[a] Mean ± SEM. Number of animals is shown in parentheses.
* $P < 0.05$ vs control.
** $P < 0.01$ vs control.

IRS levels in control rats only in Experiment 1.

Experiment 3 (Table II and Fig. 1). Administration of rGH to MSG-treated rats significantly increased body weight ($P < 0.01$) and nasal-anal length ($P < 0.05$) over pretreatment values (Table II). However, MSG-treated rats given rGH still were smaller and obese in comparison to control rats and did not differ in size or obesity from MSG-treated rats injected with vehicle.

As in the first two experiments, IRS concentrations were significantly reduced ($P < 0.05$) in the ME and ARC and significantly increased in the pancreas ($P < 0.01$) and antrum ($P < 0.05$) of MSG-treated rats when compared to IRS levels measured in these tissues from control rats (Fig. 1). Plasma and PVN IRS levels were not affected by neonatal MSG treatment in this experiment. Treatment of MSG animals with rGH did not influence IRS levels in the pancreas and ARC when compared to levels measured in the pancreas and ARC of MSG-treated rats receiving vehicle. Surprisingly, rGH treatment of MSG rats caused a further increase in antral IRS levels. Furthermore, treatment of MSG rats with rGH partially restored ME IRS levels to control values, however, ME IRS levels in MSG rats given rGH did not differ significantly from ME IRS levels measured in vehicle-treated MSG rats. The effects of rGH replacement on IRS levels in the duodenum, jejunum, and colon were not determined since IRS levels in these tissues were not significantly altered by neonatal MSG treatment in the first two experiments (Table I).

Discussion. The results of the present study extend previous observations on the effects of neonatal MSG treatment on MBH IRS levels in that IRS levels were consistently reduced in the ARC and ME and in some experiments, in the PVN. Furthermore, these data provide new evidence which demonstrates a pronounced effect of MSG treatment on IRS levels in specific organs of the GEP system. Interestingly, changes in pancreatic and antral IRS levels were in a direction opposite to that of changes in ARC, ME, and PVN IRS concentrations. The divergent responses of IRS levels in the hypothalamus and GEP system may relate to different regulatory mechanisms controlling secretion of somatostatin in these tissues. To this end, substance P decreased IRS release from a perfused rat stomach preparation (31) while increasing IRS release from the perfused rat hypothalamus (32). Further, neurotensin increased hypothalamic IRS release (32, 33) while having no effect on gastric IRS release (31). Finally, fasting of rats for 72 hr increased

TABLE II. EFFECTS OF rGH ON OVERALL BODY GROWTH AND OBESITY IN RATS TREATED NEONATALLY WITH MSG[a]

Group	Body wt (g)		Nasal–anal ln (cm)		Lee index	
	Preinjection	Postinjection	Preinjection	Postinjection	Preinjection	Postinjection
Control (8)[b]	427 ± 15.3[c]	427 ± 14.5	23.50 ± 0.98	23.81 ± 1.05***	0.320 ± 0.001	0.316 ± 0.002
MSG (6)	325 ± 12.6**	335 ± 13.2**†	20.97 ± 0.24**	21.32 ± 0.21**	0.328 ± 0.005	0.326 ± 0.003*
MSG + rGH (6)	303 ± 16.8**	335 ± 15.1**†	19.73 ± 0.55**	21.00 ± 0.35**.***	0.340 ± 0.004**	0.330 ± 0.001**

[a] The 2 to 3-month-old rats treated with MSG or 10% NaCL (control) during the first 10 days of life were injected sc every 6 hr for 10 days either with 200 μl vehicle (0.1 *N* NaOH in 1% BSA–PBS) or rGH (40 μg/injection).

[b] Number of animals per group is shown in parentheses.

[c] Mean ± SEM.

* $P < 0.05$ vs control.

** $P < 0.01$ vs control.

*** $P < 0.05$ vs pre-GH value.

† $P < 0.01$ vs pre-GH value.

IRS levels in various GEP organs without altering hypothalamic IRS concentrations (34, 35).

The decrease in hypothalamic IRS levels and possibly IRS release into the portal vessels in MSG-treated rats may be due to the deficiency in GH secretion reported in these animals (12) since: (1) treatment of rats hypophysectomized for 12 days previously with bovine GH restored depressed IRS levels in the hypothalamus (28), and (2) GH stimulated IRS release from hypothalamic fragments *in vitro* (18, 19). Failure of rGH to substantially reverse MSG-induced alterations in IRS levels in specific hypothalamic areas in this study may have been due to inadequate duration of treatment, replacement with insufficient amounts of rGH, or a generalized hyporesponsiveness of these tissues to rGH due to the prolonged absence (3–5 months) of normal circulating GH levels. Although rGH replacement in MSG rats caused a further increase in antral IRS levels without affecting pancreatic IRS levels it remains to be determined whether GH plays a physiologic role in the regulation of somatostatin secretion in the GEP system.

In one of three experiments, peripheral plasma IRS levels were significantly elevated in comparison to IRS levels in control rats thus confirming a previous report (17). The inconsistency in MSG-induced alterations in plasma as well as PVN IRS levels in the present study is not totally surprising since previous studies have reported inconsistent effects of MSG treatment on other hormone concentrations in tissue and blood (5, 7, 8, 36). This may in part be due to the varying degrees of MSG-induced destruction of neural tissues which may result in a specific effect of MSG treatment being more or less pronounced between groups of rats. In addition, inconsistent effects of MSG treatment on plasma IRS levels as well as the variability of these levels in control rats especially when comparing levels in Experiments 1 and 2 with levels in Experiment 3 may have been due to contamination of trunk plasma with IRS from other sources such as the severed spinal cord.

Since somatostatin release into the pituitary portal vessels may be reduced in GH-deficient, MSG-treated rats, the present data

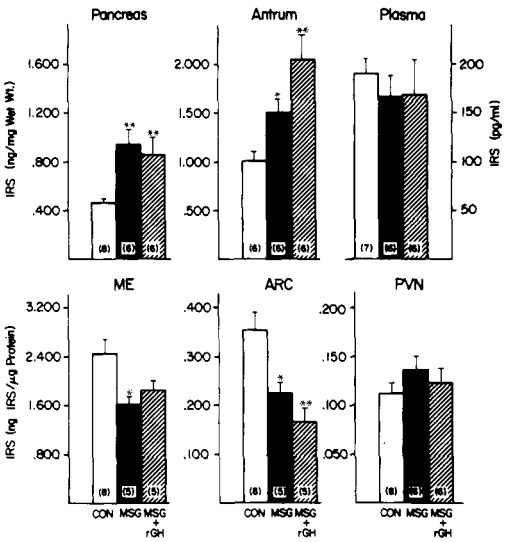

FIG. 1. Effect of rGH (40 μg/injection every 6 hr for 10 days) on MSG-induced alterations in tissue and plasma IRS levels. Separate groups of rats treated neonatally either with 10% NaCl (CON) or MSG were injected with 200 μl vehicle (0.1 *N* NaOH in 1% BSA–PBS) every 6 hr for 10 days. Note different scales on *Y* axis for tissue and plasma IRS measurements. * = $P < 0.05$ vs CON; ** = $P < 0.01$ vs CON.

suggest, albeit indirectly, that the hypersomatostatinemia observed in some MSG-treated rats may be due to increases in pancreatic and antral IRS secretion. In this regard, Shapiro *et al.* (34) reported that decreases in hepatic portal IRS levels after a 72-hr fast were reflected in decreased levels of IRS in inferior vena caval serum. However, pancreatic IRS levels were higher after fasting than prior to fasting thus pointing to the need for direct measurements of IRS release from the pancreas and antrum before conclusive statements concerning tissue IRS release can be made.

In summary, the effects of neonatal MSG treatment can now be extended to include alterations in D-cell function in the pancreas and antrum of adult rats. Although a direct effect of MSG treatment cannot be ruled out, elevations in GEP IRS levels may relate to the altered metabolic state of these animals as a consequence of known perturbations in the secretion of other hormones from the hypothalamus, anterior pituitary gland, and possibly the GEP system. Whether changes in GEP IRS levels are a cause or a result of the metabolic disturbances seen in MSG-treated rats will require additional studies.

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