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Neonatal MSG Reduces Hypothalamic DA, β-Endorphin, and Delays Weight Gain in Genetically Obese ($A^{viable\ yellow}/a$) Mice

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CAPUTO, F. A., S. F. ALI, G. L. WOLFF AND A. C. SCALLET. *Neonatal MSG reduces hypothalamic DA, β-endorphin, and delays weight gain in genetically obese ($A^{viable\ yellow}/a$) mice.* PHARMACOL BIOCHEM BEHAV **53**(2) 425–432, 1996. – Neonatal treatment with monosodium glutamate (MSG) decreases proopiomelanocortin (POMC) peptides and results in obesity. The yellow mouse is a model of obesity induced by the viable yellow (A^{vy}) gene at the *agouti* locus on Chromosome 2, which results in overproduction of a POMC receptor antagonist. Thus we hypothesized that MSG, when imposed on the genetically susceptible model, would alter the development of obesity. Both yellow obese (A^{vy}) and black lean *(a/a)* males were injected on Postnatal Days 1, 3, 5, 7, and 9 with 2.0 mg/g body weight MSG or saline SC. Their food intake, growth parameters, and neurochemical status were examined. Paradoxically, MSG interacted with the yellow phenotype to delay the rapid rate of weight gain characteristic of this model ($p < 0.05$). Food intake was decreased ($p < 0.05$) in both phenotypes treated with MSG, as was hypothalamic content of dopamine ($p < 0.05$) and of the POMC peptide, β-endorphin ($p < 0.001$). The yellow obese phenotype was more sensitive than the black lean phenotype to the neurochemical effects of early postnatal MSG administration. Recent reports suggest the *agouti* locus protein is an antagonist of the receptor for another POMC peptide, melanocyte-stimulating hormone (MSH). Therefore, the balance of functional activity between various POMC peptides appears to be an important factor in the development of both acquired and genetic obesity.

MSG Development Obesity Food intake Genetic Mice Hypothalamus Viable yellow
β-Endorphin Dopamine (DA) Serotonin (5-HT) (A^{vy}) *Agouti*

OBESITY is a morbid condition characterized by both genetic and environmental factors in its development. Animal models exist for both aspects of the disease.

Early postnatal administration of monosodium glutamate (MSG) produces selective neuronal necrosis within the preoptic and arcuate nuclei of the hypothalamus and median eminence. Brain circumventricular organs (CVOs) such as the area postrema (AP) and subfornical organ (SFO), as well as medial habenular nuclei and neurons of the rostral hippocampus have also shown damage after perinatal MSG administration (21,26). This results in a syndrome characterized by obesity, stunted growth, neuroendocrine disturbances, and an increase in body weight that is not accompanied by hyperphagia (4,18,26,29,30,38). In addition to the lack of hyperphagia, MSG appears not to induce hyperinsulinemia or insulin resistance during the development of obesity in mice (8,38). However, rats have been reported to become hyperinsulinemic after early postnatal MSG administration (32,39).

Proopiomelanocortin (POMC, synthesized only in neurons of or near the CVOs) is the precursor of three groups of biologically active peptides: the adrenocorticotropins, the melanotropins, and the endorphins. POMC-derived peptides are known to be influenced by MSG treatment. The content of adrenocorticotropic hormones (ACTH) and β-endorphin-like material in the mediobasal hypothalamus, medial preoptic nucleus, and amygdala, as determined by immunoassay, have been reported as markedly reduced after neonatal MSG administration (6,17,19,42). However, there was no change in ACTH or β-endorphin levels in whole pituitary (6), anterior lobe of the pituitary, or in plasma ACTH concentrations (19). Neonatal MSG also has been reported to reduce hypothalamic dopamine (DA) levels (25).

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α-Melanocyte stimulating hormone (α-MSH), cleaved from ACTH, induces eumelanin synthesis by melanocytes in mammals and stimulates melanocyte aggregation in fish, amphibians, and reptiles capable of rapid coloration changes. It has also been shown to be reduced in the rat hypothalamus after early neonatal treatment with MSG (10,42).

The viable yellow mouse is a model of obesity induced by the autosomal dominant A^{vy} gene at the *agouti* locus on Chromosome 2. The *agouti* locus specifies a protein, secreted by the hair follicle cells, which binds to a surface or intracellular MSH receptor and prevents eumelanin synthesis (7,24,41). In brain, it has recently been demonstrated that the *agouti* protein is an antagonist to the melanocortin-4 receptor, which mediates the central neuroendocrine and autonomic effects of POMC peptides (22). This model is distinct from other models of obesity because it produces a moderate obesity with minimal diabetes due to overexpression of the *agouti* gene (40). The POMC-derived peptide, α-MSH, regulates tyrosinase activity in melanocytes by activating adenyl cyclase. The activation of adenyl cyclase thus induces an increase in transcription of the tyrosinase gene and an increase in tyrosinase activity (15,20). Tyrosinase is the rate-limiting enzyme involved in the synthesis of two types of melanins; phaeomelanin, which produces a red/yellow coloration and eumelanin, which produces a brown/black coloration. When tyrosinase levels are low in the murine melanocyte, the majority of tyrosine is converted via a dopaminergic pathway to phaeomelanin (13). At higher levels of tyrosinase, excess 3,4-dihydroxyphenylalanine (DOPA) may be diverted to a pathway leading to the synthesis of eumelanins.

Because both MSG administration and the A^{vy} gene result in a decrease in the hypothalamic bioavailability of α-MSH and DA (42), as well as in obesity, one might expect to see interactions upon combined treatment with these factors. Thus we hypothesized that MSG given to an A^{vy} mouse might be either ineffective if it acted on hypothalamic neurochemistry via the same pathway as the viable yellow gene, or additive if different paths were involved. Therefore, the aim of the present study was to evaluate the effects of MSG on yellow obese (A^{vy}/a) and lean black *(a/a)* mice with regard to the role of POMC peptides and DA in the induction and maintenance of obesity and coat coloration. Thus, body weight gain, food intake, various growth parameters, and neurochemical status were examined after neonatal treatment of each genotype with MSG or saline.

METHODS

Animal Housing and Maintenance

C57BL/6NNctr females were mated to yellow YS/Wff-C3Hf/Nctr-A^{vy} males. The F1 male hybrids were used throughout the experiment.

On the day of birth mice were culled and cross-fostered up to a maximum of eight male pups/dam. On Postnatal Day (PND) 1, 3, 5, 7, and 9 pups were injected with either 0.9% sodium chloride (NaCl) SC or the monosodium salt of glutamic acid (MSG), 2 mg/g SC.

All pups with a particular dam received the same treatment. Pups were allowed to remain with the dam until PND 28, at which time they were placed two/cage in plastic cages. At PND 60 mice were moved to individual cages. Mice were housed in a temperature controlled room (24 ± 2°C) on a 12 : 12, light : dark schedule.

Food, Water, and Body Weight Measurements

Food pellets (NIH 31, vitamin fortified) and distilled water were available ad lib. Metal food hoppers with food pellets were weighed weekly to the nearest 0.1 g. Food intake was not corrected for spillage.

Body weight was measured weekly to the nearest 0.1 g (n = 10 mice/group). The Lee Index of Obesity has been used as a measure of obesity and has been shown to correlate well with carcass fat content (2,3). The Lee Index is calculated as the cube root of (the body weight in grams divided by the nasoanal length in cm) × 1000.

Neurochemistry

On PND 240–254 (8–8.5 months) mice (n = 5 mice/group) were quickly sacrificed by cervical dislocation. Brains were rapidly removed and dissected over ice following the guidelines of Glowinski and Iversen (11). Tissues were immediately frozen on dry ice and stored at −70°C until analyzed by high performance liquid chromatography (HPLC) combined with electrochemical detection for DA, serotonin (5-HT), and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) according to the method of Ali et al. (1).

At approximately the same age (PND 254–270, 8.5–9 months), additional groups of mice (n = 5 mice/group) were sacrificed and their brains dissected as above and stored frozen at −70°C until assayed for β-endorphin (31). β-Endorphin was measured by radioimmunoassay (RIA). Mouse hypothalami were individually weighed and sonicated in 0.5 ml of 0.1 N hydrochloric acid (HCl), centrifuged at 13,000 rpm for 10 min and the supernatant frozen. Mouse pituitaries were handled as above except they were sonicated in 1.0 ml of 0.1 N HCl.

A series of known rat β-endorphin standards with the unknown samples were prepared and incubated overnight (16–24 h) with rabbit anti-β-endorphin serum. The following day ^{125}I β-endorphin was added for an additional overnight incubation. On the final day, goat anti-rabbit IgG (GARGG) serum and normal rabbit serum (NRS) were added and the incubation was allowed to proceed at room temperature for 90 min. At the end of the incubation, a sodium phosphate buffer was added and the samples centrifuged at 3000 rpm for 20 min at 4°C. The supernatant was aspirated and the pellet counted in an Apex Automatic Gamma Counter from ICN Micromedic Systems for 1 min. All assay materials were obtained from Peninsula Laboratories Inc. IC_{50} of the assay was 29 pg/tube and the standards ran from 1–128 pg/tube.

Morphometrics

At the time of sacrifice on PND 240–254 (n = 10 mice/group), body weight, nasoanal length, tail length, and weights of liver, heart, kidneys, adrenals, testes, and whole brain were determined.

Statistics

Data were analyzed utilizing factorial analyses of variance (ANOVA) or two-way ANOVAs with repeated measures on 1 factor, using the Newman–Keuls approach for posthoc comparisons. Significance levels were set at $p < 0.05$.

RESULTS

Body Weights During Development

From postnatal week (PNW) 8-20 the MSG-treated yellow mice were significantly lighter than the saline-treated controls (Fig. 1). It was not until PNW 24 that the MSG-treated yellow mice began to catch up to the saline-treated yellow mice in body weight. Results of the body weight data reveal a significant main effect of genotype [$F(1,28) = 111.6, p < 0.05$], as well as a significant main effect of weeks [$F(6,168) = 612.1$, $p < 0.05$]. There was no significant main effect of MSG treatment [$F(1,28) = 0.9, p > 0.05$]. However, there was a significant Genotype × MSG Treatment × Weeks interaction [$F(6,168) = 4.6$, $p < 0.05$; $n = 8$ mice/group]. Thus, the early MSG treatment of the yellow obese mice severely delayed the rapid weight gain seen in the saline-treated yellow genotype.

Among the black mice, on PNW 8 the saline-treated controls were significantly heavier than the MSG-treated animals. However, by PNW 12 the situation was reversed and the MSG-treated black mice became significantly heavier than the saline-treated black mice and remained heavier until PNW 32. These results are consistent with previous reports in the literature that MSG treatment induces an increase in body weight when administered during the early postnatal period (26,28).

MSG-treated yellow mice were significantly heavier at all time points than the MSG-treated black mice. Similarly, the saline-treated yellow mice were significantly heavier than the saline-treated black mice at all time points examined.

Food Intake

Due to the significant difference in body weight between the two genotypes, the mean daily food intake was analyzed on a per gram body weight basis. As shown in Fig. 2, the saline-treated black mice, in general, consumed more food than any other group, whereas the MSG-treated yellow mice consumed the least. Statistical results show a significant main effect of genotype [$F(1,28) = 11.3$, $p < 0.05$], treatment [$F(1,28) = 41.8, p < 0.05$], and postnatal week [$F(4,112) = 5.1$, $p < 0.05$], as well as a significant Genotype × Treatment × Postnatal Week interaction [$F(4,112) = 3.4$, $p < 0.05$]. During PNWs 20, 24, and 28 the MSG-treated yellow mice ate significantly less than their saline-treated yellow littermates. Similarly, during PNW 20, the MSG-treated black mice ate significantly less than saline-treated blacks. These results are in contrast to the body weight results that show the saline-treated yellow mice to have gained the most weight and saline-treated black mice to have gained the least weight (Fig. 1).

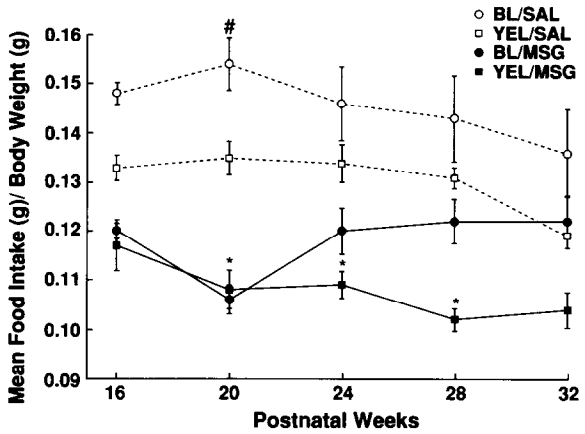

FIG. 2. Mean ± SEM food intake (g)/body weight (g) for saline-treated black (BL/SAL), saline-treated yellow (YEL/SAL), MSG-treated black (BL/MSG), and MSG-treated yellow (YEL/MSG) mice from Postnatal Weeks 16-32 ($n = 10$ mice/group). *Significantly different from YEL/SAL. #Significantly different from BL/MSG.

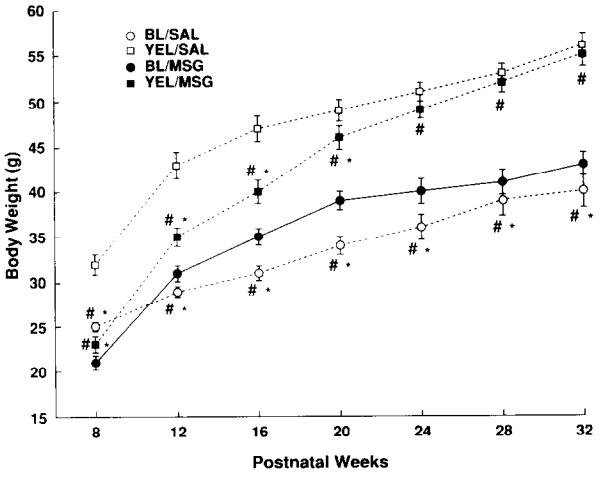

FIG. 1. Mean ± SEM body weights (g) for saline-treated black (BL/SAL), saline-treated yellow (YEL/SAL), MSG-treated black (BL/MSG), and MSG-treated yellow (YEL/MSG) mice from Postnatal Weeks 8-32 ($n = 10$ mice/group). *Significantly different from YEL/SAL. #Significantly different from BL/MSG.

Water Intake

Due to the significant difference in body weight between the genotypes, the mean daily water intake was analyzed on a per gram body weight basis. In general, the saline-treated yellow mice imbibed more water than all other groups, whereas the MSG-treated yellow mice consumed the least (Fig. 3). Statistical results show a significant main effect of treatment [$F(1,28) = 6.43$, $p < 0.05$], weeks [$F(4,112) = 3.69$, $p < 0.05$], as well as a Genotype × Treatment × Weeks interaction [$F(4,112) = 6.22$, $p < 0.05$]. There was no significant main effect of genotype. The curves generated for water intake differ from those generated from food intake (Fig. 2) and body weight (Fig. 1). Thus, body weight does not reflect the amount of food and/or water consumed.

Phenotypic Coloration

Coat coloration was not affected by neonatal administration of MSG. The proportion of yellow obese mice within the (C57BL/6xYS)F_1 hybrid population remained approximately 50%, similar to the proportion expected from $a/a \times A^{vy}/a$ matings. Thus, loss of hypothalamic α-MSH through the early administration of MSG did not appear to change eumelanin synthesis by the hair follicle melanocytes in the black mice.

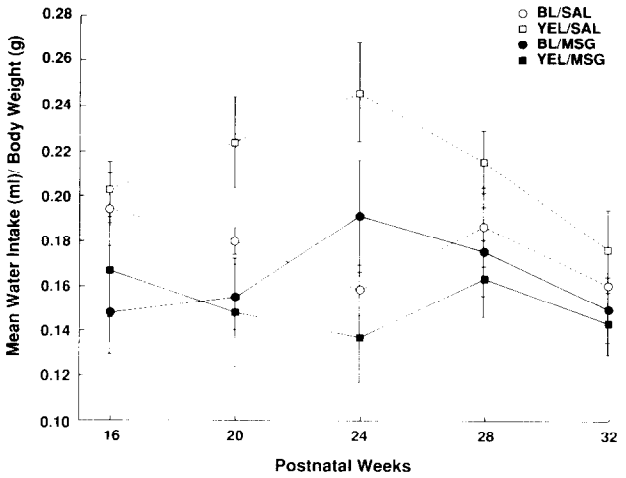

FIG. 3. Mean ± SEM water intake (ml)/body weight (g) for saline-treated black (BL/SAL), saline-treated yellow (YEL/SAL), MSG-treated black (BL/MSG), and MSG-treated yellow (YEL/MSG) mice from Postnatal Weeks 16–32 (n = 10 mice/group).

Obesity

As shown in Table 1, there was a significant main effect of MSG treatment [$F(1,36) = 97.7, p < 0.001$] and genotype [$F(1,36) = 29.1, p < 0.001$] on the Lee Index of Obesity but there was no Treatment × Genotype interaction. The MSG-treated black mice were 11% more obese than the saline-treated black mice, whereas the MSG-treated yellow mice were 9% more obese than the saline-treated yellow mice. Interestingly, the increase in the Lee Index is comparable in both genotypes despite significant differences in body weight between the four groups as shown in Fig. 1.

Body Weights at Sacrifice

At the time of sacrifice there was a significant difference in body weight between the genotypes (Table 1). The saline-treated yellow mice had the highest value with a mean body weight of 59.5 g, whereas the saline-treated black mice had the lowest body weight at 38.5 g. The black MSG-treated mice were 17% heavier than their controls; in contrast, the MSG-treated yellow mice were 10% lighter than their controls. Thus, early postnatal MSG treatment had the paradoxical effect of decreasing body weight in the genetically obese mice. There was a significant main effect of genotype [$F(1,36) = 71.2, p < 0.001$], as well as a Genotype × Treatment interaction [$F(1,36) = 14.0, p < 0.001$]. However, there was no significant main effect of MSG treatment.

Linear Growth

As expected (5,24,26) body (nasoanal) length of MSG-treated mice at the time of sacrifice was significantly reduced compared to their controls (Table 1). The MSG-treated black mice were 5% shorter than saline-treated black controls, and the MSG-treated yellow mice were 11% shorter than their saline-treated yellow controls. As expected (34,37), saline-treated yellow mice were longer than black saline-treated mice; however, MSG-treated yellow mice were of comparable length to MSG-treated black mice. Thus, it appears that the yellow phenotype may be more sensitive to the stunting effects of early postnatal administration of MSG. Statistical results reveal a significant main effect of genotype [$F(1,36) = 23.1, p < 0.001$] and MSG treatment [$F(1,36) = 83.7, p < 0.001$] and a significant Genotype × MSG Treatment interaction [$F(1,36) = 13.5, p < 0.001$].

Tail length was also significantly shortened by postnatal MSG administration [main effect of treatment $F(1,36) = 298.1, p < 0.001$]. The MSG-treated black mice had a 19% reduction compared to their controls, while the MSG-treated yellow mice had a 25% reduction when compared to their

TABLE 1

MORPHOMETRICS AT SACRIFICE (8-9 MONTHS OLD)

	Body Weight (g)	Body Length (cm)	Tail Length (cm)	Lee Index Obesity‡
Bl/MSG	45.2 ± 1.3	9.2 ± 0.1	5.9 ± 0.1	387.4 ± 3.6
Bl/Sal	38.5 ± 2.3*†	9.7 ± 0.1*†	7.3 ± 0.09†	346.1 ± 5.0
Yel/MSG	53.3 ± 1.5*†	9.3 ± 0.09*	5.6 ± 0.1*†	404.3 ± 2.8
Yel/Sal	59.5 ± 1.5	10.5 ± 0.06	7.5 ± 0.05	370.3 ± 3.5

ORGAN WEIGHTS AT SACRIFICE (8-9 MONTHS OLD)

	Brain (mg)‡	Heart (mg)‡	Adrenal (mg)‡	Kidney (mg)	Liver (g)	Testes (mg)‡
Bl/MSG	373.4 ± 5.6	155.9 ± 5.3	5.0 ± 0.5	378.6 ± 26.0	2.1 ± 0.3	154.5 ± 9.0
Bl/Sal	451.2 ± 6.1	184.1 ± 10.8	2.7 ± 0.3	460.7 ± 15.0*†	1.9 ± 0.3*	263.9 ± 7.2
Yel/MSG	373.0 ± 2.5	167.5 ± 9.6	6.0 ± 0.4	377.2 ± 24.0*	2.5 ± 0.3*	155.3 ± 9.1
Yel/Sal	457.8 ± 4.7	206.7 ± 5.9	4.2 ± 0.5	615.9 ± 16.7	6.1 ± 0.4	277.6 ± 6.1

Shown above are mean ± SEM morphometric and organ weights of saline-treated black (Bl/Sal), saline-treated yellow (Yel/Sal), MSG-treated black (Bl/MSG), and MSG-treated yellow (Yel/MSG) mice (n = 10 mice/group).

*Significantly different from Yel/Sal, $p < 0.05$.

†Significantly different from Bl/MSG, $p < 0.05$.

‡Significant main effect of treatment, $p < 0.05$.

controls [Genotype × Treatment interaction $F(1,36) = 7.9$, $p < 0.01$; Table 1].

Organ Weights

Total brain weight was found to be significantly decreased after early postnatal MSG treatment [$F(1,36) = 273.7$, $p < 0.001$]. However, brain weight was decreased comparably in both genotypes; 17% for the black mice vs. 19% for the yellow mice. As shown in Table 1, there was no significant main effect of genotype nor an interaction effect.

There was also a significant main effect of genotype [$F(1,36) = 4.3$, $p < 0.05$] and treatment [$F(1,36) = 16.8$, $p < 0.001$] on heart weight (Table 1). However, there was no significant Genotype × Treatment interaction. Again, the decrease in heart weight between the genotypes was similar, the black MSG-treated hearts being 15% smaller than their saline controls, while the yellow MSG-treated hearts were 19% smaller than their respective controls.

Kidney weight was also significantly decreased in MSG-treated mice of both genotypes (Table 1). The black MSG-treated mice had an 18% reduction in kidney weight compared to their saline-treated controls. The MSG-treated yellow mice, however, had a 39% reduction in kidney weight when compared to their controls. Statistical results revealed main effects of genotype [$F(1,36) = 13.4$, $p < 0.001$] and MSG treatment [$F(1,36) = 58.5$, $p < 0.001$] as well as a Genotype × Treatment interaction [$F(1,36) = 13.9$, $p < 0.001$]. Again, the yellow MSG-treated mice were more sensitive to the early postnatal effects of MSG administration.

Testes weight was also significantly reduced after early postnatal MSG administration [$F(1,36) = 212.3$, $p < 0.001$], but to a comparable degree in both genotypes. The black MSG-treated mice showed a 41% decrease in testes weight, while the yellow MSG-treated mice revealed a 44% decrease in testes weight when compared to their respective controls. Thus, there was no significant main effect of genotype nor Genotype × MSG Treatment interaction.

A significant difference in liver weight was also found between the two genotypes [$F(1,36) = 47.1$, $p < 0.001$] and after administration of MSG [$F(1,36) = 26.4$, $p < 0.001$]. Although the black MSG-treated mice showed an 11% increase in total liver weight, the yellow MSG-treated mice showed a 58% reduction in total liver weight when compared to their saline-treated controls [significant Genotype × MSG Treatment interaction $F(1,36) = 30.9$, $p < 0.001$; Table 1].

Early postnatal MSG administration appears to have increased adrenal weight in both genotypes but to varying degrees (Table 1). The black MSG-treated mice showed an 85% increase in adrenal weight when compared to their controls; in contrast, the yellow MSG-treated mice had only a 43% increase in adrenal weight. Results revealed significant main effects of genotype [$F(1,36) = 8.5$, $p < 0.01$] and MSG treatment [$F(1,36) = 22.8$, $p < 0.001$], but no significant Genotype × Treatment interaction.

Neurochemistry

As shown in Table 2, there was a significant main effect of MSG treatment on DA levels in the hypothalamus [$F(1,16) = 4.6$, $p < 0.05$], but there was no main effect of genotype nor a significant MSG Treatment × Genotype interaction. Both black and yellow genotypes had decreased hypothalamic DA concentrations after early postnatal administration of MSG. The MSG-treated black mice revealed a 10% decrease in hypothalamic DA levels when compared to their controls. In contrast, the yellow MSG-treated mice showed a 26% decrease in hypothalamic DA concentration when compared to their controls. Thus, the yellow phenotype appears to be more sensitive to the early postnatal effects of MSG treatment.

There was no significant difference between the two genotypes and treatment conditions or Genotype × Treatment interactions for DOPAC, HVA, 5-HT, or 5-HIAA in the hypothalamus.

Caudate levels of DA, 5-HT, and 5-HIAA were not significantly effected by MSG-treatment or genotype. However, DOPAC [$F(1,16) = 4.6$, $p < 0.05$] and HVA [$F(1,16) = 7.3$, $p < 0.05$] showed significant main effects of genotype

TABLE 2

HYPOTHALAMIC CONCENTRATIONS (NG/100 MG WET WEIGHT)

	DA*	DOPAC	HVA	5-HT	5-HIAA
Bl/MSG	31.5 ± 3.5	16.4 ± 2.8	20.9 ± 1.0	103.1 ± 4.9	24.7 ± 4.6
Bl/Sal	35.1 ± 2.6	11.7 ± 0.9	18.9 ± 2.0	88.3 ± 5.0	20.2 ± 4.9
Yel/MSG	33.7 ± 4.7	13.4 ± 0.6	20.2 ± 0.9	96.5 ± 4.0	27.7 ± 3.5
Yel/Sal	45.5 ± 3.3	13.5 ± 0.6	17.2 ± 0.7	94.4 ± 2.2	15.9 ± 2.5

CAUDATE CONCENTRATIONS (NG/100 MG WET WEIGHT)

	DA	DOPAC†	HVA†	5-HT	5-HIAA
Bl/MSG	1035 ± 0.9	97.6 ± 6.7	133.9 ± 6.8	69.2 ± 3.8	15.3 ± 2.4
Bl/Sal	1035 ± 0.6	117.8 ± 15.7	173.9 ± 23.0	83.9 ± 11.1	18.5 ± 5.2
Yel/MSG	1034 ± 0.9	89.1 ± 5.8	115.9 ± 5.9	71.6 ± 3.9	10.6 ± 3.5
Yel/Sal	1033 ± 1.8	86.8 ± 3.3	122.4 ± 6.6	62.7 ± 2.9	10.6 ± 2.7

Shown above are mean ± SEM levels of DA, DOPAC, HVA, 5-HT, and 5-HIAA in hypothalamus and caudate nucleus of saline-treated black (Bl/Sal), saline-treated yellow (Yel/Sal), MSG-treated black (Bl/MSG), and MSG-treated yellow (Yel/MSG) mice.

*Significant main effect of treatment, $p < 0.05$.

†Significant main effect of genotype, $p < 0.05$. No significant Genotype × Treatment interaction (n = 5 mice/group).

but not of MSG treatment or Genotype × Treatment interactions. Overall, the black mice showed DOPAC levels of 108 ng/100 mg wet weight, while the yellow mice had DOPAC levels of 88 ng/100 mg wet weight. DOPAC levels were decreased in the black MSG-treated mice by 17%, whereas the MSG-treated yellow mice showed a 3% increase in DOPAC levels in comparison with their saline-treated controls. HVA levels were 154 ng/100 mg wet weight for black mice and 119 ng/100 mg wet weight for yellow mice. HVA levels in black MSG-treated mice were decreased by 23%, whereas yellow MSG-treated mice had only a 5% decrease in HVA when compared to their respective controls.

As shown in Table 3, there is a significant decrease in hypothalamic β-endorphin levels in both genotypes after early postnatal MSG administration. As depicted in the top panel, β-endorphin levels per hypothalamus were decreased by 52% in MSG-treated black mice and 71% in MSG-treated yellow mice, compared to their respective controls [$F(1,15) = 31.4$, $p < 0.001$ effect of MSG treatment; no effect of genotype or interaction].

Similarly, the MSG-treated black mice had a 30% reduction in hypothalamic β-endorphin levels when expressed per mg wet tissue weight, whereas the MSG-treated yellows had a 66% reduction in β-endorphin levels compared to their respective controls [$F(1,15) = 10.5$, $p < 0.01$ effect of MSG treatment; no effect of genotype or interaction]. Again, the yellow genotype appears to be more sensitive to the early postnatal effects of MSG administration.

β-Endorphin levels per pituitary were increased by early MSG treatment [$F(1,12) = 7.0$, $p < 0.05$] to comparable levels in both genotypes. The black MSG-treated mice had a 43% increase in pituitary β-endorphin levels, while the yellow MSG-treated mice had a 36% increase, compared to their respective controls (Table 3, bottom panel). There was no effect of genotype or Genotype × Treatment interaction. Similarly, when β-endorphin levels were expressed per mg wet tissue weight, the black MSG-treated mice had a 207% increase and the yellow MSG-treated mice showed a 163% increase in β-endorphin concentration [$F(1,12) = 29.1$, $p < 0.001$]. Again, there was no significant effect of genotype or Genotype × MSG Treatment interaction.

DISCUSSION

Although administration of MSG has been reported to decrease endogenous concentrations of α-MSH (10), the proportion of mice with yellow coat coloration in this study was not different from the expected Mendelian ratio. Thus, loss of α-MSH in the brain did not appear to affect coat coloration of the black lean mice. These results confirm that local control within the surrounding hair follicle (41) is the predominant factor in coat coloration. This also suggests that the MSG-induced increase of β-endorphin in the pituitary gland was without significant effect on MSH released to plasma.

As shown in Fig. 1, early postnatal MSG treatment had a profound effect on body weight gain in the black mice. The black mice that were treated with MSG showed a typical pattern of weight gain after PNW 12 (26,28,29). However, when MSG-treatment was administered to the (C57BL/6 × YS)F_1 yellow A^{vy}/a mice, there was a delay in the development of the inherent obesity. From PNW 8–20 the MSG-treated yellow mice were significantly lighter than their controls, but began to catch up by PNW 24. Thus, early postnatal administration of MSG delayed the greater rate of weight gain characteristic of the yellow genotype. The decrease in the rate of weight gain after MSG administration is similar to the reduction in weight gain in the yellow mice after adrenalectomy reported by Hausberger and Hausberger (12).

The decreased body weight gain in the MSG-treated yellow mice does not appear to reflect differences in food or water intake. The lack of hyperphagia in MSG-treated mice (4,9, 26,38) and particularly in yellow mice (9,36,37) confirms previous reports. Furthermore, the present results are also in agreement with the lack of hyperphagia observed in obese rats after neonatal MSG administration (18,27,30,32).

Successful destruction of the arcuate nucleus of the hypothalamus by early postnatal MSG administration was implied by both the morphometric and neurochemical results. The increase in the Lee Index of Obesity in the MSG-treated groups (4,9,18,30,32), and the decreases in body and tail length are indicative of MSG-induced hypothalamic damage (4,9,27,28,38). The yellow mice were longer, as well as heavier, than their black controls. The interaction of MSG treat-

TABLE 3

β-ENDORPHIN LEVELS IN THE HYPOTHALAMUS AND PITUITARY

	Hypothalamus (pg)		Hypothalamus (pg/mg wet weight)	
	Black	Yellow	Black	Yellow
Saline	440 ± 50.3	482 ± 34.8	28.6 ± 3.3	27.7 ± 2.0
MSG	213 ± 93.7*	140 ± 10.0*	19.9 ± 8.7†	9.5 ± 0.7†
	Pituitary (pg)		Pituitary (ng/mg wet weight)	
	Black	Yellow	Black	Yellow
Saline	26.6 ± 4.7	33.7 ± 1.6	17.9 ± 1.0	24.8 ± 3.5
MSG	38.0 ± 7.4*	46.0 ± 3.4*	55.0 ± 17.2†	65.0 ± 6.6†

Shown above are mean ± SEM β-endorphin levels in the hypothalamus (left) and hypothalamus/mg wet weight (right) and pituitary (left) and pituitary/mg wet weight (right) in saline-treated black, saline-treated yellow, MSG-treated black, and MSG-treated yellow mice ($n = 5$ mice/group).

*Significant main effect of treatment, $p < 0.05$.

†Significant main effect of treatment, $p < 0.01$.

ment with genome is significant because MSG lowered body length in yellow mice more than that of black controls, down to an apparent minimum representing the decreased length achieved by MSG-treated blacks.

Immunoreactive growth hormone releasing hormone (GHRH) cells in the arcuate nucleus and GHRH immunoreactive fibers in the median eminence have been shown to be decreased after MSG treatment in the rat (5,23,43) and may be the underlying cause of these morphometric changes. This finding suggests a GHRH or growth hormone (GH) oversecretion in the yellow mice; one that is reversed by MSG lesions of the GHRH neurons. This similar pattern found in liver weights of yellow mice may reflect the high sensitivity of this tissue to GH effects.

Accordingly, a decrease in total brain, heart, kidney (4), and testes weight (4,9,29,30), as observed in the present study, have all been reported as consequences of early postnatal MSG administration. Interestingly, Shimizu et al. (33) reported significantly heavier organ weights for heart, spleen, liver, and kidney for yellow mice when compared to lean black mice, results confirmed by the present study. Adrenalectomy of the yellow mice (33) decreased heart, spleen, liver, and kidney weights, whereas the size of the brain was not effected. Thus, early postnatal administration of MSG yields results similar to those reported after adrenalectomy in the yellow genotype. Taken together with the previously mentioned findings of Hausberger and Hausberger (12), there is the suggestion of a similar mechanism of action, perhaps involving adrenocorticotropic hormone effects on GH actions.

The decrease observed in hypothalamic β-endorphin content is also consistent with the syndrome associated with early MSG administration (6,17,19,42). On the other hand, with regard to the pituitary, Hong et al. (17) reported a decrease in β-endorphin-like immunoreactivity in the pituitary of rats, whereas Bodnar et al. (6) and Krieger et al. (19) reported no change in pituitary levels of β-endorphin. All of these reports contrast with the increase in pituitary β-endorphin in mice as determined by our radioimmunoassay. The discrepancy in pituitary β-endorphin levels between the studies may be due to species or age differences.

Shimizu et al. (35) have reported that hypothalamic DOPAC, 5-HT, and 5-HIAA levels were similar between yellow obese and lean black mice, while DA concentrations (as in the present study) were increased in the yellow genotype. Also, as in Shimizu et al. (35), we observed that hypothalamic DOPAC, HVA, 5-HT, and 5-HIAA levels were comparable in both yellow and black mice, and we further observed here that DA was decreased in both genotypes after MSG treatment. Moreover, there was a strong trend for even greater decreases of DA in the MSG-treated yellow mice than in MSG-treated black mice. These results are consistent with the decreased levels of DA in the mediobasal hypothalamus (14) and arcuate nucleus of the hypothalamus (25) as reported after neonatal MSG treatment in rats. Additionally, there is evidence that MSG causes degeneration of DA neurons that project from the arcuate nucleus of the hypothalamus to the median eminence (16,25). Thus, the decreased rate of body weight gain seen in the MSG-treated yellow mice may be attributable to a greater decrease in hypothalamic DA.

The decrease in DA concentration after MSG treatment appears to be specific for the hypothalamus because we found no significant differences in DA concentration in the caudate nucleus. These results confirm those of Nemeroff et al. (25), who also reported no change in DA concentration in the caudate nucleus, forebrain, substantia nigra, or pituitary following neonatal MSG treatment in rats.

Two separate models of obesity, one caused by an environmental neurotoxin (MSG) and one by a genetic variant (the viable yellow mutation at the *agouti* locus) have each been reported to be accompanied by functional abnormalities of both DA and POMC metabolism. We hypothesized that if a final common pathway produced both types of obesity, then combining the two models should produce little or no further obesity than either treatment alone. Our results support this hypothesis by showing that the combined treatment considerably lengthened the time required to produce obesity. However, our data also indicate that the eventual obesity achieved by the combined treatments was nearly as great as would be expected from adding together the individual treatment effects. Further developmental studies will be required to explain the delay of obesity in the yellow genotype.

The previously reported effects of MSG on hypothalamic DA and the POMC peptide β-endorphin have been confirmed for both the yellow and black mouse genotypes. We failed to obtain conclusive evidence for genotypic variation in monoamines or β-endorphin, although trends for elevations of DA in the hypothalamus and β-endorphin in the pituitaries of yellow obese mice were observed. These results are generally consistent with earlier suggestions by Shimizu et al. (34,36) that increased hypothalamic DA-induced inhibition of pituitary *N*-acetylation of POMC peptides may be a factor in the yellow obesity syndrome.

We note here for the first time that the effects of MSG on both hypothalamic DA and β-endorphin was more pronounced in the yellow obese mice than the black mice. Two separate sets of treated animals, one assayed for DA and the other for β-endorphin, both showed larger decrements of these biomarkers of MSG neurotoxicity in the yellow mice compared to their black controls. The reason for this is unknown but may relate to an altered excitability of the hypothalamic neurons to the excitotoxic effects of MSG that was conferred by the overexpression of the *agouti* protein and its presumed antagonism of melanocortin-4 receptors (22). If the yellow obese mice have larger hypothalamic lesions than comparably dosed black mice, the variation in lesion size might also have affected the development of obesity in this group. These observations may also serve to suggest that a range of variability in dose–response curves for the hazardous effects of MSG-like neurotoxicants may be seen as a function of different genotypes.

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