MSG Effects on Beta-Endorphin and Alpha-MSH in the Hypothalamus and Caudal Medulla

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ALESSI, N. E., P. QUINLAN AND H KHACHATURIAN. *MSG effects on beta-endorphin and alpha-MSH in the hypothalamus and caudal medulla* PEPTIDES 9(4) 689–695, 1988.—Monosodium glutamate (MSG) was given to neonatal male rats to determine its effects on neurons containing beta-endorphin (β-END) and alpha-melanocyte stimulating hormone (α-MSH) within the basal hypothalamus (arcuate nucleus) and caudal medulla [nucleus tractus solitarius (NTS)] and on the levels of β-END and α-MSH within these areas. Immunocytochemical studies demonstrated a reduction in the number of cells within the medial hypothalamic area (arcuate nucleus) among MSG-treated animals versus saline controls. MSG did not reduce the number of cell bodies within the caudal medulla (NTS). MSG significantly reduced β-END and α-MSH immunoreactive levels in the basal hypothalamus as determined by radioimmunoassay. Whereas a significant reduction in the level of β-END occurred in the ventral caudal medulla (VCM), none occurred in the dorsal caudal medulla (DCM) In contrast, levels of α-MSH increased significantly in the DCM among animals receiving MSG compared to control animals. This study documents the contribution of beta-endorphin containing neurons of the basal hypothalamus to areas of the caudal medulla. The effect of MSG on beta-endorphin and α-MSH neurons in these areas and their differential effects on levels in the caudal medulla areas raises questions about the sites of origin of these peptides.

Beta-endorphin   Alpha-MSH   Monosodium glutamate   Arcuate nucleus   Nucleus tractus solitarius

PERIKARYA containing pro-opiomelanocortin (POMC) and its products, e.g., beta-endorphin (β-END) and alpha-MSH (α-MSH), are found in the arcuate nucleus and nucleus tractus solitarius (NTS) within the central nervous system [1, 9, 11]. β-END immunoreactive fibers are found in dorsal and ventral caudal medulla. Although the origin of these fibers is unknown, retrograde track tracing has demonstrated that cell bodies in the hypothalamus project to the NTS [19]. Radioimmunoassay studies in the adult rat, utilizing either the entire caudal medulla or dorsal caudal medulla, indicate that the major POMC products are α-MSH and β-END [1, 2, 4]. Given the potential physiological actions of either α-MSH or β-END, it is important to discern not only their quantity but where these peptides may originate, i.e., the basal hypothalamus or the caudal medulla.

In the following study, monosodium glutamate (MSG) was administered to rats to assess the effects of MSG treatment on β-END and α-MSH in the caudal medulla and the basal hypothalamus. The administration of MSG during development has been demonstrated to result in loss of perikarya in the medial basal hypothalamus (arcuate nucleus) and a reduction in the β-END and α-MSH im-

munoreactivity in the brain [5,13]. The combination of these approaches, radioimmunoassay and immunocytochemistry, were utilized to discern the relationship of the visible reduction in the number of perikarya with reduced levels of β-END and α-MSH.

METHOD

Monosodium Glutamate Treatment (MSG)

Monosodium glutamate (MSG) was administered intraperitoneally to cross-fostered male Sprague-Dawley rats at postnatal days 2, 4, 6 and 8. The dosage was 4 mg per gram body weight in a delivery volume of 100 microliters [14,15]. The control littermates received 100 microliters of 10% saline intraperitoneally at the same ages. Eighteen (40%) of forty-five MSG-treated animals survived to adulthood with the highest death rate occurring between postnatal days 6 and 12. None of the control littermates died. Litters were housed in a twelve hour light-dark cycle (light from 0600 to 1800) with food and water provided ad lib. At postnatal day 21, the MSG and control rats were weaned and placed in mixed groups of six per cage.

Tissue Collection

The rats were housed in groups of up to six per cage until the ages of 95 and 105 days at which time they were killed by decapitation Six MSG and saline animals were randomly selected from these groups and served as the basis for the radioimmunoassay studies. Following decapitation the brains were removed from the skull and regions were dissected immediately [6]. Upon dissection, the pons-medulla tissue block was frozen on dry ice. The caudal medulla was dissected using the obex as the anterior boundary and the medulla-cord junction as the posterior boundary. The caudal medulla was then separated into ventral and dorsal regions using the central canal as the plane of division The collected tissue was stored at −80°C Tissue extraction was performed as previously described [2]. The regions selected for study consisted of the hypothalamus, ventral medulla (VCM), and dorsal caudal medulla (DCM)

Radioimmunoassay of Beta-Endorphin and Alpha-MSH

The β-END radioimmunoassay (RIA) procedure was performed as previously described [3]. The assay consisted of a three-day disequilibrium procedure using 4% sheep anti-rabbit globulin serum as the second antibody. The peptide used as the standard and for iodination was human β-END 1-31 (Cat. No. 8616, Peninsula Lab, Belmont, CA) The antiserum was donated by Dr. Huda Akil (Mental Health Research Institute, University of Michigan). The IC_{50} for the assay was 8.58±0.65 femtomoles/tube and the sensitivity was 2.63±0 35 femtomoles/tube The interassay coefficient of variation was 7.52% and the intraassay coefficient of variation was 6 8%.

The β-END antiserum is 100% crossreactive with its larger molecular weight precursors, POMC or beta-lipotropin The antiserum showed no cross reactivity with gamma-endorphin (β-END 1-16), ACTH, α-MSH, gamma-MSH, [Leu] or [Met] enkephalin, dynorphin related peptides or a number of nonopioid pituitary peptides A complete characterization of the antiserum can be found in Cahill *et al.* [3] The β-END immunoreactivity measured in the RIA was the sum total of β-END, beta-lipotropin, and POMC immunoreactivities

The α-MSH assay procedure was performed as previously described [2] The assay consisted of a five-day disequilibrium procedure using 5% sheep anti-rabbit globulin serum as the second antibody. The peptide used as the standard and for iodination was α-MSH (Cat No 7251, Peninsula Laboratories, Belmont, CA). The antisera (Number 24) was donated by Dr. Thomas O'Donohue (formerly of Monsanto) The final dilution for antisera was 1/30,000 The IC_{50} for the assay was 14.86±0.61 femtomoles/tube and the sensitivity was 4 0±0.30 femtomoles/tube. The interassay coefficient of variation was 15 3% The α-MSH antisera is 100% crossreactive with α-MSH, des-acetyl α-MSH, and deaminated α-MSH. The antiserum was 0 0% crossreactive with ACTH 1-24, human ACTH 1-39, human β-END 61-91, human beta-lipotrophin and ACTH 1-10, ACTH 4-10 or ACTH 4-11 Protein levels were determined utilizing a standard procedure [12]. The Student's t-test for unpaired samples was used to assess the levels of significance between sample means

Colchicine Pretreatment

To enhance the visualization of neuronal perikarya colchicine (250 μg in 10 μl 0 9% saline) was injected intracere-broventricularly into adult rats, MSG- or saline-pretreated, from which brains were taken for immunocytochemistry. Only animals whose brains were used for immunocytochemistry were given colchicine prior to sacrifice The main action of colchicine is inhibition of microtubule synthesis, resulting in retardation of axonal transport and an accumulation of synthesized material in neuronal perikarya [7] For injection, each animal was anesthetized with sodium pentobarbital (5–10 mg per animal), the skull exposed, and colchicine injected into the right lateral ventricle. The skull opening was filled in with bone wax and the scalp closed using wound clips. Animals were allowed to recover for 48 hours, and then sacrificed as described below.

Immunocytochemistry of Beta-Endorphin

Brain sections were prepared for immunocytochemistry as described previously [10] Rats were anesthetized with sodium pentobarbital (50 mg/kg) given intraperitoneally and placed on crushed ice. The cardiovascular system was flushed with 0 9% saline followed by perfusion with ice-cold 4% formaldehyde (pH 7.4) in 0 1 M phosphate buffer, for 30 minutes. The brains were removed promptly and placed in the same fixative for an additional hour, after which they were transferred into 15% sucrose in phosphate-buffered saline (PBS) and refrigerated overnight. Tissue blocks were frozen onto brass cryostat chucks with liquid nitrogen and sectioned in a cryostat at −20°C. Sections of 20 μm thickness were thaw-mounted onto gelatin-coated slides and stored at −80°C.

Primary rabbit antiserum to β-END (gift of Dr. Stanley Watson, Mental Health Research Institute, University of Michigan) was diluted with 0.3% Triton X-100 in 0 02 M PBS, added onto the sections, and allowed first to incubate for 1 hour at 37°C, then overnight at 4°C After 3×10 minutes PBS washes, the sections were incubated with goat anti-rabbit serum (Arnel) for 30 minutes at 37°C and then at 4°C overnight. The sections were again washed in PBS, and incubated in successive steps with horseradish peroxidase (HRP) antiserum, followed by another PBS wash, and HRP enzyme (Sigma, Type VI) for 40 minutes, each at 37°C The slides were then washed in PBS and immersed in a solution of 0.03% hydrogen peroxide and 0 125 mg/ml diaminobenzidine (Sigma) for 15 minutes with constant stirring. The sections were washed in distilled water, briefly osmicated (2% osmium tetroxide), washed, dehydrated through ethanols and xylenes and mounted in Permount Observations and photography were performed using a Leitz Ortho-plan Microscope

RESULTS

Figure 1 shows the total body weights (grams) and whole brain wet weights (milligrams) of MSG and saline groups of animals The total body weights (mean±SEM) of the animals receiving MSG (340±14 grams) were significantly lower ($p < 0.005$) than animals receiving saline (419±17 grams) Brain wet weights were also significantly lower ($p < 0$ 005) in the MSG-treated animals (1,740±50 milligrams) compared to control animals (2,090±40 milligrams). The pattern of the reduced brain wet weight in MSG animals was noted for the DCM and VCM, as well (Fig. 2). The DCM showed a significant reduction ($p < 0.005$), but a greater reduction was seen in the VCM with the values 29 25±0.71 milligrams among saline control animals versus 19 38±1 5 milligrams among the MSG animals ($p < 0$ 0001). There was no significant

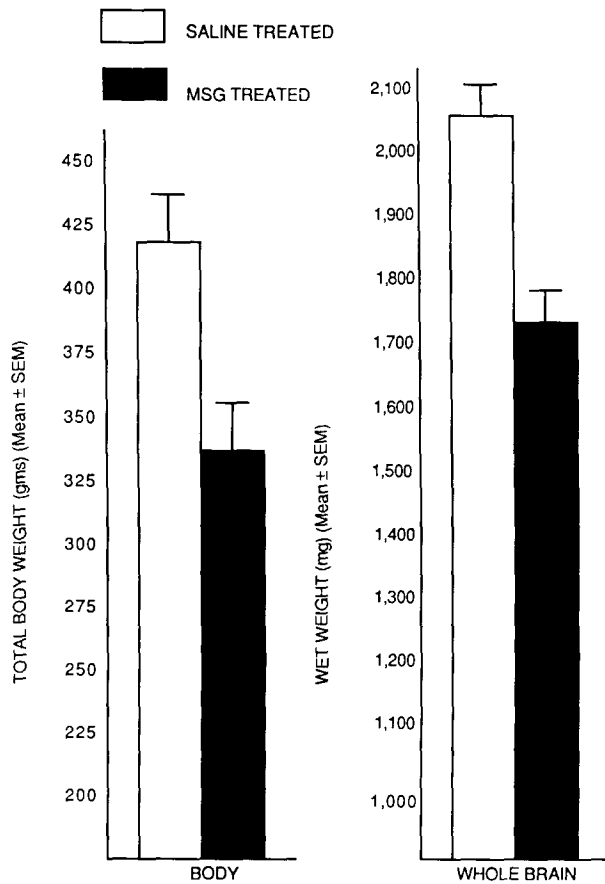FIG 1 Total body weight (grams) and whole brain wet weight (milligrams) in MSG versus control rats Unshaded bar represents saline control animals, shaded bar represents MSG animals N=6 per condition. gms=grams, mg=milligrams
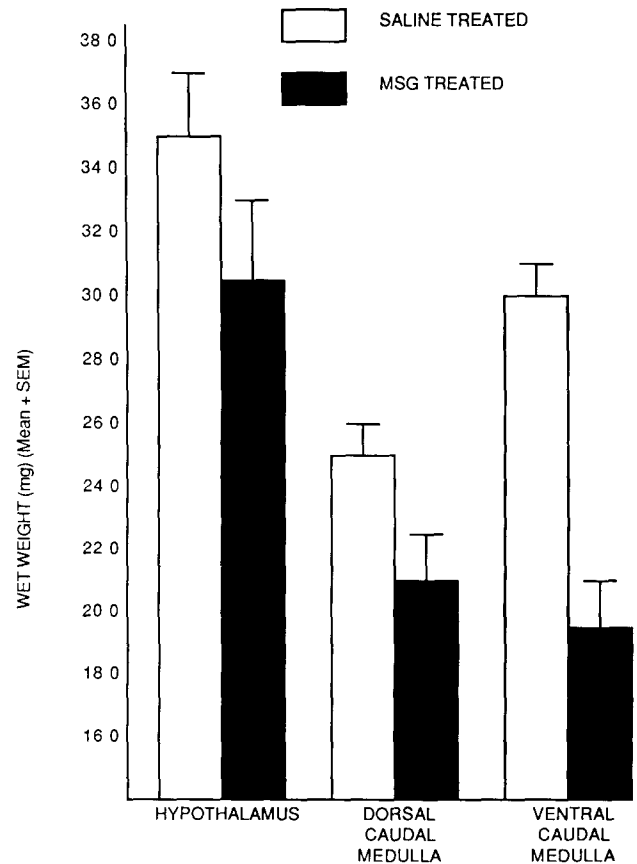

FIG 2 Wet weights (milligrams) of hypothalamus, dorsal caudal medulla, and ventral caudal medulla of MSG versus control rats Unshaded bar represents saline control animals, shaded bar represents MSG animals N=6 per area per condition mg=milligrams.

difference in hypothalamus wet weights between saline control (35.25±1.97 milligrams) and MSG- (30.13±2.55 milligrams) treated animals.

The immunocytochemical study of β-END within the hypothalamus and caudal medulla of saline control versus MSG rats is presented in Fig. 3. In Fig. 3A, the presence of β-END staining cell bodies is apparent in the arcuate nucleus of saline control animals. In Fig 3B, the reduction in cell number in the medial basal hypothalamus (arcuate nucleus) is prominent. Of note is the increase in size of perikarya contained within the lateral basal hypothalamic area. In comparison, the number of cell bodies within the caudal medulla were not reduced among MSG-treated animals versus saline control, nor did there appear to be an alteration in the size of the cell bodies, as was noted in the lateral hypothalamic β-END containing cells (Fig. 3C and D).

The values (mean±SEM) of β-END immunoreactivity in the animals receiving saline were 976.14±3.6 37 fmol/mg protein for hypothalamus, 86.20±10.92 fmol/mg protein for the VCM, and 44.49±4.51 fmol/mg protein for the DCM. The VCM had a significantly higher concentration of β-END immunoreactivity than the DCM ($p<0.005$). Among the MSG-treated animals, β-END immunoreactivity significantly decreased in the hypothalamus ($p<0.0005$) and the VCM ($p<0.025$) to 371.50±60 90 fmol/mg protein and 37.73±6.40 fmol/mg protein, respec-

tively. β-END immunoreactivity in the DCM did not significantly decrease with β-END immunoreactivity being 36.34±6.90 fmol/mg protein (see Fig. 4).

The values (mean±SEM) of α-MSH-like immunoreactive material in the animals receiving saline were 229 28±43.53 fmol/mg protein for the hypothalamus, 29.51±4.33 fmol/mg protein for the VCM, and 17.14±1.24 fmol/mg protein for the DCM. The VCM had a significantly higher concentration of α-MSH than the DCM ($p<0.025$). In the MSG-treated animals, α-MSH significantly decreased in the hypothalamus from 229.28±43.53 to 85.31±6.48 fmol/mg protein ($p<0.005$). Whereas α-MSH in the DCM significantly increased ($p<0.01$) from 17.14±1.24 to 28.28±3.79 fmol/mg protein, levels within the VCM did not significantly change ($p<$N S.) (see Fig. 5).

DISCUSSION

The administration of monosodium glutamate (MSG) during development resulted in a decrease in the levels of β-END immunoreactivity and α-MSH immunoreactivity in the medial basal hypothalamus. These results replicate earlier radioimmunoassay studies [9]. Although the β-END radioimmunoassay results were paralleled by a decrease in the number of β-END staining cell bodies in the medial hypothalamus (arcuate nucleus), cell bodies in the lateral
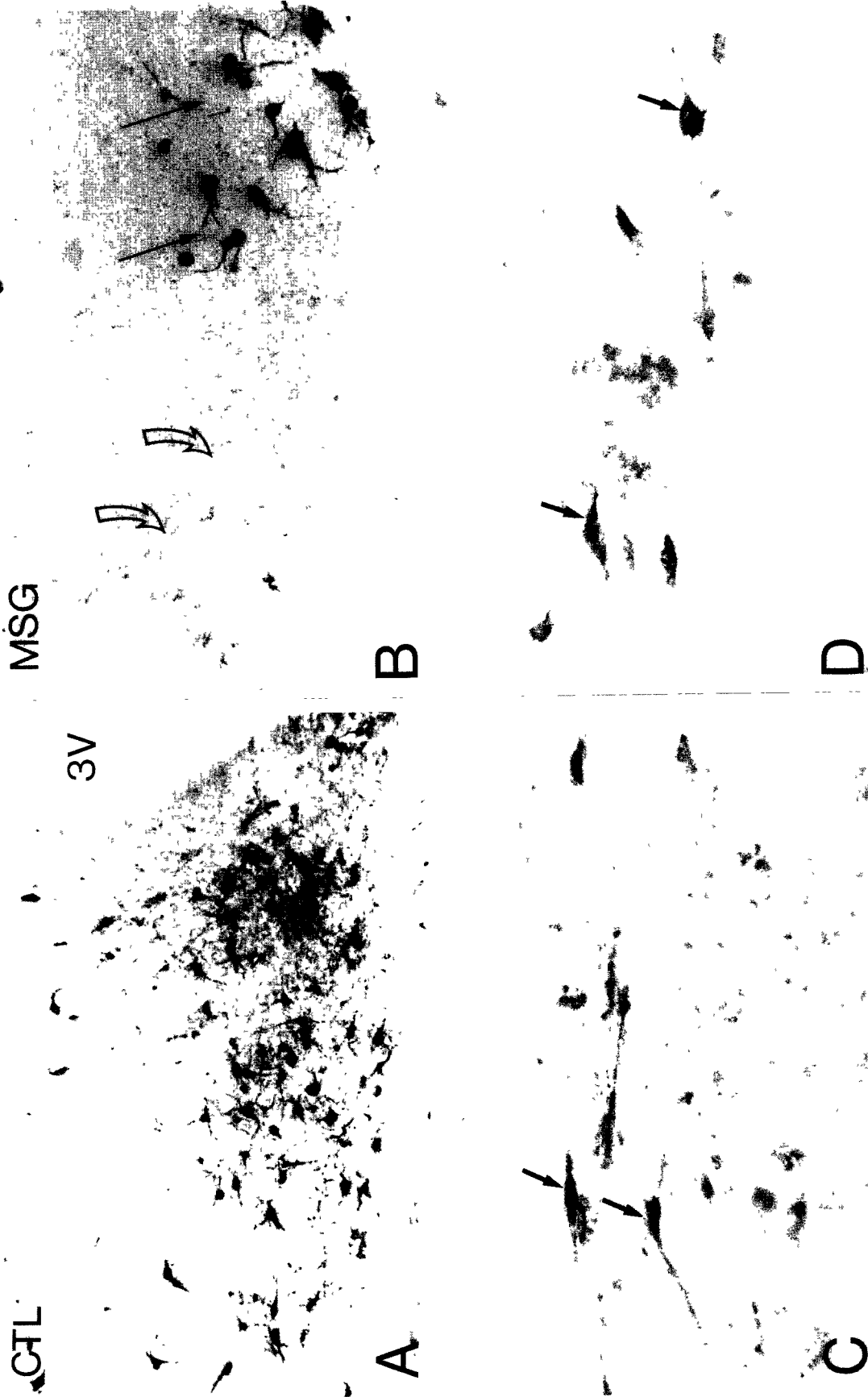
FIG 3 Panels A and B show the effect of MSG treatment on β-END immunoreactive neurons in the arcuate nucleus Note in the control animal A (CTL) cells are seen in the medal and lateral basal hypothalamus In the treated animal B (MSG) loss of cells due to cell death is completely medial (open arrows point to medal basal hypothalamus, arcuate nucleus, the principle site of cell death) Many lateral cells survive (thin areas) By contrast, MSG treatment does not seem to affect the β-END neurons of the NTS, as can be seen in panels C (CTL) and D (MSG) Individual neurons in both panels C and D are depicted by arrows Magnifications A and B=130×, C and D=506× Abbreviations CTL=control MSG=monosodium glutamate, and 3V=third ventricle
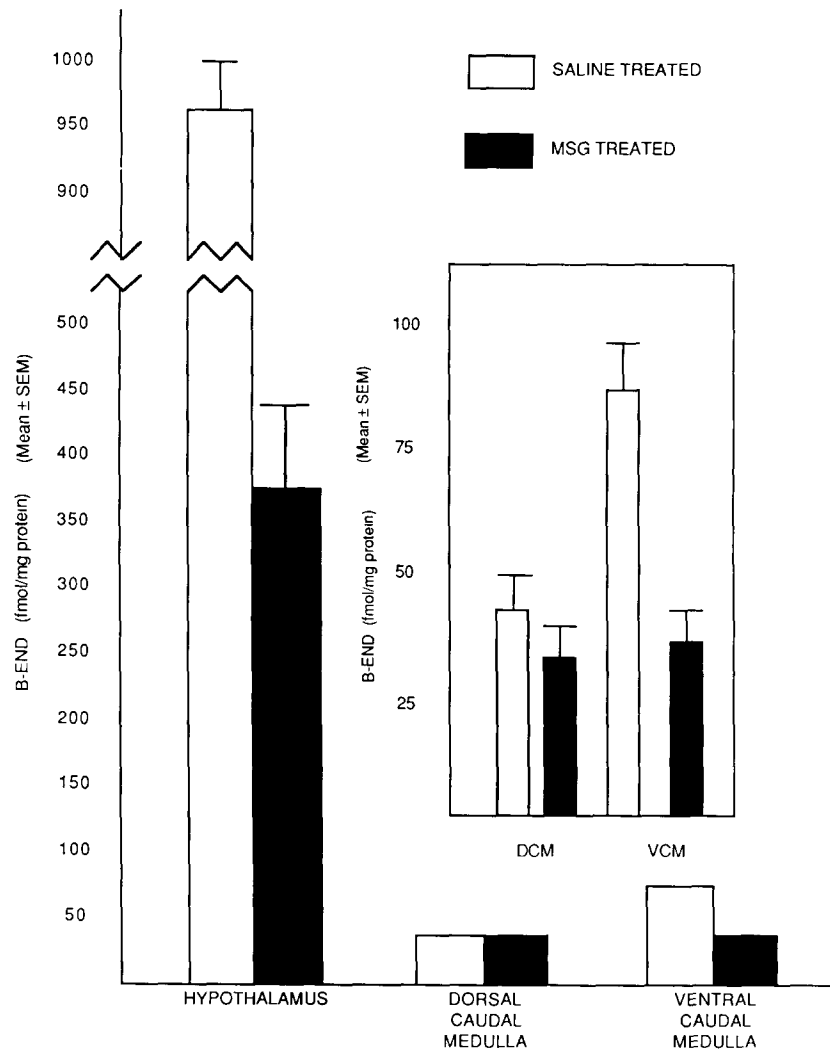
FIG. 4 Beta-endorphin-like immunoreactivity (fmol/mg protein) in MSG versus control rats Unshaded bar represents saline control animals, shaded bar represents MSG animals N=6 per area per condition fmol/mg protein=femtomoles per milligrams of protein

basal hypothalamus were spared and appeared to have increased in size Although β-END staining cell bodies in the dorsal caudal medulla did not appear to be affected either in number or size, there were significant alterations in the levels of β-END immunoreactivity using radioimmunoassay techniques. Of note, β-END decreased significantly in the ventral caudal medulla (VCM) without a concomitant decrease in the dorsal caudal medulla (DCM).

The lack of a decrease in the levels of β-END immunoreactivity in the DCM would support the lack of a decrease in cell number in the nucleus tractus solitarius as demonstrated by immunocytochemistry. If all the material within the VCM originated from the DCM, e g , nucleus tractus solitarius cell bodies, one would expect that the levels of β-END in the VCM in MSG-treated versus control animals would not decrease, as well. The finding of a significant drop in β-END levels within the VCM suggests that this material originates from an additional site other than the dorsal caudal medulla, such as the arcuate nucleus. With cardiovascular and respiratory centers present in VCM areas,

the hypothalamus could modulate physiological functioning originating from these areas via these projections. Such "distant" versus "proximal" innervations would allow subtle but important modulations

While alterations in the levels of α-MSH immunoreactivity in the hypothalamus paralleled those seen with β-END, alterations within the caudal medulla were quite different. Among MSG-treated animals, a drop in α-MSH levels comparable to β-END levels in VCM was not noted. In fact, α-MSH levels in the VCM remained unchanged, while α-MSH levels in the DCM rose significantly. One can only speculate as to why the alterations in α-MSH levels are different than those of β-END. Two speculations are: First, the distribution of end products of pro-opiomelanocortin (POMC)-containing cells within the dorsal caudal medulla (nucleus tractus solitarius) may be regionally specific. This same logic may also apply to material originating from outside the dorsal caudal medulla, i.e., the arcuate nucleus or possibly other areas of the brain. This might explain on an anatomical basis why there are differences in levels fol-
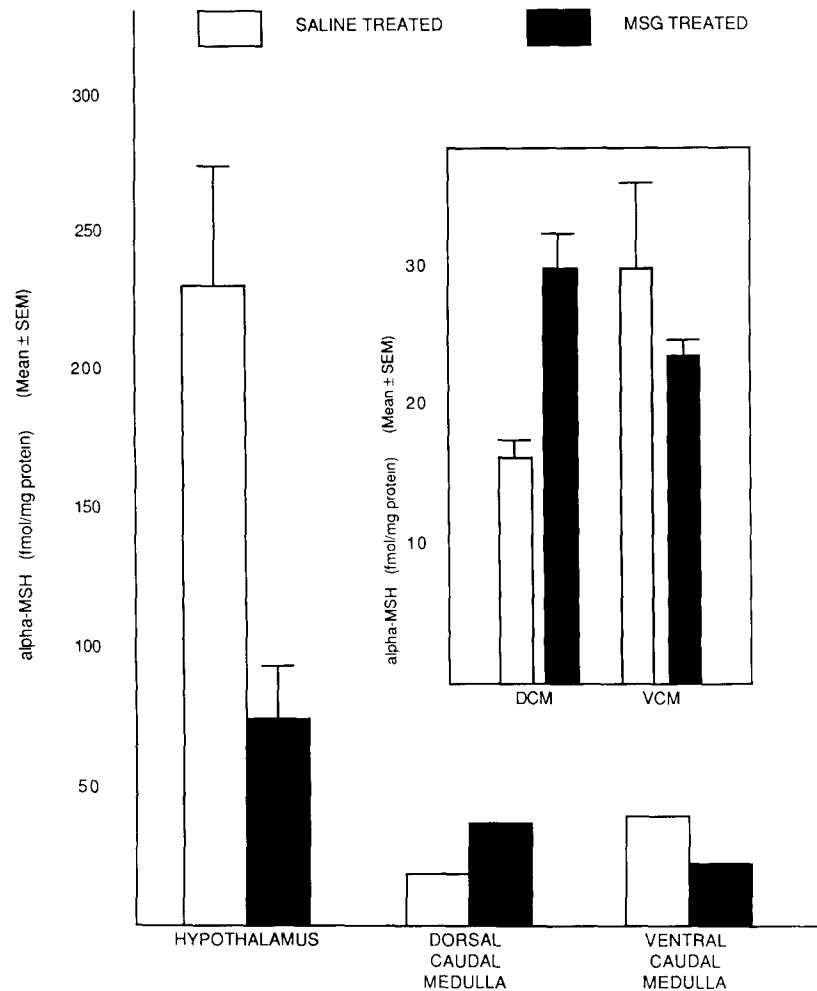
FIG 5 Apha-MSH-like immunoreactivity (fmol/mg protein) in MSG versus control rats Unshaded bar represents saline control animals, shaded bar represents MSG animals N=6 per area per condition fmol/mg protein=femtomoles per milligrams of protein

lowing the lesioning of the medial hypothalamus Second, on a biochemical basis, the differences may be due to an alteration in the rates of metabolism, either in production or in breakdown.

The alterations in the levels of α-MSH are further complicated by the identification of the alpha-2 group within the lateral hypothalamic area [9,16] These cell bodies have been identified as being a separate group which contain α-MSH, yet no other POMC-derived material [20]. Studies have suggested that this cell group contains α-MSH-like material, not "true" α-MSH [9] The implication of this finding is that the α-MSH identified within the caudal medulla, both DCM and VCM, may represent a combination of POMC-derived α-MSH, originating from the dorsal caudal medulla (NTS) and the medial basal hypothalamus (arcuate nucleus), and an α-MSH-like material originating from the alpha-2 cell group within the lateral hypothalamus

Although these explanations might explain steady state levels of α-MSH in MSG-lesioned animals or a differential decrease, as was found with β-END, there remains the question of why α-MSH levels increased in DCM among MSG-treated animals versus control animals Any explanation is

flawed by the lack of knowledge of the site of origin of the α-MSH immunoreactive material, whether "true α-MSH" or "α-MSH-like." Nevertheless, there are two explanations that can be put forth to explain why post MSG levels of α-MSH increased: (1) The increase represents the impact of the earlier lesion, yet does not reflect current need; and (2) The increase is reflective of an increased need of α-MSH for the current homeostasis of the system. In regard to the first explanation, α-MSH has been reported to be a trophic agent in the brain [8, 17, 18]. Cells within the dorsal caudal medulla (NTS) responsible for the modulation of respiratory and cardiovascular functioning, might be influenced by the trophic action(s) of α-MSH during development. Therefore, increased levels of α-MSH in the DCM could be explained on the basis of the importance of these cells receiving exposure to α-MSH during development. Multiple sites, i.e., medial and lateral hypothalamus, and medulla, as well as the alpha-2 cell group within the lateral hypothalamus, could represent a built-in redundancy constituting a fail safe system, which would ensure that α-MSH or an α-MSH-like material reach the nucleus tractus solitarius neurons during development. Such an alteration resulting from a lesion of

other primary sites may remain throughout the life of the animal. Further studies are obviously needed to determine the anatomy and biochemistry of these peptides and their roles during development and in the physiological functioning of the adult

In conclusion, future studies of β-END material within the caudal medulla, especially studies which attempt to characterize β-END forms in this area, must take into account the significant contribution of medial basal hypothalamic POMC-containing neurons to the levels of β-END in this area. Since this study was not designed to determine the origins of the projections to these areas nor to differentiate α-MSH from α-MSH-like material, the hypotheses concerning the alterations in the levels of α-MSH are based on limited information Further studies of the α-MSH

cells and medial basal hypothalmic POMC-containing neurons are needed to clarify the role of these cell groups during development, i.e , do they produce a "trophic" maternal necessary for the development of caudal medulla neurons, and to determine their biochemical composition and role as physiological modulators in the adult

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