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INCREASE IN DELTA, BUT NOT MU, RECEPTORS IN MSG-TREATED RATS

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Summary

Neonatal treatment of rats with Monosodium Glutamate (MSG) has been demonstrated to destroy cell bodies of neurons in the arcuate nucleus including the brain beta-endorphin (B-END) system. The effects on opiate receptors of the loss of B-END is unknown. Seven to nine month old rats treated with MSG on the first two postnatal days and litter matched untreated control rats were decapitated and their brains dissected into several regions. Opiate receptor assays were carried out with [^{3}H] morphine (mu receptor ligand) and [^{3}H] DADL (delta receptor ligand) for each brain region for both MSG-treated and control rats simultaneously. Scatchard plot analyses showed a selective increase in delta receptors in the thalamus only. No corresponding change in mu receptors in the thalamus was found. The cross-competition IC_{50} data supported this conclusion, showing a loss in the potency of morphine in displacing [^{3}H] DADL in the thalamus of MSG treated rats.

Injection of monosodium glutamate (MSG) in neonatal animals produces destruction of cell bodies in the arcuate nucleus of the hypothalamus (1). Immunocytochemical studies demonstrate a loss of the cell bodies and degeneration of the fibers from the ACTH/B-END system from arcuate nucleus in brain after MSG treatment (2). This destruction of the ACTH/B-END neurons has been confirmed by RIA measurements of ACTH and B-END in the brain (3). MSG lesions have no clear effect upon pituitary ACTH or B-END content (2).

B-END binds to opioid receptors with good affinity (4). The mu and delta subtypes of opiate receptors are most easily demonstrated in brain tissue binding studies (5,6). The mu receptor preferentially binds morphine, while the delta receptor preferentially binds enkephalins and is believed to be the receptor for methionine enkephalin and leucine enkephalin. B-END binds well to both mu and delta receptors in vitro, with a slight preference towards the mu receptor. Whether B-END binds

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to mu or delta receptors or neither *in vivo* is unclear at this point. The destruction from the B-END fibers of the arcuate nucleus might be expected to have an effect upon opiate receptors in the terminal regions of the brain B-END system. Since MSG-treated rats show depletion of brain B-endorphin without changes in other endogenous opiates, specific changes in opiate binding accompanying this lesion should provide further insights into opiate receptor subtype and regulation within this system.

MATERIALS AND METHODS

Neonatal rats were treated with MSG according to the following schedule: 2 grams MSG/kilogram body weight on the first two postnatal days; repeat treatment of 4 grams MSG/kilogram were done on post natal days 4, 6, and 8 (12). Male MSG rats and age and sex matched controls were killed at approximately 9 months of age. Female MSG lesioned rats and age/sex matched controls were decapitated at approximately 7 months of age. After decapitation, the brains were rapidly removed and dissected on ice into the following brain regions: hypothalamus, hippocampus, striatum, frontal cortex, thalamus, and midbrain. Tissue samples from all animals of the same group were pooled for homogenization and subsequent opiate receptor assays. Some animals were perfused and their brains removed for immunohistochemical verification of the destruction of B-END cell bodies.

Opiate receptor binding assays were done on each brain region of MSG lesioned and control animals simultaneously with both [^{3}H] morphine (Amersham) and [^{3}H] D-alanine-2-D-leucine-5-enkephalin (DADL-enkephalin, Amersham). The assays were performed using a modification of the opiate receptor binding assay of Akil et al (7). Each group contained 7 animals. The tissues were homogenized in 0.05 M Tris HCl buffer, pH 7.4 (at 25°C) with 5% DMSO (dimethylsulfoxide) to minimize freezing effects. The brain homogenates were frozen at -70°C until ready for use. Frozen homogenates were placed directly into a 37° water bath and incubated for 45 minutes to promote dissociation of endogenous ligands from the receptors. After centrifugation, the tissue was resuspended at a concentration of 50 mg tissue/ml 0.05 M tris buffer. Aliquots of tissue (0.3ml) were incubated with [^{3}H]-DADL enkephalin and [^{3}H]-morphine at concentrations ranging from 0.5 nM to 25 nM in a total assay volume of 0.5 ml. One micromolar morphine and one micromolar DADL enkephalin were used to define nonspecific binding for [^{3}H]-morphine and [^{3}H]-DADL enkephalin respectively. Because of the low ratio of specific to nonspecific binding at high concentrations of [^{3}H] ligand, a mixture of [^{3}H] ligand and unlabelled ligand was used to calculate bound and free concentrations at higher concentrations of morphine and DADL enkephalin. In addition, samples were incubated with varying concentrations of unlabelled morphine with [^{3}H]-DADL enkephalin and unlabelled DADL enkephalin with [^{3}H]-morphine to determine IC_{50} for both ligands against both [^{3}H] ligands. After a 2 hr, incubation at 4°C, samples were rapidly filtered under vacuum using Whatman GF/B glass fiber filters. The filters were washed twice with 4.5 ml of cold tris buffer, dispersed by shaking in 10 ml of Beckman aqueous scintillation cocktail and counted in a

Beckman LS9000 liquid scintillation counter. Biorad protein assays were used to determine that differences between MSG and control are not due to dilutional error. A given brain region for both MSG and control rats was completely studied in one experiment on one day.

Dissociation constants (K_D) and receptor concentration (Bmax) were obtained from Scatchard plot analysis of the data for each ligand in each brain region using computer generated multiple linear regression analysis.

RESULTS

Immunohistochemical analysis of brains from selected MSG-lesioned and control animals showed a consistent loss of B-END, immunolabelled in the arcuate of MSG-treated animals. The Scatchard analysis shows differences in only two brain regions, thalamus and striatum. Morphine and DADL enkephalin data show little if any change in the K_D between MSG treated and control groups. However, there are changes in the Bmax. In striatum, an increase in mu receptors (B_{max} control = 247pM; B_{max} MSG = 304 pM) was not accompanied by any changes in delta receptors. Due to the limited amount of striatal tissue available, we were unable to replicate this study. In thalamus, the number of delta receptors increased in the MSG lesioned rats (B_{max} control = 140 pM; B_{max} MSG = 244 pM) This increase could be seen in the first group of MSG rats (9 months old) and was replicated in the 7 month old MSG rats. The cross-competition experiments further support this observation (Table 1). Morphine, which ordinarily is more potent in thalamus in displacing [^{3}H] DADL than DADL enkephalin itself, lost much of its potency in the MSG group. The IC_{50} of morphine changes from 1.8 to 34nM. This loss of potency is consistent with an increase in delta receptors, such that the pattern of IC_{50} displacement is more characteristic of delta receptor-rich areas (e.g., striatum or cortex) than the mu receptor-rich thalamus.

TABLE 1. Thalamus

IC_{50} (nM) in Cross Competition

	^{3}H Morphine		[^{3}H] DADL	
	Control	MSG	Control	MSG
Morphine*	1.3	1.8	2.5	34
DADL*	28	32	15	20

*Mean of two experiments

DISCUSSION

The use of multiple ligands and brain regional analysis is a sensitive and specific technique for detecting changes in opiate receptors. Most previous attempts to find opiate receptor changes have utilized whole brain and one ligand, generally morphine, dihydromorphine or etorphine. If opiate receptors exist in subtypes *in vivo*, the regulation of each subtype may be separate. Thus there is no reason to expect, *a priori*, that the use of only one ligand could demonstrate a selective change in one receptor subtype. In the case of the MSG lesion, the use of [^{3}H]-DADL, a delta receptor ligand, was critical for the demonstration of the increase in B_{max}, since there were no changes in mu receptors. In addition the use of brain regional analysis was crucial since the change was confined to the thalamus, which is only 10% by weight of the whole brain. Since MSG lesions are confined to the brain B-END system, it is more appropriate to seek changes in specific terminal areas than in the whole brain. This report demonstrates an up regulation of opiate receptors in response to a chemical lesion destroying the arcuate nucleus B-END system. It is not known if this up-regulation represents a supersensitivity to delta-agonists *in vivo*.

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REFERENCES

1. J.W. OLNEY, Science 164 719-721 (1969)
2. R.J. BODNAR, G.M. ABRAMS, E.A. ZIMMERMAN, D.T. KRIEGER, G. NICHOLSON, J.S. KIZAR, Endocrinology 30 280-284 (1980)
3. D.T. KRIEGER, A.S. LIOTTA, G. NICHOLSEN and J.S. KIZER, Nature 278 562-563 (1979)
4. H. AKIL, W.A. HEWLETT, J.D. BARCHAS and C.H. LI, Eur. J. Pharmacol. 64 1-8 (1980)
5. K.J. CHANG and P. CUARTRECASAS, J. Biol. Chem. 254 2610-2618 (1979)
6. J.A.H. LORD, H.J. WATERFIELD and H.W. KOSTERLITZ, Nature 267 495 (1977)
7. W.H. HEWLETT, H. AKIL and J. BARCHAS, submitted