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EFFECT OF MONOSODIUM GLUTAMATE ON BRAIN TYROSINE HYDROXYLASE, DOPAMINE-β-HYDROXYLASE, TRYPTOPHAN HYDROXYLASE AND MONOAMINE OXIDASE IN RAT

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ABSTRACT: *The effects of monosodium glutamate (MSG) on the activities of tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH), tryptophan hydroxylase (TPH) and monoamine oxidase (MAO) in various regions (cerebral cortex, striatum, midbrain, pons and medulla of rat brain have been determined. It was observed that up to 1mM MSG had no significant effects on the activities of brain tyrosine hydroxylase, dopamine β-hydroxylase, tryptophan hydroxylase and monoamine oxidase in all regions of rat brain. These results indicated that MSG itself exerted no direct effect on the important enzymes synthsizing and metabolizing the monoaminergic neuronal system.*

Keywords: *MSG, tyrosine hydroxylase, dopamine-β hydroxylase, tryptophan hydroxylase, monoamine oxidase, brain, rat.*

INTRODUCTION

Glutamic acid is one of the most common amino acids found in nature as its free form and is present virtually in all proteins. Monosodium glutamate (MSG) is widely used as a flavor agents in cooking, presumably as a taste stimulant and a flavor enhancer. L-Glutamic acid, the free acid of MSG is a constituent of protein and also occurs as a free form in a variety of vegetable, meat and seafood (Hac *et al.*, 1949; Maeda *et al.*, 1949; Maeda *et al.*, 1958).

There has been extensive research on the physiology, biochemistry and toxicology of glutamic acid for more than 30 years. Unlike the other essential neutral or basic amino acids, the acidic amino acids, such as glutamate and aspartate, can be synthesized in brain cells at high rates commensurate with the metabolic demands for these compounds. Consequently, the rate of transport of the acidic amino acids from blood to brain is much lower than the rate observed for the neutral or basic amino acid (Oldendorf, 1971). Despite the relative independence of the brain from the levels of circulating acidic amino acids, it was suggested that these compounds would be neurotoxic when plasma levels are elevated by the administration of large doses (Olney, 1976). The rate of synthesis of several putative neurotransmitters (i.e., serotonin, catecholamine, histamine) is affected by the levels of precursor neutral amino acids in brain cells (tryptophan, tyrosine, histidine)(Fernstrom and Wurtman, 1971; Schwartz *et al.*, 1972; Wurtman *et al.*, 1974).

To investigate the influence of MSG on catecholaminergic and tryptaminergic neuronal systems, the effect of MSG on tyrosine hydroxylase (a synthesizing and marker enzyme of dopamine neuron), dopamine-β hydroxylase (a synthesizing and marker enzyme of noradrenergic neurons), tryptophan hydroxylase (a synthesizing and marker enzyme of serotonin neuron) and monoamine oxidase (a metabolizing enzyme of monoamine neurons), in various brain regions of rat have been determined.

MATERIALS & METHODS

Chemicals used in this work were obtained as follows; (^{14}C)-phenylethylamine hydrochloride (PEA, 50.2 mCi/mmol), L-(^{14}C)-tyrosine (502.1 mCi/mmol) from New England Nuclear of Boston, MA, USA; Catalase, DL-6-methyl-5,6,7,8-tetrahydropterine 2HCl (6-MPH_4), tyrosine, mercaptoethanol, N-ethylmaleimide, pargyline hydrochloride, sodium fumarate, ascorbic acid, tyramine hydrochloride, phenylethylamine, tryptophan, dithiothreitol, α-methyl-tyrosine, from Sigma Chemical Co., St Louis, MO, USA. All other chemicals used were of the highest grade commercially available.

Sprague-Dawley rats (150-200g) were decapitated and the brains were dissected on an ice-cooled glass plate. Four regions were separated; (1) cortex (2) striatum (3) midbrain (4) pons & medulla oblongata as described by Glowinski & Iversen (1966). Individual regions from 4-5 rats were pooled. The tissues were weighed and homogenized with 50 mM Tris-HCl (pH 7.5) and centrifuged at 20,000g for 20 min. The supernatant fraction was assayed for tyrosine hydroxylase, dopamine-β-hydroxylase, tryptophan hydroxylase. The mitochondrial fraction was assayed for monoamine oxidase.

TH activity was assayed by the method of Reis et al. (1975). The assay mixture, which contained 50 μl of enzyme, 7.5 μl of 1 M sodium acetate buffer, pH 5.8, 5 μl of 200 μM tyrosine, 2.5 μl of L-(^{14}C)-tyrosine (0.25 μCi), 5 μl of 15 μM 6-MPH_4 in 420 mM mercaptoethanol, 1 μl of catalase (100 μ), 4 μl of distilled water, were incubated for 20 min at 37 °C. The reaction was stopped by the addition of 1 ml of 0.4 N perchloric acid containing 10 ug carrier L-dopa and centrifuged at 1000g for 10 min; the supernatant was added to 20 ml beaker containing 5 ml of 2% EDTA (W/V), 1.5 ml of 0.35 M KH_2PO_4. The mixture was adjusted to pH 8.6-8.9 with 1N NaOH. The suspension was then poured over a column containing 200 mg of activated alumina and was washed with 20 ml of distilled water. ^{14}C-DOPA was eluted with 4 ml of 0.5 N acetic acid. The radioactivity of the total eluate was counted in 15 ml of Bray's solution in New England Nuclear Scintillation counter.

DBH was measured by dual-wavelength spectrophotometry described by Kato et al. (1974). Incubation mixture (total volume 1 ml) contained (in final concentrations); enzyme, 0.2 M sodium acetate buffer, pH 5.0, 30 mM N-ethylmaleimide, 10 μM $CuSO_4$, 500 μM pargyline hydrochloride, 10 mM sodium fumarate, 10 mM ascorbic acid, 490 μg (490U) of catalase, 20 mM tyramine hydrochloride. Incubation was carried out for 45 min at 37 °C.

MAO activity was determind radiochemically, as described by Fowler et al. (1979), at 37 °C and pH 7.4 with incubation for 10 min using (^{14}C) phenylethylamine diluted with unlabelled phenylethylamine as substrate. The final phenylethylamine concentration was 0.1 mM.

Tryptophan hydroxylase activity was assayed by the method of Friedman et al (1972). Incubation mixture contained; enzyme, 120 mM Tris-acetate buffer, pH 7.5, 2 mM dithiothreitol, catalase, 400 μM tryptophan, 200 μm 6-MPH_4. Incubation was caried out for 30 min at 37 °C. The reaction was stopped with 100 μl of 70% perchloric acid and the precipitated proteins were centrifuged out. In the supernatant, formation of 5-hydroxytryptophan was determind by fluorometry (excitation at 312 nm and emission at 540 nm).

In all of the above enzyme assays, the effect of adding monosodium glutamate at 0.1 mM and 1 mM (in final concentrations) was studied. Specific inhibitors; α-methyl-p-tyrosine (α-MPT)(1 mM) for tyrosine hydroxylase, fusaric acid (1 mM) for DBH, p-chlorophenylamine(PCPA)(1 mM) for tryptophan hydroxylase, pargyline (1 mM) for MAO B were used as positive controls.

Protein was determined according to the method of Lowry et al. (1951).

Student t-tests were used to determine the statistical significance of differences.

RESULTS

As shown in Table 1, monosodium glutamate (MSG) had no effect on tyrosine hydroxylase (TH, a synthesizing and marker enzyme of dopamine neurons) activities of various regions of brain up to 1 mM concentration. In contrast, α-methyl-p-tyrosine, a competitive-inhibitor of TH, showed 44-67% inhibition at 1 mM concentration.

Table 2 shows that MSG had no effect on dopamine-β-hydroxylase (DBH, a synthesizing and marker enzyme of noradrenergic neurons) activities of various brain regions up to 1 mM concentration. Enzyme activity in the presence of 1 mM fusaric acid, used as a blank, was completely inhibited.

Table 1. Effect of MSG on tyrosine hydroxylase activity in various regions of rat brain.

Groups / Brain regions	Tyrosine Hydroxylase Activity (DOPA formed pmol / mg protein / hr)			
	Control	MSG 0.1 mM	MSG 1 mM	α-MPT 1 mM
Cortex	85.0 ± 3.6	83.4 ± 2.4	84.8 ± 5.9	37.8 ± 10.1
Striatum	379.5 ± 22.4	331.1 ± 62.7	358.0 ± 16.6	126.9 ± 12.4
Midbrain	195.2 ± 38.2	227.1 ± 30.5	240.5 ± 31.2	92.4 ± 16.1
Pons & Medulla	21.1 ± 1.9	26.3 ± 4.3	30.0 ± 6.7	11.9 ± 1.1

The values represent mean ± S.E.M. of 3 different duplicate determinations.

Table 2. Effect of MSG on dopamine-β-hydroxylase activity in various regions of rat brain

Groups / Brain regions	Dopamine-β-hydroxylase activity (Octopamine formed pmol / mg protein / min)		
	Control	MSG 0.1 mM	MSG 1 mM
Cortex	26.1 ± 3.5	23.2 ± 4.2	24.6 ± 4.2
Striatum	20.6 ± 3.7	29.2 ± 1.8	26.4 ± 3.1
Midbrain	49.8 ± 5.5	47.9 ± 5.1	48.1 ± 3.4
Pons & Medulla	71.7 ± 6.9	55.8 ± 7.0	61.1 ± 6.1

The values represent mean ±S.E.M. of different duplicate determinations.

As shown in Table 3, MSG had no effect on tryptophan hydroxylase (TPH, a synthesizing and marker enzyme of serotonin neurons) activities up to 1 mM concentration. In contrast, p-chlorophenyl-amine showed 46-51% inhibition at 1 mM concentration.

Monoamine oxidase (a metabolizing enzyme of monoamine neurons) activities in various regions of rat brain were not significantly affected by the presence of MSG up to 1 mM concentration. On the contrary, pargyline, an MAO inhibitor, almost completely inhibited MAO activity, even at 0.1 mM concentration (Table 4).

Table 3. Effect of MSG on tryptophan hydroxylase activity in various regions of rat brain

	Tryptophan hydroxylase activity (5-hydroxytryptophan formed pmol/mg protein/30 min)			
Groups		MSG		PCPA
Brain regions	Control	0.1 mM	1 mM	1 mM
Cortex	54 ± 9	54 ± 9	72 ± 15	31 ± 6
Striatum	90 ± 2	90 ± 15	94 ± 12	35 ± 9
Midbrain	829 ± 20	883 ± 20	816 ± 7	401 ± 19
Pons & Medulla	508 ± 11	497 ± 24	492 ± 17	259 ± 41

The values represent mean ± S.E.M. of 3 different duplicate determinations.

Table 4. Effect of MSG on MAO B activity in various regions of rat brain

	Monoamine oxidase B activity (nmol/mg protein/h)			
Groups		MSG		Pargyline
Brain regions	Control	0.1 mM	1 mM	0.1 mM
Cortex	29.66 ± 1.14	27.02 ± 1.48	28.51 ± 1.35	0.08 ± 0.03
Striatum	31.32 ± 2.01	29.09 ± 1.52	24.32 ± 1.49	0.18 ± 0.07
Midbrain	31.60 ± 1.26	29.03 ± 2.12	29.19 ± 0.98	0.06 ± 0.03
Pons & Medulla	24.24 ± 0.67	25.66 ± 0.77	23.41 ± 0.92	0.13 ± 0.05

The values represent mean ± S.E.M. of 3 different duplicate determinations.

DISCUSSION

The metabolism of glutamate in various tissues of central nervous system has been studied extensively since the appearance of neurophysiological and neurochemical evidence that glutamate may function as an excitatory neurotransmitter. Interest has developed for the metabolism of glutamate as it relates specifically to this putative synaptic function (Shank and Aprison, 1979). It is postulated that the levels of essential amino acids in brain tissues may affect cerebral protein synthesis and thereby may affect enzyme synthesis.

In brain, glutamate is one of the most active neuroexcitatory substances and also functions as the precursor for gamma aminobutyric acid, a very important inhibitory transmitter. Initial reports on the biochemical deficits resulting from local injection of kainic acid into the neostriata of rats were of particular interest because the morphological and biochemical changes resulting from its injection were very similar to those reported for Huntington's chorea (Fibiger

1978). Costa *et al.*, (1979) suggested that glutaminergic afferents to striatum stimulate the GABA and Ach metabolism, presumably because glutamate functions as an excitatory transmitter.

Since the necrosis of the hypothalamic neurons following the administration of MSG to the neonate mouse was reported (Olney 1969), there have been several attempts to assess the toxicity of this substance in a variety of species.

It was suggested that the biochemical changes observed in extrapyramidal nuclei, such as cholinergic, serotonergic, dopaminergic systems were also the result of their excitatory action at the glutamate receptor (Olney *et al.*, 1975).

The results of present study reveal that MSG did not affect the enzyme activities of TH, DBH, TPH, and MAO at the concentration up to 1 mM in various brain region. From this result, it is suggested that the neurotoxicity observed with MSG may not be a direct effect of MSG on important enzymes involved in monoamine neurotransmitter metabolism.

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