Some biochemical effects of sub-acute oral administration of L-arginine on monosodium glutamate-fed Wistar albino rats: body weight changes, serum cholesterol, creatinine, and sodium ion concentrations

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Some biochemical effects of sub-acute oral administration of L-arginine on monosodium glutamate-fed Wistar albino rats 1: body weight changes, serum cholesterol, creatinine, and sodium ion concentrations

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The present study aimed to determine whether monosodium glutamate (MSG) could induce biochemical effects at low dose levels, and to examine the possible role of L-arginine (L-ARG) on MSG-induced effects. Thus, MSG, at a dose of 5 mg kg\(^{-1}\) of body weight (BW) was administered to adult male albino rats by oral intubation daily for 28 days. MSG treatment significantly increased BW, serum cholesterol (CHOL), and creatinine concentrations, but decreased serum sodium ion (Na\(^+\)) levels. L-ARG 20 mg kg\(^{-1}\) of BW co-administered with MSG, significantly increased BW, serum CHOL, and creatinine concentrations, but reduced serum Na\(^+\) concentrations. Data show that these effects induced by MSG at a dose of 5 mg kg\(^{-1}\) were enhanced by L-ARG. Thus, L-ARG at 20 mg kg\(^{-1}\) may exacerbate MSG-induced adverse effects in rats.

Keywords: monosodium glutamate; L-arginine; cholesterol; creatinine; body weight; sodium ion; nitric oxide

Introduction

Nitric oxide (NO) is a free radical that regulates many physiological processes including gene expression (Aram et al. 2008). Yang (2005) observed that the synthesis of NO is involved in mediation of most of the beneficial effects of L-arginine (L-ARG), and that L-ARG plays a critical role in NO synthesis. These observations and the rising hope of potential therapeutic use of NO in diverse fields of medicine led to a recent increase in the dietary and therapeutic use of L-ARG.

Monosodium glutamate (MSG), the sodium salt of glutamate (GLU), is a food additive generally used to improve flavor in foods. Flavorings are vital in savory food manufacturing and could play an important nutritional role by providing needed appeal (Loliger 2000). This may explain the continued use of MSG as a flavor enhancer amidst the raging controversy on the safety of its use in humans. Indeed, evidence of MSG-induced toxicity was reported (Paull and Lechan 1974;
Takasaki 1978; Praputpittaya and Wililak 2003) but studies of the possible interactive effects of amino acids, especially those that are consumed in food or used as medicine, on MSG-induced toxicity are lacking.

L-ARG together with MSG may be present in human diet. Since L-ARG may exert therapeutic effects through its major metabolite, NO, it is necessary to study the possible effects of simultaneous use of ARG and MSG so as to establish whether ARG might aggravate, reduce or fail to alter the potential adverse effects of MSG. Therefore, the present study was aimed at examining the potential interactive effects of ARG, a widely used amino acid, on MSG-induced effects in rats.

Materials and methods

Chemicals

Monosodium glutamate (Ajinomoto brand) was purchased from a regular foodstuff market atNsukka. L-ARG was obtained from Sigma Chemical Co., St. Louis, MO. USA. Other chemicals were of certified analytical grade.

Animals and treatments

The animals used in this work were adult male albino rats obtained from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. Twelve adult male albino rats with mean body weight (BW) of 93 ± 0.5 g were kept for 1 week to acclimatize. Then, the animals were randomly assigned to three groups of four rats each. Group II rats were fed MSG (5 mg kg\(^{-1}\) BW) alone, whereas group III rats were fed ARG (20 mg kg\(^{-1}\)) with MSG (5 mg kg\(^{-1}\)). Group I rats were given distilled water (1 mL kg\(^{-1}\)). Treatment was by daily oral intubations for 28 days. The rats were housed in well-cleaned stainless steel cages at room temperature (28 ± 2°C) and exposed to a 12 h light/dark cycle under humid tropical conditions. Animals were provided with rat feed and drinking (tap) water ad libitum for the duration of the experiment.

Blood collection and preparation

The procedures employed for the blood collection were described previously (Egbuonu et al. 2009). Briefly, blood samples of the rats sacrificed following anesthesia 24 h after the 28 days treatment were collected individually with sterile capillary tubes into properly labeled polystyrene centrifuge tubes by ocular puncture technique. The blood samples thus collected were allowed to clot. Then, the serum was removed by centrifugation, collected individually and stored in a deep freezer for determination of the serum cholesterol (CHOL), creatinine, and sodium ion (Na\(^+\)) concentrations. The weight of rats was measured on first and last days.

Assay of CHOL concentration

The assay of serum CHOL concentration was by a slight modification of the colorimetric method of Zlatkis, Zak, and Boyler (1953). To a 0.1 mL of serum sample 5 mL of ethanol was added. The content was shaken and centrifuged for
5 min and the resultant supernatant was used. Then, 2 mL of chromogen was added to all tubes. The tubes were allowed to stand for 40 min after which absorbance was read at 550 nm.

**Assay of serum creatinine concentration**
The serum creatinine concentration was determined by the method of Wilding and Kennedy (1977). To a 0.1 mL of serum sample 0.8 mL of acid tungstate was added. The content was shaken and then centrifuged and the resultant supernatant was used. Then 0.2 mL of picric acid and 0.1 mL of sodium hydroxide (NaOH) (1.4 mol L\(^{-1}\)) was added into all the tubes and read at 500 nm.

**Determination of serum sodium ion (Na\(^+\)) concentration**
The determination of Na\(^+\) concentration was by the flame emission photometric estimation.

**Statistical analysis**
All analyses were performed by one-way analysis of variance (ANOVA) using the SPSS for Windows version 11.0 package. The least significant difference (LSD) test was used for the multiple comparisons of means. Differences were considered significant at \(p < 0.05\) level of significance.

**Results**

**BW change**
Table 1 shows a significant increase in the MSG-fed rats (group II) and in rats that were fed with L-ARG and MSG together (group III). A quantitative rise was noted in ARG + MSG-fed rats compared to MSG-fed alone (Table 1).

**CHOL concentration**
The results presented in Table 2 show a significant increase in serum CHOL concentration in groups II and III.

### Table 1. Effects of MSG and MSG + ARG on BW change.

<table>
<thead>
<tr>
<th>Group</th>
<th>BW change (kg)</th>
<th>Relative value (%)</th>
<th>Difference from control (%)</th>
<th>Difference from MSG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.0850 ± 0.01</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSG alone</td>
<td>0.1020 ± 0.01*</td>
<td>120*</td>
<td>+20*</td>
<td></td>
</tr>
<tr>
<td>MSG plus ARG</td>
<td>0.1095 ± 0.01*</td>
<td>128.82*</td>
<td>+28.82*</td>
<td>+8.82**</td>
</tr>
</tbody>
</table>

Notes: Results are mean ± SEM for four rats in each group.
*Significantly different from control (\(p < 0.05\)).
**Significantly different from MSG-treated rats (\(p < 0.05\)).
Serum creatinine concentration

The serum concentration of creatinine was elevated in the MSG-treated rats (group II). This was further quantitatively potentiated in rats co-treated with L-ARG and MSG (group III). On comparison with the MSG-fed rats, there was 2-fold increase in the serum creatinine concentration in rats fed L-ARG with MSG (Table 3).

Serum sodium ion (Na$^+$) concentration

The serum Na$^+$ concentration, as determined by the flame emission photometric estimation, was found to be significantly decreased in rats administered MSG. Further, simultaneous administration of L-ARG and MSG to rats also reduced serum Na$^+$ concentrations (Table 4).

Discussion

In the present study, the observed increase in the BW in rats that were fed MSG alone is consistent with previous report of Mozes et al. (2004). MSG has the potential to affect the receptors that regulate adiposity in the brain (Dawson et al. 1997). Thus, the increase in BW by the MSG treatment may indicate a potential to enhance weight gain, perhaps by stimulating the receptors in the brain that favor the

Table 2. Effects of MSG and MSG+ARG on CHOL concentration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum CHOL concentration (mg per 100 mL)</th>
<th>Relative value (%)</th>
<th>Difference from control (%)</th>
<th>Difference from MSG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>76.94 ± 0.04</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSG alone</td>
<td>80.69 ± 0.75*</td>
<td>104.87*</td>
<td>+4.87*</td>
<td></td>
</tr>
<tr>
<td>MSG plus ARG</td>
<td>80.83 ± 0.11*</td>
<td>105.05*</td>
<td>+5.05*</td>
<td>+0.18**</td>
</tr>
</tbody>
</table>

Notes: The results are mean ± SEM for four rats in each group.
*Significantly different from control (p < 0.05).
**Significantly different from MSG-treated rats (p < 0.05).

Table 3. Influence of MSG and MSG+ARG on serum creatinine concentration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum creatinine concentration (mg per 100 mL)</th>
<th>Relative value (%)</th>
<th>Difference from control (%)</th>
<th>Difference from MSG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1.92 ± 0.05</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSG alone</td>
<td>3.99 ± 0.05*#</td>
<td>207.81*</td>
<td>+107.81*</td>
<td></td>
</tr>
<tr>
<td>MSG plus ARG</td>
<td>9.18 ± 0.15*</td>
<td>478.12*</td>
<td>+378.12*</td>
<td>+270.31**</td>
</tr>
</tbody>
</table>

Notes: The results are mean ± SEM for four rats in each group.
*Significantly different from control (p < 0.05).
**Significantly different from MSG-treated rats (p < 0.05).
#From Egbuonu et al. (2009).
deposition of fat in adipose tissue. The possible BW enhancing potential of MSG may be harmful since increase in BW is associated with health risks (Roe 1991; Hanley et al. 2005) hence, the use of MSG for possible BW enhancement should not be encouraged. Our result indicates that the potential weight gain risks in rats treated with ARG and MSG are not lower, suggesting that L-ARG may affect the influence of MSG on BW gain.

Elevated serum CHOL concentration has been associated with the development of cardiovascular diseases (CVD), such as atherosclerosis (Nagata, Ishiwaki, and Sugano 1982; Martin et al. 1986; Posner et al. 1991; Kromhout et al. 1995) and non-insulin dependent diabetes mellitus (NIDDM) (Depres et al. 1990). Thus, the observation of an increase in serum CHOL concentration in rats given MSG and a combination of ARG with MSG at the tested dose may be suggestive of possible risks of CVD and NIDDM, possibly due to impaired CHOL metabolism. Simultaneous administration of L-ARG and MSG increased the effect on serum CHOL concentration relative to MSG alone suggesting that the possible hypercholesterolemic potential of MSG and the attendant risks may be enhanced by L-ARG.

In this study, it was observed that the serum creatinine concentration increased in rats that were fed MSG alone and in rats that were fed ARG with MSG, indicating a decreased renal capacity to excrete creatinine as against the suggestion by Panda (1989). The elevation of the serum creatinine concentration by MSG and ARG + MSG appears to suggest the possible up-regulation of protein catabolism and concomitant rise in the synthesis of creatinine that needs to be excreted with urine (formed via the reactions of the urea cycle). The deamination of MSG may produce the toxic ammonium ion (NH\textsuperscript{+}\textsubscript{4}) that needs to be detoxified and excreted via the reactions of the urea cycle. Furthermore, since urea synthesis converts toxic NH\textsuperscript{+}\textsubscript{4} to non-toxic urea, the possibly impaired renal function to excrete creatinine as shown by the increased serum concentration of creatinine observed in the MSG-fed rats may lead to inefficient detoxification of the ammonium ion that may be accumulated, consequently promoting ammonium ion intoxication. Thus, the use of MSG alone or together with ARG at the tested doses may be of no benefit in the cases of renal disorders. Our observations were anticipated since L-ARG was shown to participate in creatinine biosynthesis via the synthesis of creatine phosphate (Rodwell 2003). The possible increase in creatine phosphate due to L-ARG may, as a

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum sodium ion concentration (mmol L\textsuperscript{-1})</th>
<th>Relative value (%)</th>
<th>Difference from control (%)</th>
<th>Difference from MSG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1081.00 ± 2.67</td>
<td>100</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MSG alone</td>
<td>958.00 ± 1.41*#</td>
<td>88.62*</td>
<td>-11.38*</td>
<td>-11.40*</td>
</tr>
<tr>
<td>MSG plus ARG</td>
<td>957.75 ± 1.17*</td>
<td>88.60*</td>
<td>-11.40*</td>
<td>-0.02**</td>
</tr>
</tbody>
</table>

Notes: The results are mean ± SEM for four rats in each group.
*Significantly different from control (p < 0.05).
**Significantly different from MSG-treated rats (p < 0.05).
#From Egbuguonu et al. (2009).
consequence, increase synthesis of creatinine leading to the observed elevation in the serum concentration.

The serum Na\(^+\) concentration was observed to decrease in the MSG and ARG + MSG-treated rats. This may indicate decreased Na intake (probably due to overload of Na\(^+\) from the MSG-treatment), severe dehydration, or diabetes mellitus (Bush 1991). The decrease in the serum Na\(^+\) concentration observed in the MSG-fed rats was not as expected since MSG has Na moiety in its chemical composition which may be easily ionized to release Na constituent with a possible resultant increase in the serum Na\(^+\) concentration. However, severe dehydration may be occurring in the present study with increased serum creatinine concentration which may enhance the renal capacity to synthesize and excrete more urea leading to elevated water loss and consequent dehydration. In addition, diabetes mellitus (the non-insulin dependent type) may result from the elevated serum CHOL concentration as suggested by Depres et al. (1990). In summary, the results of the present study demonstrated that MSG induced alterations in rats with a dose of 5 mg kg\(^{-1}\) L-ARG did not significantly enhance these MSG-induced effects.

References


Takasaki, Y 1978. Studies on brain lesion by administration of monosodium L-glutamate to mice 1. *Brain lesion in infant mice caused by administration of monosodium L-glutamate*. *Toxicology* 9: 293–305.

