Effect of Inhibition of Nitric Oxide Synthase on Food Intake of Chicks Fed Diets Differing in Arginine Content

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In order to investigate whether food intake suppression elicited by inhibition of nitric oxide (NO) synthase (NOS) is affected by dietary levels of L-arginine (Arg), chicks (one week old) were prefed either an Arg-deficient (AD), Arg-moderate deficient (AM), Arg-standard (AS) or Arg-excess (AE) diet for 3 days. Birds were then intraperitoneally administered NO-nitro-L-arginine methyl ester HCl (L-NAME), a NOS inhibitor, or saline and given the same diet used during the prefeeding stage. Food intake was reduced by L-NAME over 2 h after feeding, compared to the saline control group, and the relative suppressive effect of L-NAME increased as dietary Arg levels decreased.

Key words: nitric oxide, NO-nitro-L-arginine methyl ester HCl, feeding behavior, dietary arginine contents

Introduction

It has been long known that dietary L-arginine (Arg) is required for optimum nitrogen retention and normal growth for mammalian species (Milner et al., 1974; Morris and Rogers, 1978; Deshmukh and Shope, 1983) and chickens (Sugahara et al., 1984; Kino and Okumura, 1986) and alleviates the adverse effect induced by dietary excesses of single amino acids in the chicken (Yanaka and Okumura, 1981). Arg is antagonistic to lysine and thus, dietary increase of one requires an increase in the other (Jones, 1964). In addition to its nutritional roles, Arg stimulates secretion of growth hormone (Saito and Saito, 1982), insulin (Mulloy et al., 1982) and glucagon (Rocha et al., 1972) in mammalian species. In rats, Arg also has a number of important beneficial effects for injured animals (Barbul et al., 1983) and acts as a cytoprotector against gastric injury produced by hydrochloric acid (Takeuchi et al., 1993).

Recently, much attention has been directed toward Arg because it is a precursor of nitric oxide (NO), a compound with diverse biological activities (Moncada, 1992; Knowles and Moncada, 1994). NO was originally identified as a factor which produced endothelium relaxation via stimulation of guanylate cyclase resulting in synthesis of cGMP (Ignarro et al., 1987; Moncada, 1992). NO has also been identified as a neurotransmitter mediating smooth muscle relaxation in response to stimulation of...
It has been reported that inhibition of NO synthase (NOS) depresses food intake in chickens (Choi et al., 1994) and rodents (Morley and Flood, 1991; Morley and Flood, 1992; Calignano et al., 1993; Squadrito et al., 1993; Morley and Flood, 1994; Squadrito et al., 1994). However, the mechanism by which NO regulates food intake is unclear. In the central nervous system of rats, food deprivation increases NOS activity which is reduced by Arg analogues (Squadrito et al., 1994). The anorexia caused by NOS inhibition in the rat appears mediated by 5-HT receptors, because the response is reversed by 5-HT receptor antagonism (Squadrito et al., 1993, Squadrito et al., 1994). We recently found that NO may interact with the $\alpha_{2}$ adrenergic system to modulate food intake in chickens (Choi et al., 1995).

The physiological role of NO and NOS activity appear to exist in the avian gut (Martinez et al., 1993; Li et al., 1994) and brain (Bruning, 1993; Panzica et al., 1994). Food intake may be partly modulated by the NO system outside the central nervous system because intraperitoneal (i.p.) injection of a NOS inhibitor, N$^6$-nitro-L-arginine methyl ester HCl (L-NAME), depressed food intake of the chicken (Choi et al., 1994). The present study, therefore, was done to investigate whether the reduced food intake induced by NOS inhibition is affected by dietary concentrations of Arg. Amino acid concentrations in the plasma were investigated in chicks fed diets with various levels of Arg.

Materials and Methods

Experiment 1. Determination of amino acids in plasma of chicks given different levels of dietary Arg.

Day-old single comb White Leghorn male chicks, purchased from a local supplier (Hattori Hatchery Co. Ltd., Nagoya, Japan), were housed in a room with continuous light and constant-temperature (28±1°C). They were given free access to commercial diet (CP 21.5%; ME 11.84 MJ/kg, Marubeni Shiryo Co. Ltd., Tokyo, Japan) and water for 1 week. Chicks were then distributed into 4 groups of 7 birds each with similar mean body weights and given Arg-deficient (AD), Arg-moderate deficient (AM), Arg-standard (AS) or Arg-excess (AE) diet (Table 1) for 3 days. After birds were slightly anesthetized by diethyl ether, blood from a jugular vein was collected into chilled heparinized tubes, and centrifuged at 3000 × g for 10 min. The plasma was stored at −20°C until analysis. Determination of amino acids was performed using a high speed amino acid analyzer (Hitachi L-8500, Hitachi, Tokyo, Japan) after deproteinization with an equal volume of 3% sulfosalicylic acid.

Experiment 2. Effect of dietary levels of Arg on food intake of chicks treated with L-NAME.

Birds (seven days of age) reared under the same regime as Experiment 1 were allotted to 4 groups of 14 birds each and fed on AD, AM, AS or AE diet for 3 days. Thereafter, all chicks were fasted for 3 h with free access to water. Then, half of each group was injected i.p. with L-NAME (100 mg/kg body weight) (Choi et al., 1994) while
Table 1. Composition (g/kg) of Arg-deficient (AD), Arg-moderate deficient (AM), Arg-standard (AS), or Arg-excess(AE) diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>AD</th>
<th>AM</th>
<th>AS</th>
<th>AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>452.0</td>
<td>450.3</td>
<td>448.4</td>
<td>398.4</td>
</tr>
<tr>
<td>L-arginine. HCl</td>
<td>0.0</td>
<td>6.0</td>
<td>12.1</td>
<td>62.1</td>
</tr>
<tr>
<td>L-glutamic acid</td>
<td>108.5</td>
<td>104.2</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Amino acid mixture</td>
<td>89.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>200.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>30.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>56.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antacid mixture</td>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1.5</td>
<td></td>
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</tr>
</tbody>
</table>

* Yokota et al. (1989).
† Fujipro-R, Fuji Oil Co. Ltd., Osaka, Japan.
‡ Yang et al. (1989).
§ Al(OH)₃ : NaHCO₃ = 1 (wt) : 1 (wt).

the remaining half were injected with saline. The chicks were then given access to their previous diets for 2 h. L-NAME was purchased from Wako Pure Chemicals, Osaka, Japan.

Statistical Analysis

Data were subjected to one- or two-way ANOVA by General Linear Model procedure using a commercially available package (SAS, 1985), and comparison of differences between means were made by Duncan’s new multiple range test. The results are indicated as means ± SEM.

Results and Discussion

Fig. 1 shows plasma Arg concentrations (μ mol/ml) of chicks given AD, AM, AS or AE diets for 3 days. Plasma Arg concentrations were decreased as dietary Arg amounts decreased. Regression analysis showed that Arg concentration in plasma was $0.06666 + 0.01738 \times$ (R² = 0.85977, P < 0.0001), where X is Arg concentrations in the diet.

Fig. 2 shows the 2 h food intake of AD, AM, AS and AE diets in chicks administered with L-NAME (100 mg/kg body weight) or saline. The 2 h food intake was significantly (P < 0.001) influenced by dietary Arg contents. The birds given the AD diet showed the lowest food intake and those given the AS showed the highest food intake. The administration of L-NAME significantly (P < 0.01) depressed the 2 h food intake compared with the saline control. No significant interaction between dietary Arg contents and L-NAME was observed. Since the mean body weight of the four dietary groups were different (64.1 ± 1.5 g in the AD group, 77.9 ± 2.1 g in the AM group, 92.2 ± 1.5 g in the AS group and 85.5 ± 1.1 g in the AE group), relative food intake of each diet in L-NAME- to saline-treated groups as 100% was analyzed and also shown in
Fig. 1. Plasma Arg concentrations (μmol/ml) of chicks given Arg-deficient (0 g Arg/kg diet), Arg-moderate deficient (6 g Arg/kg diet), Arg-standard (12.1 g Arg/kg diet) or Arg-excess (62.1 g Arg/kg diet) diet for 3 days. Values represent the means±SEM of 7 birds.

Fig. 2. The 2 h food intake of Arg-deficient (0 g Arg/kg diet), Arg-moderate deficient (6 g Arg/kg diet), Arg-standard (12.1 g Arg/kg diet) or Arg-excess (62.1 g Arg/kg diet) diet in chicks administered with N⁵-nitro-L-arginine methyl ester HCl (L-NAME) (100 mg/kg body weight) or saline. Values represent the means±SEM of 7 birds.

Table 2. The effect of L-NAME on the inhibition of food intake was dose dependent and caused a greater percent suppression as the dietary Arg content decreased.

The present study showed that the decreased food intake caused by NOS inhibition can be modulated by dietary Arg levels. The result that peripheral administration of L-NAME decreased food intake of normal diet (AS diet) was in accordance with previous reports in meat-type chickens (CHOI et al., 1994) and rodents (MORLEY and
Table 2. The suppressive effect of \(N^G\)-nitro-L-arginine methyl ester HCl on food intake of Arg-deficient (AD), Arg-moderate deficient (AM), Arg-standard (AS), or Arg-excess (AE) diet

<table>
<thead>
<tr>
<th>Diets</th>
<th>AD</th>
<th>AM</th>
<th>AS</th>
<th>AE</th>
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<tr>
<td></td>
<td>99.1±4.8(^A)</td>
<td>43.5±17.4(^B)</td>
<td>33.0±13.0(^B)</td>
<td>6.5±8.6(^B)</td>
</tr>
</tbody>
</table>

Relative food intake of the \(N^G\)-nitro-L-arginine methyl ester HCl-treated group in each diet was expressed as a percentage of saline-treated group as 100\%. Values represent the means±SEM of 7 birds. \(^A\)Significantly different at \(P<0.05\).

FLOOD, 1991; MORLEY and FLOOD, 1992; SQUADRITO et al., 1994). On the other hand, the decrease in food intake caused by NOS inhibition was reversed by acute administration of large amounts of Arg (MORLEY and FLOOD, 1991; MORLEY and FLOOD, 1992; SQUADRITO et al., 1994). The present study clearly indicates that the anorexigenic effect of L-NAME was attenuated by enhancing plasma Arg concentration through dietary Arg supplementation. To confirm, however, the involvement of NO itself, the direct measurement of NO production or the use of \(N^G\)-nitro-D-arginine methyl ester HCl as a negative control of L-NAME remains to be studied.

Acknowledgements

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References


アルギニン含量が異なる飼料を摂取したヒナの飼料摂取量
に及ぼす一酸化窒素合成酵素の阻害効果

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一酸化窒素合成酵素の阻害により抑制される飼料摂取量が飼料アルギニン含量により影響を受けるかどうかを
調べるために、1週齢のヒナに飼料アルギニン水準が
欠乏，弱欠乏，標準ならびに過剰の4飼料を3日間与え
た。その後，一酸化窒素合成酵素の阻害剤であるN\textsuperscript{±}-ニ
トロ-L-アルギニンメチルエステル塩酸塩（L-NAME）を
腹腔投与し，同飼料を与えた。どの飼料においても2
時間の飼料摂取量はL-NAMEにより減少したが，L-NAMEの相対的な効果は飼料アルギニン含量の低下に
伴い増加した。

キーワード：一酸化窒素，N\textsuperscript{±}-ニトロ-L-アルギニンメ
チルエステル塩酸塩，摂食行動，飼料アル
ギニン含量