Glutamate ionotrophic and metabotropic receptors affect feed intake in broiler cockerels

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Abstract: In this study the role of the glutamatergic system on feed intake in 24-hour-feed-deprived broiler cockerels was investigated. ICV injection of 0, 0.675, 1.25, and 2.5 nmol of glutamate reduced feed intake dose-dependently, and increased the latency time to start feeding. Pretreatment with 2.5 nmol HQCA, an ionotropic glutamate antagonist resulted in both an increase in feed intake and a decrease in latency of birds to start feeding. Pretreatment with 2 nmol of MSPG, a metabotropic glutamate receptor antagonist, severely reduced feed intake and increased the latency to start feeding. These findings suggest, for the first time, that glutamate, acting as a neurotransmitter, is involved in feed intake regulation in broiler cockerels. This effect is probably mediated by both ionotropic and metabotropic receptors. It appears that both postsynaptic and presynaptic glutamate receptors are involved.

Key words: feed intake, broiler, glutamate, HQCA, MSPG.

Introduction

L-glutamic acid is the major excitatory neurotransmitter in the central nervous system (CNS) (Dingledine and McBain, 1994; Ozawa et al., 1998; Gaviraghi, 2000; Danbolt, 2001), probably involved in normal brain activities as well as CNS developmental processes including cell migration, differentiation and death (Danbolt, 2001). Many neurons, and even glial cells, bear glutamate receptors (Glur) on their plasma membrane (Danbolt, 2001). Glutamate is the most abundant amino acid in the mammalian and avian brain, a part of which is located in the extracellular fluid (ECF). ECF concentration of glutamate determines the degree of receptor activation in the brain (Danbolt, 2001). Over-activation of these receptors can be detrimental, and there is not an extracellular enzyme to metabolize glutamate, therefore, its uptake by the presynaptic membrane is the most efficient mechanism to inactivate this neurotransmitter (Edmonds et al., 1995; Danbolt, 2001).

There are two major families of glutamate receptors, the ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors. The former is subdivided into N-methyl-D-aspartic acid (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors (Reid and Bliss, 2000), and the latter is subdivided into group I, group II and group III receptors. iGluRs are ion channels allowing Na⁺ or Na⁺ and Ca²⁺ to enter the cell and activate biochemical processes, while mGluRs are coupled with G proteins, activating phospholipase C (group I) or inhibiting adenyl cyclase (groups II and III) (Danbolt, 2001).

Systemic, ICV or local injections of glutamate or its agonists into the lateral hypothalamus elicits a dose-dependent stimulation of feed intake in mammals (Stricker-Krongrad et al., 1992; Reddy et al., 1986; Stanley et al., 1993; Stanley et al., 1996; Stanley et al., 1997; Burns et al., 1997; Stratford et al., 1998; Mistlberger et al., 1999). Meanwhile, systemic, ICV or local injections of some Glur antagonists into the median raphe nucleus...
Table 1: Cumulative feed intake (g) of broiler cockerels following ICV injection of 3 different doses of glutamate (p<0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF²</td>
<td>21.38</td>
<td>31.48</td>
<td>38.29</td>
<td>43.08</td>
<td>46.64</td>
<td>52</td>
<td>55.5</td>
<td>60.2</td>
</tr>
<tr>
<td>Glutamate(2.5 μmol)</td>
<td>0 b</td>
<td>0 b</td>
<td>0 b</td>
<td>6.6</td>
<td>16.93</td>
<td>26</td>
<td>32.99</td>
<td>39.91</td>
</tr>
<tr>
<td>Glutamate(1.25 μmol)</td>
<td>0 b</td>
<td>4.3</td>
<td>13.56</td>
<td>28.46</td>
<td>34.29</td>
<td>42.25</td>
<td>51.44</td>
<td>59.66</td>
</tr>
<tr>
<td>Glutamate(0.675 μmol)</td>
<td>22.3 a</td>
<td>33.79 a</td>
<td>38.99 a</td>
<td>44.14 a</td>
<td>49.16 a</td>
<td>54.43 a</td>
<td>58.43 a</td>
<td>61.86 a</td>
</tr>
</tbody>
</table>

*: each column with different superscripts are significantly different (p<0.05).
1: aCSF: artificial Cerebrospinal Fluid.

(Wirthafer and Trifunovic, 1988), accumbence nucleus (Maldonado-Irizarry et al., 1995; Stratford et al., 1998) and ventral striatal and ventral pallidal areas (Da Silva et al., 2003) increases feed intake. It has also been shown that stimulation of eating by intrahypothalamically injected Neuropeptide Y is dependent upon NMDA receptor activation (Lee and Stanley, 2005). Evidence has shown that NMDA receptors may mediate some aspects of eating and satiety (Duva et al., 2005). These findings suggest that several central and peripheral glutamatergic circuits are involved in feed intake regulation. Although glutamate receptors are widely distributed in the avian brain, there is little information on the impact of glutamate and GluRs on feeding behavior in the domestic fowl (Zenow Lucia, 2000, Baghbanzadeh and Babapour, 2001). Da Silva (2003) showed that glutamatergic inputs to cells containing NMDA and/or AMPA receptors located in the lateral hypothalamus (LHY) could modify both the beginning of a feeding bout and its duration. The present study was conducted to investigate the acute effects of ICV injection of glutamate, its vesicular uptake inhibitor and its ionotropic and metabotropic antagonists, on feed intake in broiler cockerels.

Methods

Animals

Day-old Ross 208 broiler cockerels were housed in heated batteries until 2 weeks-of-age after which they were transferred to individual cages and provided with continuous light. Birds were given free access to a broiler starter ration containing 21% protein and 2900 kcal/kg metabolizable energy in individual feeders. The room temperature was maintained at 22 ± 2 °C. This experiment was carried out at Faculty of Veterinary Medicine, University of Tehran.

Surgery

At 3 weeks-of-age, those birds with the approximate weight of 750 g were chosen and anesthetized with 25 mg/kg iv injection of sodium pentobarbitone (Sagattal, Rhone Merieux, Lyon, France). A 16-mm-long, 23-gauge, thin-walled stainless-steel guide cannula was implanted stereotaxically into the right lateral ventricle, according to the technique described previously by Denbow et al. (Denbow et al., 1981). The stereotaxic coordinates were AP=6.7, L=0.7, H=3.5–4 mm below the dura mater with the head oriented as described previously (Van Tienhoven and Juhasz, 1962). Three anchoring screws were fixed in the calvaria surrounding the cannula and acrylic cement (Pars Acryl) was used to secure the cannula. An orthodontic # 014 wire (American Orthodontics), trimmed to the exact length of the guide cannula, was inserted into the guide cannula while the chicks were not being used for experiments. Penicillin (Hayan Pharmaceuticals, Iran) was applied to the incision to prevent infection. The birds were allowed at least 5 days recovery following surgery.

Experiments

Three experiments were conducted to determine the effects of the glutamatergic system on feed intake. In each, eight birds were used in a replicated 4x4 Latin square design in which birds and days were the blocking factors. All solutions were injected at 2-day intervals so that each bird received each solution.
Table 2 - Cumulative feed intake (g) of broiler cockerels following ICV injection of HQCA (p<0.05).

<table>
<thead>
<tr>
<th>Treatment / Time</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
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<tbody>
<tr>
<td>aCSF+aCSF</td>
<td>16.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>aCSF+Glu&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HQCA&lt;sup&gt;3&lt;/sup&gt;+aCSF</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.48&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.75&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HQCA+Glu</td>
<td>12.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.55&lt;sup&gt;d&lt;/sup&gt;</td>
<td>47.09&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

<sup>a</sup>: each column with different superscripts are significantly different (p<0.05).
1- aCSF: artificial Cerebrospinal Fluid.
2-Glu: Glutamate.
3-HQCA: 3-hydroxy-2-quinozaline carboxylic acid.

Table 3 - Cumulative feed intake (g) of broiler cockerels following ICV injection of MSPG (p<0.05).

<table>
<thead>
<tr>
<th>Treatment / Time</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
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</thead>
<tbody>
<tr>
<td>aCSF&lt;sup&gt;1&lt;/sup&gt;+aCSF</td>
<td>18.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>aCSF+Glu&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MSPG&lt;sup&gt;3&lt;/sup&gt;+aCSF</td>
<td>20.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>69.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MSPG+Glu</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>: each column with different superscripts are significantly different (p<0.05).
1- aCSF: artificial Cerebrospinal Fluid.
2-Glu: Glutamate.
3-MSPG: (RS)-α-Methyl-4-sulphonophenylglycine (MSPG).

during the 7-day experimental period. Injections were made with a 29-gauge, thin-walled stainless-steel injection cannula which extended 1.0 mm beyond the guide cannula. The injection cannula was connected to a 10-μl Hamilton syringe connected to a 50-cm length of polyethylene tube. All solutions were prepared in artificial cerebrospinal fluid (aCSF) (7.25g NaCl, 0.22g KCl, 2.18g NaHCO<sub>3</sub>, 0.29g CaCl<sub>2</sub>/2H<sub>2</sub>O, 0.25g MgSO<sub>4</sub>/6H<sub>2</sub>O, 0.17g KH<sub>2</sub>PO<sub>4</sub>, 1.8g D-Glucose, all dissolved in 1L of sterile deionised water (Anderson and Heisey, 1972) that served as the control. Beginning several days before starting the experiments, the birds were restrained daily to acclimatise to the procedure. Birds were deprived of feed for 24 hours prior to injection. Solutions were injected over a 30-s period and the injection cannula remained in place for an additional 30 s before removal. All injections were made early in the morning, and birds were returned to their cage after injection. Proper location of the guide cannula was verified by intracerebroventricular injection of methylene blue and anatomically slicing the frozen brain tissue at the end of the experiments.

In Experiment 1, 0.675, 1.25, and 2.5 nmol of glutamate (Sigma-Aldrich Chemie GmbH, Germany) was injected in a volume of 10 μl into the right lateral ventricle. Fresh food was supplied at the time of injection, and food intake (g) was recorded at 15, 30, 60, 90, 120, 150 and 180 min postinjection (PI).

In Experiment 2, birds received 2.5 nmol of 3-hydroxy-2-quinozalinecarboxylic acid (HQCA) (Tocris Bioscience, Northpoint, UK), an ionotropic glutamate receptor antagonist, before injecting 2.5 nmol of glutamate. Each bird was given two 5-μl injections 15 min apart as described in Table 3. Food intake (g) was measured as in Experiment 1. The dose of HQCA was determined from preliminary trials.

Experiment 3 was conducted similar to experiment 3, except that the chicks were injected with 2nmol of (RS)-α-methyl-4-sulphonophenyl glycine (MSPG) (Tocris Bioscience, Northpoint, UK), a metabotropic glutamate receptor antagonist.

**Statistical analysis**
Cumulative feed intake (g) was subjected to two-way analysis of variance at each time period, and a Duncan’s Multiple Range Test and Dunnett test used for comparisons between means.
Results and discussion

The ICV injection of glutamate in 24-hour fasted broiler cockerels significantly decreased feed intake (p<0.05). This decrease was dose-dependent (Table 1). This effect was still evident 180 minutes after injection.

Concentrations of glutamate greater than 2.5 μmol resulted in convulsions, and the birds were very excited and vocal, so this dose was chosen for subsequent experiments. Although studies question the role of glutamatergic mechanisms in birds, recent results indicate that a decrease in glucose levels in hypothalamic slices caused a decrease in glutamate concentration in synaptic terminals, which is consistent with our results (Madl and Royer, 1999). Furthermore, our results agree with Zeni et al. (2000) who showed a decrease in feed intake immediately after ICV injection of glutamate in pigeons.

The ICV injection of HClCa attenuated the impact of glutamate on feed intake. Injection of HClCa alone increased feed intake to an intermediate level. This suggests a critical role of inotropic receptors in the central control mechanisms of feed intake in domestic fowl.

The ICV injection of (Rs)-α-Methyl-4-sulphonophenylglycine (MSPG), a metabotropic glutamate receptor antagonist, severely inhibited feed intake even after 240 minutes post injection. The injection of MSPG alone significantly (p<0.05) increased feed intake.

These findings not only support a role for ionotropic receptors in feed intake regulation, which is in agreement with Stanley et al. (1996, 1997) and Zuni et al. (2001), but also imply a dual role for glutamate metabotropic receptors (mGluR). The present study is the first one investigating the role played by mGluRs in feed intake regulation. Considering the apparently opposite role of group II &III mGluRs in comparison with group I mGluRs, and the presynaptic position of the latter group, further investigation is necessary.

References

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تأثیر گیبرنده‌های یونیوریوپیک و متابوتریوپیک گلوتامات بر انجام غذا در جوجه خروس‌های گوشته

علي باغانی واده، و همای شاه‌پور

یک عصری گروه گردو گلیم، دانشکده پزشکی دانشگاه تهران، تهران، ایران.

در این مطالعه تأثیر سیستم گلوتاماترژیکی بر انجام غذا در جوجه خروس‌های گیبرنده‌های 22 ساعت از خوردن غذا محور و مورد اندازه‌گیری داد. تزریق در داخل

بطن مغز سفر - 777۷٪ و 75٪ تانولوگلوتامات از آن غذا را به صورت وابسته به مقدار، کاهش و تأخیر در آغاز خوردن غذا را افزایش داد. پیش تزریق در 25٪ تانولوگلوتامات از ۲ - هیدروکسی این - ۲ - کیتون علی این کروکسیلک اسید، آناناکوتین گیبرنده‌های یونیوریوپیک گلوتامات به طور معمول دارای فعالیت افزایشی غذا و کاهش دارای آغاز غذا دیر است. پیش تزریق در 2 - نانومول آف - میل - ۲ - سولفونوفیل کلیسین، آناناکوتین گیبرنده متابوتریوپیک گلوتامات به شدت افزایش گرفت و زمان آغاز غذا دیر در افزایش داد. پیش فهای همه این اولین بر توانان داده‌های که گلوتامات به عنوان یک مادهی عصبی در تنظیم مارکی انجام غذا در جوجه خروس گوشته دخالت دارد. این اثر احتمالا توسط هر دو گیبرنده یونیوریوپیک و متابوتریوپیک تبدیل می‌گردد.

په مرا و درد در این قطعات هر دو تنش غیره‌های یک گیبرنده گلوتامات.

وژه‌های کلی دی انجام غذا جوجه خروس گوشته گلوتامات.