

# Glutamate ionotropic 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 2nmol 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<sup>+</sup> or Na<sup>+</sup> and Ca<sup>2+</sup> to enter the cell and activate biochemical processes, while mGluRs are coupled with G proteins, activating phospholipase C (group I) or inhibiting adenylyl 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; Mistleberger *et al.*, 1999). Meanwhile, systemic, ICV or local injections of some GluR antagonists into the median raphe nucleus

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**Table 1- Cumulative feed intake (g) of broiler cockerels following ICV injection of 3 different doses of glutamate (p<0.05).**

Treatment Time (min)	15	30	45	60	90	120	150	180
aCSF <sup>1</sup>	21.38 <sup>a*</sup>	31.48 <sup>a</sup>	38.29 <sup>a</sup>	43.08 <sup>a</sup>	46.64 <sup>a</sup>	52 <sup>a</sup>	55.5 <sup>a</sup>	60.2 <sup>a</sup>
Glutamate(2.5 μmol)	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	6.6 <sup>b</sup>	16.93 <sup>b</sup>	26 <sup>b</sup>	32.99 <sup>b</sup>	39.91 <sup>b</sup>
Glutamate(1.25 μmol)	0 <sup>b</sup>	4.3 <sup>c</sup>	13.56 <sup>c</sup>	28.46 <sup>c</sup>	34.29 <sup>c</sup>	42.25 <sup>c</sup>	51.44 <sup>a</sup>	59.66 <sup>a</sup>
Glutamate(0.675 μmol)	22.3 <sup>a</sup>	33.79 <sup>a</sup>	38.99 <sup>a</sup>	44.14 <sup>a</sup>	49.16 <sup>a</sup>	54.43 <sup>a</sup>	58.43 <sup>a</sup>	61.86 <sup>a</sup>

\* : each column with different superscripts are significantly different (p<0.05).

1- aCSF : artificial Cerebrospinal Fluid.

(Wirtshafter 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 (Zeni 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  $22 \pm 2$  °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 4×4 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).**

Treatment / Time	15	30	45	60	90	120	150	180
aCSF <sup>1</sup> +aCSF	16.02 <sup>a*</sup>	20.1 <sup>a</sup>	24.46 <sup>a</sup>	28.8 <sup>a</sup>	36.69 <sup>a</sup>	41.75 <sup>a</sup>	47.03 <sup>a</sup>	50.59 <sup>a</sup>
aCSF+Glu <sup>2</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	10.46 <sup>b</sup>	15.3 <sup>b</sup>	19.66 <sup>b</sup>	31.09 <sup>b</sup>
HQCA <sup>3</sup> +aCSF	0 <sup>b</sup>	3.53 <sup>c</sup>	8.98 <sup>c</sup>	15.48 <sup>c</sup>	24.39 <sup>c</sup>	24.98 <sup>c</sup>	34.44 <sup>c</sup>	42.75 <sup>c</sup>
HQCA+Glu	12.64 <sup>a</sup>	19.3 <sup>a</sup>	23.11 <sup>a</sup>	27.03 <sup>a</sup>	32.7 <sup>a</sup>	38.09 <sup>a</sup>	41.55 <sup>d</sup>	47.09 <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- quinoxaline carboxylic acid.

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

Treatment / Time	15	30	45	60	90	120	150	180
aCSF <sup>1</sup> +aCSF	18.06 <sup>a*</sup>	25.68 <sup>a</sup>	30.8 <sup>a</sup>	34.9 <sup>a</sup>	40.2 <sup>a</sup>	44.1 <sup>a</sup>	46.9 <sup>a</sup>	50.2 <sup>a</sup>
aCSF+Glu <sup>2</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0.54 <sup>b</sup>	3.44 <sup>b</sup>	22.2 <sup>b</sup>	33.7 <sup>b</sup>	36.4 <sup>b</sup>	41.6 <sup>b</sup>
MSPG <sup>3</sup> +aCSF	20.95 <sup>a</sup>	28.95 <sup>a</sup>	35.3 <sup>a</sup>	40.9 <sup>c</sup>	50 <sup>c</sup>	56.2 <sup>c</sup>	62 <sup>c</sup>	69.4 <sup>c</sup>
MSPG+Glu	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	1.3 <sup>d</sup>	2.4 <sup>d</sup>

\* : each column with different superscripts are significantly different (p<0.05).

1- aCSF: artificial Cerebrospinal Fluid.

2- Glu: Glutamate.

3- MSPG: (RS)- $\alpha$ -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- $\mu$ l 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 acclimate 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, 0.675, 1.25, and 2.5 nmol of glutamate (Sigma-Aldrich Chemie GmbH,

Germany) was injected in a volume of 10  $\mu$ l 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-quinoxalinecarboxylic acid (HQCA) (Tocris Bioscience, Northpoint, UK), an ionotropic glutamate receptor antagonist, before injecting 2.5 nmol of glutamate. Each bird was given two 5-  $\mu$ l 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)- $\alpha$ -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 Dunnet 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  $\mu\text{mol}$  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 glucostatic mechanisms in birds, recent results indicate that a decrease in glucose levels in hippocampal 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 HQCA attenuated the impact of glutamate on feed intake. Injection of HQCA 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)- $\alpha$ -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 Zeni *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

1. Anderson, D.K., Heisey, R. (1972) Clearance of molecules from cerebrospinal fluid in chickens. *A J of Physiol.* 222: 645-648.
2. Baghbanzadeh, A., Babapour, V. (2001) CNS glutamatergic control of food intake in domestic fowl. *Appetite.* 37: 267.
3. Bellochio, E.E., Reimer, R.J., Fremeau, R.T.Jr, Edwards, R.H. (2000) Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. *Science.* 289:957-960.
4. Burns, G.A., Ritter, R.C. (1997) The non-competitive NMDA antagonist MK-801 increases food intake in rats. *Pharmacol. Biochem. and Behav.* 56:1145-9.
5. Danbolt, N.C. (2001) Glutamate uptake. *Prog. Neurobiol.* 65: 1-105.
6. Da Silva, A.A., Marino-Neto, J., Paschoalini, M.A. (2003) Feeding by microinjections of NMDA and AMPA-kainate receptor antagonists into ventral striatal and ventral pallidal areas of the pigeon. *Brain Res.* 966: 76-83.
7. Da Silva, A.A., Campanella, L.C.A., Caldas Ramos, M., Serralvo Faria, M., Paschoalini, M.A., Mariano-Neto, J. (2006) Ingestive effects of NMDA and AMPA-Kainate receptor antagonists microinjections into the lateral hypothalamus of the pigeon (*Columba livia*). *Brain Res.* 75-82.
8. Denbow, D.M., Cherry, J.A., Siegel, P.B., Van Krey, H.P. (1981) Eating, drinking and temperature response of chicks to brain catecholamine injections. *Physiol. and Behav.* 27: 265-9.
9. Dingledine, R., McBain, C.J. (1994) Excitatory amino acid transmitters in Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 5<sup>th</sup> Ed., Raven Press. New York, 367-387.
10. Duva, M.A., Siu, A., Stanley, B.G. (2005) The NMDA receptor antagonist MK-801 alters lipoprivic eating elicited by 2-mercaptoacetate. *Physiol. And Behav.* 83: 787-791.
11. Edmonds, B., Gibb, A.J., Colquhoun, D. (1995) Mechanism of activation of glutamate receptors and the time course of excitatory synaptic currents. *Annual Rev. Physiol.* 57: 495-519.
12. Gaviraghi, G. (2000) Excitatory amino acid



- receptors. *Pharmaceutica Acta Helvetiae*. 74: 219-220
13. Lee, S.W., Stanley, B.G.(2005) NMDA receptors mediate feeding elicited by neuropeptide Y in the lateral and perifornical hypothalamus. *Brain Res*. 1063: 1-8.
  14. Madl, J.E., Royer, S.M.(1999) Glutamate in synaptic terminals is reduced by lack of glucose but not hypoxia in rat hippocampal slices. *Neuroscience*; 94:417-430.
  15. Maldonado-Irizarry, C.S., Swanson, C.J., Kelly, A.E. (1995) Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. *J.of Neuroscience*; 15:106779-88.
  16. Mistlberger, R.E., Antle, M.C.(1999) Neonatal monosodium glutamate alters circadian organization of feeding, food anticipatory activity and photic masking in the rat. *Brain Res*. 842: 73-83.
  17. Ozawa, S., Kamiya, H., Tsuzuki, K.(1998) Glutamate receptors in the mammalian central nervous system. *Progress in Neurobiol*. 54: 581-618.
  18. Pearson, R.(1972) The biochemistry of avian brain in *The Avian Brain*, Academic press, 57.
  19. Reddy, V.M., Meharg, S.S., Ritter, S.(1986) Dose related stimulation of feeding by systemic injections of monosodium glutamate. *Physiol. Behavior*; 38:4465-9.
  20. Reid, C.A., Bliss, T.V.P.(2000) Learning about kainate receptors. *Tips*. 21:159-160.
  21. Stanley, B.G., Ha, L.H., Spears, L.C., Dee, M.G. (1993) Lateral hypothalamic injections of glutamate, kainic acid, DL-alpha-amino-3-hydroxy-5-methylisoxazole propionic acid or N-methyl-D-aspartic acid rapidly elicits intense transient eating in rats. *Brain Res*.613: 188-95.
  22. Stanley, B.G., Willett III, V.L., Donias, H.W., Dee II MG, Duva. M.A.(1996) Lateral hypothalamic NMDA receptors and glutamate as physiological mediators of eating and weight control. *A. J. Physiol*. R443-R449.
  23. Stanley, B.G., Butterfield, B.S., Grewal, R.S.(1997) NMDA receptor coagonist glycine site: evidence for a role in lateral hypothalamic stimulation of feeding. *A. J. of Physiol*. R790-R796.
  24. Stratford, T.R., Swanson, C.J., Kelley, Ann.(1998) Specific changes in food intake elicited by blockade or activation of glutamate receptors in the nucleus accumbens shell. *Behav. Brain Res*. 93: 43-50.
  25. Stricker-Krongrad, A., Beck, B., Nicholas, J.P., Burler, C.(1992) Central effects of monosodium glutamate on feeding behavior in adult long-evans rats. *Pharmacol., Biochem. and behave*. 43: 3881-6.
  26. Van Tienhoven, A., Juhasz, L.P.(1962) The chicken telencephalon, diencephalons and mesencephalon in stereotaxic coordinates. *J. Comp. Neurol*. 118: 185-97.
  27. Wirtshafter, D., Trifunovic, R.(1988) Stimulation of ingestive behaviors following injections of excitatory amino acid antagonists into the median raphe nucleus. *Pharmacol., Biochem. and Behav*. 30: 2529-33.
  28. Zeni Lucia, A.Z.R., Seidler, H.B.K., De Carvalho, N.A.S., Freitas, C.G., Mariano-Neto, J. Paschoalini, A.(2000) Glutamatergic control of food intake in regions: effects of central injections of glutamate, NMDA and AMPA receptor agonists and antagonists. *Pharmacol., Biochem. & Behav*. 65: 67-74.



## تأثیر گیرنده های یونوتروپیک و متابوتروپیک گلو تامات بر اخذ غذا در جوجه خروس های گوشتی

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در این مطالعه تأثیر سیستم گلو تاماترژیک بر اخذ غذا در جوجه خروس هایی که ۲۴ ساعت از خوردن غذا محروم بوده اند مطالعه گردید. تزریق داخل بطن مغزی صفر، ۰/۶۷۵، ۱/۲۵ و ۲/۵ نانومول گلو تامات اخذ غذا را به صورت وابسته به مقدار، کاهش و تاخیر در آغاز "خوردن غذا" را افزایش داد. پیش تزریق ۲/۵ نانومول از ۳- هیدروکسی- ۲- کینوزالین کربوکسیلیک اسید، آنتاگونیست گیرنده های یونوتروپیک گلو تامات به طور معنی داری به افزایش اخذ غذا و کاهش زمان آغاز خوردن منجر گردید. پیش تزریق ۲- نانومول آلفا - متیل- ۴- سولفونوفنیل گلیسین، آنتاگونیست گیرنده متابوتروپیک گلو تامات به شدت اخذ غذا را کاهش و زمان آغاز غذا خوردن را افزایش داد. این یافته ها برای اولین بار نشان داده اند که گلو تامات به عنوان یک میانجی عصبی در تنظیم مرکزی اخذ غذا در جوجه خروس گوشتی دخالت دارد. این اثر احتمالاً توسط هر دو گیرنده یونوتروپیک و متابوتروپیک تعدیل می گردد. به نظر می رسد که در این فرآیند هر دو نوع گیرنده های پیش سیناپسی و پس سیناپسی دخالت داشته باشند.

واژه های کلیدی: اخذ غذا، جوجه گوشتی، گلو تامات.

