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Glutamatergic Control of Food Intake in Pigeons: Effects of Central Injections of Glutamate, NMDA, and AMPA Receptor Agonists and Antagonists

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ZENI, L. A. Z. R., H. B. K. SEIDLER, N. A. S. CARVALHO, C. G. FREITAS, J. MARINO-NETO AND M. A. PASCHOALINI. *Glutamatergic control of food intake in pigeons: Effects of central injections of glutamate, NMDA, and AMPA receptor agonists and antagonists.* PHARMACOL BIOCHEM BEHAV **65**(1) 67–74, 2000.—The possible involvement of glutamatergic mechanisms in the control of food intake was studied in free-feeding and in 24-h food-deprived (FD24) pigeons for 1 h after intracerebroventricular (ICV) treatment with glutamate (Glu, 0, 50, 150, 300, and 600 nmol). Glu injections dose dependently induced decreases (30–65%) in food intake (FI) and feeding duration (FD), and increases in latency to start feeding (LSF) in FD24 animals, but not in free-feeding ones. None of these treatments affected noningestive behaviors (locomotion, sleep, and preening). In FD24 pigeons, ICV treatments with *N*-methyl-D-aspartic acid (NMDA, 0.1, 1, 4, 8, or 16 nmol) or D,L-α-amino-3-hydroxy-isoxazole proprionic acid (AMPA, 0.1, 1, 4, or 8 nmol) decreased FI and FD, but left LSF unchanged compared to vehicle-treated FD24 controls. Kainic acid (0.1, 0.5, and 1 nmol), or [*trans*-(IS,3R)-ACPD-($5NH_4OH$)] (ACPD, 0.1, 1, 4, 8, and 16 nmol) left unchanged the ingestive profile of FD24 pigeons. Pretreatment with the NMDA receptor antagonist MK-801 (15 nmol) and the AMPA-kainate receptor antagonist CNQX (390 nmol), 20 min before an ICV injection of Glu (300 nmol) induced a partial blockade of the Glu-induced decreases in FI and FD and completely inhibited the Glu-induced increase in LSF in FD24 pigeons. ICV injections of MK-801 (30 nmol) and of CNQX (780 nmol) increased FI and FD and reduced LSF in free-feeding pigeons. A lower dose of MK-801 (15 nmol) increased FI and FD, but not LSF. Conversely, a lower dose of CNQX (390 nmol) reduced LSF without changing FI or FD. These findings indicate the involvement of Glu as a chemical mediator in the regulation of food intake in the pigeon, possibly acting on multiple central mechanisms in this species through NMDA- and AMPA-sensitive Glu receptors. © 1999 Elsevier Science Inc.

Glutamate NMDA AMPA Food intake Avian

GLUTAMATE (Glu), considered to be a major excitatory neurotransmitter in the central nervous system, was recently demonstrated to be an endogenous agent involved in the neural control of food intake and body weight in mammals. Systemic, intracerebroventricularly (ICV) or local administration of Glu or Glu agonists into the lateral hypothalamus can evoke a dose-related stimulation of food intake in mammals (17,18,24–26,28,29) that can be mediated by *N*-methyl-D-arpartate (NMDA) as well as by other excitatory amino acid (EAA) receptor subtypes. However, systemic (1,2), ICV (23) and local injections of a number of EAA receptor antagonists into the median raphe (30,31) or into the nucleus accumbens (11,14) can elicit increases in food intake, indicating that eating behavior can be modulated by multiple EAA-mediated circuits located both centrally and at the periphery (1). Adult feeding behavior can also be affected by neonatal treatment with high Glu doses, which induce obesity in addition to other behavioral and metabolic disturbances thought to be related

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to excitotoxic damage and cell loss in hypothalamic and circumventricular districts [e.g., (13,15)].

Avian central mechanisms of food intake control appear to include the participation of catecholaminergic (3,4,7) and serotonergic (27) components that respectively mediate hyper- and hypophagic responses parallel to those observed in mammals. Although these data may support suggestions for the conservative nature of the evolution of food intake control systems in amniotes (12), there is evidence that continuous, long-term artificial selection for increased body weight has resulted in dramatic changes in central monoaminergic control of feeding in avian forms. Chicken lineages selected for fast growth appear to have increased responsiveness to food-stimulating compounds, and a decreased responsiveness for hypophagic drugs, while those selected for low adult weight show an inverse profile of sensitivity to feeding-affecting substances [e.g., (5,12)]. On the other hand, domestic pigeons have not been submitted to artificial selection directed at specific growth or feeding attributes, and are highly responsive to catecholamine-induced feeding as well as to 5-HT–induced hypophagia (3,27). These data emphasize the need to consider strain differences, and warrant the use of the domestic pigeon as a suitable subject in studies exploring the roles of different neuronal messengers in food intake control systems in birds.

Glu receptors have been found to be widely distributed in the avian CNS (8,16), and can be involved in learning and memory processes as well as in neuroendocrine control mechanisms in pigeons and fowl (6,9,19). Neonatal Glu injections in domestic fowl have also been shown to induce neuronal loss (22) and changes in monoaminergic and EAA content in hypothalamic areas (21), as well as an increase in abdominal fat deposition (20). However, no data on the role of central EAA circuits in the control of feeding behavior in adult avian forms are available in the literature. The purpose of this study was to examine the acute effects of central injections of Glu or of its agonists on NMDA, AMPA, kainate, or metabotropic EAA receptors in the feeding behavior of the domestic pigeon. We further explore this issue by investigating the effects of pretreatment with antagonists of NMDA and AMPA receptors on the eating responses to Glu injections, as well as the feeding effects of central injections of these antagonists in free-feeding pigeons.

METHOD

General Procedures

All the experimental procedures described below were conducted in strict adherence to the recommendations of the "Principles of Animal Care" (NIH, 1985) and of the "Ethical Principles of Animal Experimentation" of the Brazilian College of Animal Experimentation (COBEA, 1991). Male adult pigeons (*Columba livia*, 320–360 g body weight), were kept in individual cages, at 22–24°C with free access to food and water. At least 7 days before the experiments each animal was anesthetized with Equithesin (0.15 ml/100 g), and a guide cannula was stereotaxically implanted into the right lateral ventricle. The cannula was placed 1.0 mm lateral to the midline, 6.0 mm anterior to the interauricular line, and lowered to a depth of 6.0 mm below the surface of the skull, according to the coordinates of a stereotaxic atlas for the pigeon's brain (10). The contact of the tip of the cannula with the ventricular space was indicated by a pressure drop in a saline-filled manometer attached to the cannula. The cannula was anchored to the skull with jeweler's screws and fixed with dental cement. The cannula was maintained patent between experiments by an inner removable stylet. All ICV injections (always in a volume of 1 μl) were made through an inner cannula connected by a polyethylene tubing to a 10-μl microsyringe over a period of 60 s. A further 60-s period was allowed to permit the solution to diffuse from the tip of the cannula into the ventricle. All experimental procedures were performed between 1000 and 1500 h.

Experiment 1—Effects of Different Glu Doses on Free-Feeding and Food-Deprived Animals

This series of experiments was designed to examine the effects of ICV injections of different Glu (L-glutamic acid, monosodium salt, Sigma Chemical Co.) doses on food intake on a group of free-feeding (free access to food and water, $n = 9$) and on a group of 24-h food-deprived (FD24, $n = 9$) pigeons. Twenty-four hours before the beginning of the experiment, FD24 animals were deprived of food, but with water ad lib. After this 24 h of food deprivation, Glu (50, 150, 300, or 600 nmol, diluted in sterile artificial cerebrospinal fluid (CSF), consisting of 155 mM Na^+, 3.7 mM K^+, 2.5 mM Ca^{++}, 2.1 mM Mg^{++}, 140 mM Cl^-, and 23 mM HCO_3^-) or vehicle was administered through the intraventricular cannula. All animals received all doses of Glu with at least a 7-day interval between treatments, following a counterbalanced Latin square design with four repetitions.

Immediately after the injection, the animals were returned to their cages, food was reintroduced, and behavioral recordings were started. During the first hour after drug injection, latency for the first episode and duration of feeding, preening, locomotion, alert immobility, and sleep-like behaviors were recorded on paper protocols. Feeding was defined as a bout of pecking movements directed at the feeder. Duration of this behavior included brief interpecking intervals (<3 s), during which the animal adopts an upright posture, shows swallowing and beak movements, and then start pecking again. A sleeping pigeon might sit or stand on one or both legs, with chest and neck plumage puffed up and one or both eyelids closed [see (3)]. Sleep was recorded when the pigeon puffed up its feathers with at least one eye closed. Alert immobility was recorded when the animal showed an upright posture with both eyes open, in the absence of sleep signs (above) as well as of locomotory and ingestive behaviors. Food pellets were delivered in plastic cups with three holes of 6 cm each at the top, providing easy access to food, little spillage of the pellets, and reliable food weighing. Water was provided in plastic bottles closed at the top, with a spout that projected through the cage wires. At the end of the recording period, any food pellet that eventually spilled on the cage floor were recovered and weighed with the food remaining in the feeder. The difference between food weight at the beginning and at the end of the recording period was taken as the amount of food consumed.

Experiment 2—Effects of Glutamatergic Receptor Agonists on Food-Deprived Animals

The results of Experiment 1 indicated that Glu treatment reduced food intake in FD24 pigeons but not in free-feeding ones. As a first step to identify EAA receptor subtype(s) possibly involved in this effect, naive 24FD pigeons ($n = 8$ for each drug dose) were treated ICV with the Glu agonists *N*-methyl-D-aspartic acid (NMDA, 0.1, 1, 4, 8, or 16 nmol), D,L-α-amino-3-hydroxy-isoxazole proprionic acid (AMPA, 0.1, 1, 4, or 8 nmol), kainic acid (KA, 0.1, 0.5, and 1 nmol), [*trans*-(IS,3R)-ACPD-($5NH_4OH$)] (ACPD, metabotropic receptor agonist, 0.1, 1, 4, 8, and 16 nmol), or vehicle (artificial

CSF). Each animal was tested twice (a single dose of one of the above agonists or its vehicle injected in random sequence), and the recordings were carried out 7 days apart. The dose ranges of Glu (Experiment 1) and of Glu agonists were derived from those reported to affect feeding behavior after intrahypothalamic injections in rats (24). Higher doses of KA (4 and 8 nmol) and AMPA (16 nmol) were also tested. Although these treatments reduced food intake, they also induced a frantic locomotor activity and/or malaise, preventing a proper evaluation of feeding behavior. These doses of AMPA and KA were thus discarded during data analysis. All drugs were obtained from Research Biochemicals International (Natick, MA), and were freshly dissolved in artificial CSF. Food deprivation, injection, and behavioral recording procedures were carried out as described in Experiment 1.

Experiment 3—Effects of Pretreatment with Glutamatergic Receptor Antagonists

Data of Experiment 2 suggested that NMDA- and AMPA-sensitive receptors can mediate at least some of the aspects of the food intake reductions observed after Glu ICV injections. To further examine this possibility, two groups of experimentally naive FD24 pigeons ($n = 9$ per group) were pretreated with the NMDA receptor antagonist (+)-MK-801 (hydrogen maleate salt, 15 nmol), the AMPA–kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione complexed with 2-hydroxypropyl-β-cyclodextrin (CNQX:HBC complex, dissolved to obtain 390 nmol of CNQX in 1 μl of solution), or vehicle (artificial CSF), 20 min before ICV injection of Glu (300 nmol). This Glu dose was selected as the test dose because it was found to induce a 50% decrease in food intake in FD24 animals without affecting other noningestive behavioral parameters (see results of Experiment 1). All drugs were freshly dissolved in artificial CSF. Each experimental group was tested in three recording sessions after injection of vehicle + vehicle, vehicle + Glu, or one of the two antagonists studied + Glu. The sequence of tests followed a balanced design. Food deprivation, injection, and recording procedures were carried out as described for Experiment 1.

Experiment 4—Effects of Glutamatergic Receptor Antagonists on Free-Feeding Animals

Experiments 2 and 3 indicated that the ionotropic NMDA- and AMPA-sensitive receptors can mediate the hypophagic effects of central Glu injections in FD24 pigeons. In view of the present results and of data indicating food intake increases elicited by systemic or central EAA receptor antagonists in mammals (see introduction), it appears conceivable that treatments with these antagonists alone can induce changes in eating behavior of free-feeding pigeons. To test this hypothesis, we examined the effects of ICV injections of MK-801 (15 and 30 nmol), CNQX (390 and 780 nmol), or vehicle (artificial CSF) in two groups of naive free-feeding animals ($n = 8$ per group). The animals within a group were tested with vehicle and the two doses of one of the EAA antagonists. The treatments were conducted 7 days apart, and followed a counterbalanced Latin squared design. Injection and recording procedures were carried out as described in Experiment 1.

Histological Analysis

At the end of the experiments, Evans blue dye was injected through the cannula to determine its precise position. The pigeons were then deeply anesthetized with Equithesin and perfused transcardially with saline followed by 10% formalin. The brains were removed and subsequently cut with a vibratome in the transverse plane (75 μm), and the presence of the dye inside the lateral ventricle was taken as indicative of correct cannula positioning.

Data Analysis

Before any comparisons between experimental groups, the presence of a Gaussian distribution of the data was tested by the Levene and the Kolmogorov-Smirnov tests. Except for food intake, data for the other behavioral parameters showed a distribution significantly different from normal. Thus, data for food intake were analyzed by parametric one-way ANOVA followed by intergroup comparisons using Duncan's post hoc test, and the behavioral data were analyzed by Kruskal-Wallis (K-W) nonparametric ANOVA, followed by the post hoc Mann–Whitney test. Because the strong numerical differences in food intake and behavioral data observed between FD24 and free-feeding groups in Experiment 1 could hamper a proper evaluation of dose-related effects by two-way ANOVA (using nutritional status vs. drug dose as factors), these data were examined by parametric or nonparametric one-way ANOVA procedures performed separately for each nutritional condition group (FD24 or free-feeding). A value of $p < 0.05$ was accepted as significant in all these statistical procedures.

RESULTS

Experiment 1—Effects of Different Glu Doses on Free-Feeding and FD24 Pigeons

Glutamate injected into the lateral ventricle of 24-h food-deprived (FD24) pigeons significantly, $F(4, 40) = 8.17$, $p = 6 \times 10^{-6}$, and dose dependently decreased the amount of food ingested (Fig. 1). Free-feeding animal showed no changes in food intake after any of the Glu treatments, $F(4, 40) = 1.10$, $p = 0.36$. Glu-induced reductions in food intake of FD24 animals ranged from 30% at the lowest dose to 65% after the 600 nmol dose. Total mean duration was also significantly decreased, K-W $H(4, 45) = 18{,}06$, $p = 0.001$, after ICV injections of Glu in FD24 animals, but not in free-feeding ones (Table 1). The latency to start eating was strikingly (3- to 10-fold) and dose dependently increased in FD24 birds, K-W $H(4,48) = 20.53$, $p = 0.0004$, and remained unchanged in free-feeding animals (Table 1) after Glu treatments. Other behavioral indexes were unaffected by Glu treatments in both free-feeding (data not shown) and FD24 animals (Table 3); malaise or visible motor impairment was not observed after these Glu treatments.

Experiment 2—Effects of Glutamatergic Receptor Agonists on FD24 Pigeons

Significant changes in ingestive behavior were observed after NMDA and AMPA treatment, while the KA and ACPD doses used in the present study left unaffected feeding and the other behavioral indexes examined (Fig. 2, Tables 2 and 3). NMDA treatment induced decreases in food intake, $F((5, 74) = 4.97$, $p = 5 \times 10^{-5}$ (see Fig. 2), and in feeding duration, K-W $H(4, 80) = 12.36$, $p = 0.031$, that were significant after the 8- and 16-nmol doses (Table 2). These two doses decreased food intake by 41 and 58% compared to vehicle-treated animals, values similar to those observed after the higher Glu doses (see Experiment 1). AMPA treatment also

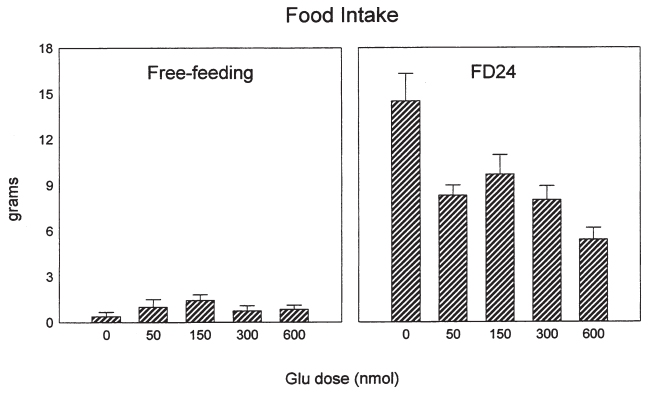

FIG. 1. Effects of intracerebroventricular injections (1 μl) of glutamate on food intake in free-feeding and in 24-h food-deprived (FD24) pigeons (n = 9 per treatment) 1 h after treatment. Data are reported as means ± SEM. *$p < 0.05$ (multiple range Duncan's test), compared to vehicle-treated animals.

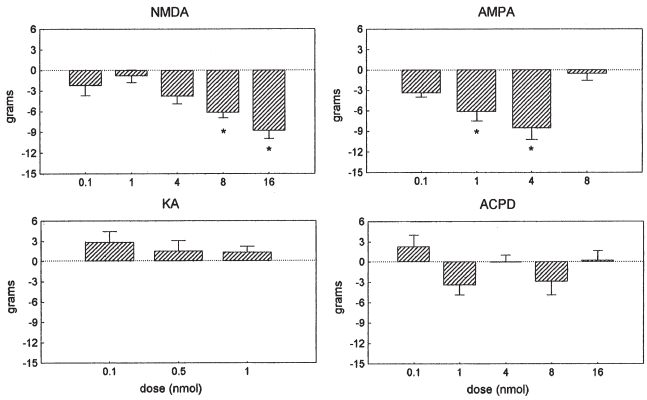

FIG. 2. Effects of intracerebroventricular injections (1 μl) of the glutamatergic receptors agonists NMDA, AMPA, KA, and ACPD on food intake in 24-h food-deprived pigeons (n = 8 per drug dose). Data are reported as means ± SEM of the differences in intake between each drug dose and the vehicle treatments corresponding to the same dose group. Mean food intake (pooled for all vehicle data for each drug) after vehicle treatment was 15.1 ± 1.1 g for NMDA, 16.5 ± 1.7 g for AMPA, 13.4 ± 1.6 g for KA, and 14.3 ± 1.0 g for ACPD experiments. *$p < 0.05$ (multiple range Duncan's test), compared to vehicle-treated animals.

induced significant and potent decreases in food intake, $F(4, 59) = 6.58$, $p = 0.0002$, but only at the two intermediate doses used in the present experiment (1 and 4 nmol, with reductions of 37 and 52%, respectively), showing a U-shaped dose–response curve. The three lower doses of AMPA elicited similar decreases in feeding duration that were significantly different from vehicle data, K-W $H(4, 64) = 21.14$, $p = 0.0003$, while the highest dose left this behavioral index unchanged (Table 2). Interestingly, the short latency to start eating characteristic of the FD24 pigeons was not significantly modified by any of the NMDA or AMPA doses examined (Table 2). The noningestive behavioral profile remained unchanged after both NMDA and AMPA treatments (Table 3).

Experiment 3—Effects of Pretreatment With Glutamatergic Receptor Antagonists

Confirming the results of Experiment 1, ICV injection of Glu (300 nmol) in FD24 pigeons pretreated with vehicle led to a nearly 50% reduction in food intake (Fig. 3) that was associated with significant reductions in feeding duration and increases in latency to start feeding. Pretreatment with MK-801 or with CNQX induced a partial but significant blockade of the hypophagic effects of ICV injected Glu (Fig. 3). In

TABLE 1

EFFECTS OF i.c.v. INJECTIONS OF GLUTAMATE (GLU) ON MEAN DURATION AND MEAN LATENCY OF FEEDING IN FREE-FEEDING (FF) AND IN 24-H FOOD-DEPRIVED (FD24) PIGEONS

State	Glu Dose (nmol)	Feeding Duration (s)	Feeding Latency (s)
FF	Vehicle	12 ± 9	2875 ± 480
	50	46 ± 29	2261 ± 532
	150	25 ± 8	2512 ± 431
	300	18 ± 8	2282 ± 522
	600	45 ± 16	1996 ± 411
FD24	Vehicle	250 ± 25	36 ± 13
	50	168 ± 20*	92 ± 28
	150	168 ± 14*	120 ± 32*
	300	117 ± 14*	145 ± 34*
	600	87 ± 15*	445 ± 164*

Values are expressed as means ± SEM. n = 9 per treatement.
*$P < 0.05$ compared to vehicle-treated pigeons (Mann–Whitney U- test).

TABLE 2

EFFECTS OF i.c.v. INJECTIONS OF GLUTAMATE AGONISTS ON MEAN DURATION AND MEAN LATENCY OF FEEDING IN 24-H FOOD-DEPRIVED PIGEONS

Drug	Dose (nmol)	n	Feeding Duration (s)	Feeding Latency (s)
NMDA	Vehicle	40	212 ± 16	67 ± 18
	0.1	8	244 ± 57	11 ± 5
	1	8	189 ± 17	7 ± 4
	4	8	178 ± 31	22 ± 11
	8	8	138 ± 18*	115 ± 50
	16	8	130 ± 18*	37 ± 5
AMPA	Vehicle	32	203 ± 12	46 ± 22
	0.1	8	103 ± 13*	10 ± 5
	1	8	135 ± 16*	7 ± 3
	4	8	102 ± 13*	10 ± 4
	8	8	185 ± 11	11 ± 5
KA	Vehicle	24	146 ± 14	35 ± 7
	0.1	8	163 ± 17	26 ± 14
	0.5	8	135 ± 11	10 ± 5
	1	8	113 ± 11	454 ± 403
ACPD	Vehicle	40	146 ± 13	31 ± 8
	0.1	8	162 ± 14	7 ± 4
	1	8	134 ± 35	12 ± 3
	4	8	167 ± 29	13 ± 4
	8	8	103 ± 18	32 ± 14
	16	8	171 ± 70	27 ± 8

Values are expressed as means ± SEM. n = 8 per treatment. Vehicle means are derived from pooled control data of all doses for each drug.
*$P < 0.05$ compared to vehicle-treated pigeons (Mann–Whitney U- test).

TABLE 3

EFFECTS OF i.c.v. INJECTIONS OF GLU (EXPERIMENT 1), OF GLUTAMATERGIC AGONISTS (EXPERIMENT 2), OF GLU ANTAGONISTS 20 MIN BEFORE GLU INJECTIONS IN 24 H FOOD-DEPRIVED PIGEONS (EXPERIMENT 3) OR OF GLU ANTAGONISTS INJECTED IN FREE-FEEDING PIGEONS (EXPERIMENT 4) ON MEAN DURATION OF NONINGESTIVE BEHAVIORS

Treatment	Locomotion (s)	Alert Immobility (s)	Sleep (s)	Preening (s)
Experiment 1				
Vehicle	55 ± 7	2466 ± 97	346 ± 106	213 ± 48
Glu (300 nmol)	63 ± 21	2795 ± 191	427 ± 150	118 ± 40
Glu (600 nmol)	92 ± 16	2573 ± 166	287 ± 100	198 ± 62
Experiment 2				
Vehicle of NMDA	33 ± 9	2632 ± 235	225 ± 107	120 ± 47
NMDA (16 nmol)	65 ± 26	2379 ± 319	183 ± 44	314 ± 222
NMDA (8 nmol)	41 ± 13	2638 ± 218	43 ± 43	322 ± 94
Vehicle of AMPA	27 ± 13	3265 ± 60	130 ± 19	52 ± 29
AMPA (8 nmol)	27 ± 8	3032 ± 103	199 ± 77	208 ± 88
AMPA (4 nmol)	98 ± 38	3098 ± 127	42 ± 16	245 ± 116
Vehicle of KA	48 ± 14	2986 ± 162	150 ± 117	254 ± 121
KA (1 nmol)	66 ± 20	3012 ± 133	152 ± 88	232 ± 98
KA (0.5 nmol)	64 ± 29	3228 ± 77	50 ± 21	130 ± 63
Vehicle of ACPD	86 ± 29	3209 ± 93	189 ± 70	62 ± 25
ACPD (16 nmol)	99 ± 65	3092 ± 163	197 ± 67	111 ± 82
ACPD (8 nmol)	33 ± 9	3411 ± 32	62 ± 26	32 ± 17
Experiment 3				
Vehicle + Vehicle	55 ± 8	2789 ± 195	361 ± 92	29 ± 10
Vehicle + Glu	59 ± 21	2553 ± 163	328 ± 98	203 ± 32
MK-801 + Glu	37 ± 5	2803 ± 165	379 ± 153	77 ± 31
CNQX + Glu	35 ± 9	3080 ± 93	127 ± 57	48 ± 31
Experiment 4				
Vehicle of MK-801	38 ± 5	2412 ± 123	392 ± 110	274 ± 58
MK-801 (30 nmol)	39 ± 5	2964 ± 224	197 ± 77	282 ± 130
Vehicle of CNQX	22 ± 3	2153 ± 302	312 ± 95	266 ± 82
CNQX (780 nmol)	38 ± 5	2593 ± 260	393 ± 197	245 ± 78

Vehicle means are derived from pooled control data of all doses for each drug. Values are expressed as the mean ± SEM. No significant differences were found between treatments and their respective vehicle data, when the data were analyzed by Kruskal-Wallis nonparametric ANOVA.

these experiments, food intake [MK-801: $F(2, 24) = 15.97$, $p = 4 \times 10^{-5}$; CNQX: $F(2, 24) = 17.13$, $p = 2 \times 10^{-5}$] and total meal duration [MK-801: K-W $H(2, 27) = 7.76$, $p = 0.02$; CNQX: K-W $H(2, 27) = 5.58$, $p = 0.049$] were both significantly higher than those of vehicle + Glu-treated animals and lower than those of vehicle + vehicle-treated pigeons. All of these antagonists potently and significantly blocked the increase in the latency to start feeding upon food presentation evoked by the Glu injection [MK-801: K-W $H(2, 27) = 9.37$, $p = 0.009$; CNQX: K-W $H(2, 27) = 12.84$, $p = 0.0016$], so that FD24 animals pretreated with these drugs ran to the feeder as fast as vehicle + vehicle-treated FD pigeons (Fig. 3). The duration of noningestive behaviors was not modified by these treatments (Table 3).

Experiment 4—Effects of Glutamatergic Receptor Antagonists on Free-Feeding Animals

In free-feeding pigeons, MK-801 and CNQX treatments significantly increased both food intake, $F(2, 21) = 9.64$, $p = 0.001$, and $F(2, 21) = 11.99$, $p = 0.0003$, respectively, and feeding duration, K-W $H(2, 24) = 7.10$, $p = 0.028$, and K-W $H(2, 24) = 11.52$, $p = 0.003$, respectively. The two MK-801 doses examined induced similar and potent increases (nearly sixfold) in food intake and feeding duration that were equivalent to those induced by the higher CNQX dose (Fig. 4). On the other hand, the latencies to start feeding were significantly decreased after the two CNQX doses, K-W $H(2, 24) = 14.45$, $p = 0.0007$, and the higher MK-801 dose, K-W $H(2, 24) = 5.94$, $p = 0.04$. It appears that while the higher doses of MK-801 and of CNQX enhanced all the aspects of feeding behavior examined, the lower dose of MK-801 increased food intake without affecting latency to start eating. Conversely, the lower dose of CNQX reduced the latency to start eating without affecting food intake or duration of feeding behavior.

DISCUSSION

The present study examined for the first time the possible involvement of glutamatergic circuits in acute food intake control mechanisms in an avian species. Data from Experiments 1 and 2 indicate that ICV injection of Glu, NMDA, or AMPA can induce short-term (1 h after food presentation) and dose-depend decreases (30–65%) in FI and FD in FD24 animals. Besides feeding behavior effects, Glu or other EAA receptor agonist injection evoked no noticeable changes in locomotion, preening, or sleep behaviors even at the higher doses used in the present experiments. Furthermore, Glu at

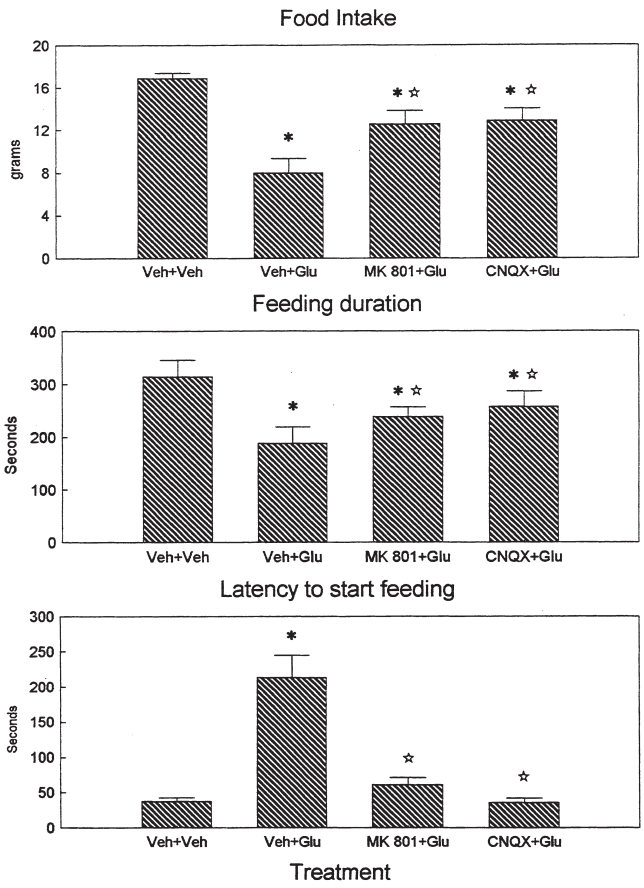

FIG. 3. Effects of ICV injections (1 μl) of Glu (300 nmol) or vehicle (Veh) on feeding behavior in 24-h food-deprived pigeons ($n = 9$ per treatment) pretreated ICV 20 min before with the glutamatergic receptor antagonists MK-801 (15 nmol), CNQX (390 nmol), or vehicle. Data are reported as means ± SEM. Data from vehicle–vehicle and vehicle–Glu treatments were pooled for all subjects in both experimental groups. * and ☆$p < 0.05$ (multiple range Duncan's test for food intake data, Mann–Whitney *U*-test for duration and latency data), compared to vehicle–vehicle (Veh + Veh) and vehicle–Glu (Veh + Glu), respectively.

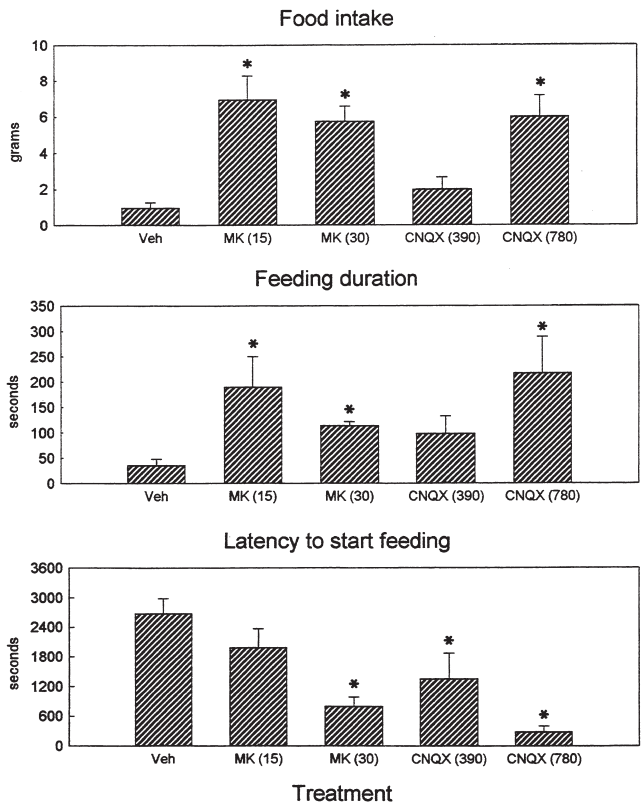

FIG. 4. Effects of ICV injections (1 μl) of MK-801 (15 or 30 nmol), CNQX (390 or 780 nmol), or vehicle (Veh) on feeding behavior of free-feeding pigeons ($n = 8$ per treatment). Data are reported as means ± SEM. Data from vehicle treatments were pooled for all subjects in both experimental groups. *$p < 0.05$ (multiple range Duncan's test for food intake data, Mann–Whitney *U*-test for duration and latency data), compared to vehicle data.

the doses presently used was unable to evoke any perceptible change in ingestive or noningestive behaviors in free-feeding animals. These results suggest that the food intake inhibition induced in FD24 pigeons by these EAA receptor agonists is unlikely to be related to nonspecific motor, sensory, or arousal effects, or to be associated with the possible cumulative (in Experiment 1) or acute (in Experiment 2) neurotoxic consequences of these treatments. The present findings indicate that activation of EAA ionotropic receptors obtained by ICV injection of Glu or Glu agonists in NMDA- and AMPA-sensitive receptors, but not in kainate or metabotropic ones, can produce a decrease in feeding behavior induced by food deprivation. In Experiment 3, ICV injections of MK-801 (a noncompetitive NMDA antagonist) and of CNQX (an antagonist of AMPA and of kainate receptors) alleviated the Glu-induced reductions in food intake of FD24 pigeons. Furthermore, ICV injections of MK-801 and of CNQX (Experiment 4) elicited eating behavior in free-feeding pigeons. In view of the absence of hypophagic effects of KA in Experiment 2, it is possible that the CNQX-induced effects on feeding were mediated mainly by AMPA receptors.

An apparently coherent scenario emerges from the present data, indicating that Glu-mediated circuits influenced by our ICV treatments can play a general inhibitory role in feeding behavior in this species, and that their effects may be mediated by AMPA and NMDA receptors. Furthermore, our data suggest that at least part of the feeding-related Glu circuits may be tonically active in free-feeding pigeons, so that blockade of AMPA or NMDA receptors may both anticipate the beginning of a feeding bout and increase food intake and feeding duration.

It is noteworthy that only Glu treatments were able to increase the latency to start feeding in FD24 pigeons. Both AMPA and NMDA treatments reduced food intake and feeding duration, but both left unchanged the latency to start eating in these animals. However, blockade of AMPA or of NMDA receptors (Experiment 3) alleviated the Glu-induced decrease in food intake and feeding duration, but completely reversed the Glu-induced increase in latency to start eating. Although it is presently difficult to reconcile these results with those of Experiments 1 and 2, it appears conceivable that both NMDA and AMPA receptor subtypes can be differentially involved in Glu-mediated actions on the multifarious aspects of feeding behavior that are operative in food-deprived pigeons. Some aspects of the effects of CNQX and MK-801 treatments on free-feeding animals (see below) can support to this general notion and warrant further investigation.

It is interesting to note that the hyperphagic effect of MK-801 is apparent even in free-feeding pigeons feed with their usual chow. Peripheral injections of MK-801 do not initiate feeding in satiated rats, but increase food intake after food deprivation or after presentation of a highly palatable food (2), and their hyperphagic effects are best observed if this drug is injected early during a meal (1). These data lead to the suggestion that MK-801 possibly delays satiation by interfering with nutrient-related signals that cause the end of a meal, and that NMDA receptors may participate in satiation mechanisms. Our data indicating that a low dose of MK-801 administered to free-feeding pigeons produces increased food intake without significantly curtailing the latency may suggest that NMDA receptors are involved in satiation mechanisms of pigeons possibly comparable to those observed in rats. In pigeons, however, its effects in ending a feeding bout appears to be independent of food palatability or of food deprivation, as seen in rats (1,2).

At the lower dose used in the present experiments, the effects of MK-801 became evident only after eating bouts were naturally or spontaneously started, and were apparently independent of the mechanisms that induce food intake. This may indicate that at low doses MK-801 could be blocking NMDA receptor-mediated Glu inputs that are initiated by feeding-related signals that contribute to terminating a feeding bout. In contrast, our lower dose of CNQX significantly reduced the latency to start eating but left feeding duration and amount unchanged, suggesting that this treatment was able to interfere only with an AMPA receptor-mediated Glu mechanism that tonically contributes to inhibition of eating. These data indicate the existence of interrelated but distinct glutamatergic mechanisms, mediated by AMPA and NMDA receptors, involved respectively in initiation of feeding and in regulation of food intake and the duration of feeding bouts in the pigeon.

The present findings contrast with those obtained in mammals indicating that Glu or Glu agonist injections can elicit feeding (17,24–26,28,29). The hypophagic doses of EAAr agonists used in the present study are similar to those necessary to induce feeding when injected locally into the lateral hypothalamus (24,25) and are much lower than the Glu doses necessary to acutely induce feeding when injected ICV [3 mg/brain in rats, see (28), compared to the 8.5–101 μg doses of Glu used in the present study]. Although our results cannot reveal the existence of similar feeding-inducing Glu mechanisms in pigeons, their presence in areas other than those reached by our ICV injections cannot be ruled out. Experiments using local injections of Glu and Glu agonists into hypothalamic areas of food-restricted pigeons are currently underway in our laboratory to further probe the localization of circuits involved in the presently reported hypophagic effects of Glu, as well as the possible presence of eating-inducing Glu-mediated mechanisms in this species.

On the other hand, enhanced eating behavior was observed after systemic [(1,2); see discussion above] and after ICV (23) injections of NMDA antagonists in rats. In the later study, ICV injections of 7-chlorokynurenic acid (an antagonist selective for strychnine-insensitive glycine binding site of the NMDA receptor/channel complex) increased feeding with a rapid onset in free-feeding rats, and this response was blocked by pretreatment with D-serine, an agonist at this receptor. Furthermore, injections of the AMPA–kainate receptor antagonists CNQX, NBQX, and DNQX into the medial nucleus accumbens evoked a site-specific, intense, and almost immediate feeding response in free-feeding rats (11,14). MK-801 injections into this region of the n. accumbens failed to elicit feeding (14). Moreover, injection of NMDA and AMPA receptor antagonists into the median raphe can induce increases in food and water intake that were thought to be related to generalized arousal changes stimulated by these treatments (30,31). These results indicate that different aspects of eating behavior may be modulated by multiple central or peripheral NMDA- and AMPA-mediated circuits having tonic inhibitory effects on food intake in mammals, and that at least part of these mammalian glutamatergic mechanisms that inhibit feeding may have a parallel in the avian feeding control systems.

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