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Activation of lateral hypothalamic mGlu1 and mGlu5 receptors elicits feeding in rats☆

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ABSTRACT

Metabotropic glutamate receptors (mGluRs) have been popular drug targets for a variety of central nervous system (CNS) disease models, ranging from seizures to schizophrenia. The current study aimed to determine whether mGluRs participate in lateral hypothalamic (LH) stimulation of feeding. To this end, we used satiated adult male Sprague-Dawley rats stereotaxically implanted with indwelling bilateral LH guide cannulas to determine if injection of (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), a broad mGluR group I and II agonist, would elicit feeding. Administration of 100 nmol ACPD induced feeding with a short latency. Similarly, unilateral LH injection of the selective mGluR group I agonist (S)-3,5-dihydroxyphenylglycine (DHPG) elicited significant feeding beginning 60 min post-injection and continuing until 4 h postinjection. Administration of the mGluR5 agonist, (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) produced a smaller delayed feeding response. These delayed but prolonged eating responses suggest that activation of LH mGluR1 and/or mGluR5 might be sufficient to elicit feeding. To determine which subtypes were involved, LH DHPG injections were preceded by LH injection of either the group I antagonist n-phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxamide (PHCCC), the mGluR1 antagonist 6-amino-n-cyclohexyl-n,3-dimethylthiazolo[3,2-a]benzimi dazole-2-carboxamide hydrochloride (YM-298198) or the mGluR5 antagonist 3-((2-methyl-4-thiazolyl)ethynyl) pyridine (MTEP), and food intake was measured. PHCCC blocked DHPG-elicited feeding, and each of the other antagonists produced significant feeding suppression. These findings suggest roles for mGluR1 and/or mGluR5 in lateral hypothalamic circuits capable of stimulating feeding behavior.

1. Introduction

Glutamate is a major excitatory neurotransmitter in the mammalian nervous system and previous findings strongly suggest a role for ionotropic glutamate receptors in feeding, especially within the lateral hypothalamus (LH; Stanley et al., 2011). Specifically, injections of glutamate or glutamate receptor agonists n-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) or kainic acid (KA) into the LH induce short latency feeding responses, while NMDA receptor antagonists suppress both the NMDA-elicited and natural feeding (Stanley et al., 1993a, 1993b, 1996). Cannula and microdialysis probe mapping studies showed the most sensitive site for these agonists to elicit feeding is in the tuberal LH (Duva et al., 2002; Stanley et al., 1993a). In addition, increases in extracellular glutamate levels within the LH coincide with feeding initiation, whereas LH glutamate levels fall rapidly as the animal eats and becomes satiated (Rada et al., 1997). This increase in glutamate release is feeding specific and does not occur with drinking (Thongkhao-on et al., 2008). This indicates that glutamate plays a crucial role in inducing food intake in the LH. However, the role of metabotropic glutamate receptors (mGluRs) within the LH has yet to be demonstrated.

The family of mGluRs is composed of eight structurally related receptors, divided into three groups on the basis of sequence homology, agonist selectivity, intracellular signaling mechanisms and differential targeting in neurons (Pin and Duvoisin, 1995). Group I mGluRs, mGluR1 and mGluR5, are positively coupled to phospholipase C (PLC), activation of which leads to the formation of IP3 and diacylglycerol, intracellular release of Ca^{2+} and stimulation of protein kinase C (PKC), while group II receptors, consisting of

☆ All efforts in the preceding experiments were made to minimize animal suffering. Experiments were approved by the institutions Animal Care and Use Committee.

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mGluR2 and 3, and group III receptors, consisting of mGluR4,6,7, and 8, are negatively coupled to adenylyl cyclase and cyclase respectively. Different subtypes in each mGluR group have different functional roles. For example, within group 1 mGluRs, mGluR1 modulate intracellular Ca^{2+} concentration, while mGluR5 are additionally linked to potentiation of NMDA receptors (Mannaioni et al., 2001). Support for the interaction of mGluRs and NMDA receptors comes from structural localization of these receptors. Group I mGluRs are concentrated in a ring around the periphery of the post synaptic density near NMDA receptors (Nusser et al., 1994; Luján et al., 1997). Therefore, as NMDA receptors have previously been implicated in feeding stimulation and group I mGluRs are linked to NMDA receptors, group I mGluR involvement in food intake merits investigation. This study aims to determine if LH mGluRs provide a functional role in feeding and to identify which receptors may mediate this behavior.

2. Materials and methods

2.1. Subjects and stereotaxic surgery

Adult male Sprague-Dawley rats weighing 350–500 g at the time of surgery were used. These rats were bred in the University of California, Riverside, Psychology Department vivarium and were descended from rats obtained from Charles River, Inc. They were individually housed in a temperature controlled vivarium (21 °C) on a 12:12 h light:dark cycle, and allowed free access to standard Purina rat chow pellets and water.

Surgery to implant an 18 mm long, 26 gauge (o.d. = 0.46 mm) stainless steel indwelling guide cannula within the LH was performed under barbiturate anesthesia (Nembutal, 50 mg/kg body weight, i.p.). The stereotaxic coordinates were: 6.1 mm anterior to the interaural line, +1.8 mm lateral to the midsagittal sinus (±1.8 mm when implanting bilaterally), and 8.2 mm ventral to the surface of the skull. Coordinates were initially based on the atlas of Paxinos and Watson (2007) then modified empirically; the incisor bar was placed at −3.3 mm during all surgeries. Cannulas were held in place by dental cement affixed to four stainless steel screws imbedded in the skull and protected with a plastic guard. To prevent clogging a 33-guage stainless steel obturator was placed within the cannula. The animals were given a minimum of seven days to recover from surgery before proceeding with testing. During this timeframe all animals were handled to adapt them to testing procedures. Procedures were approved by the University of California Riverside Institutional Animal Care and Use Committee. Three days prior to testing, the standard pellet food was replaced with a milk-mash diet consisting of Purina rat chow (500 g), sucrose (400 g), and Carnation evaporated milk (354 ml).

2.2. Central injections

Tests consisted of unilateral or bilateral injection within the LH. Solutions were injected via a 33-gauge needle that projected 1.0 mm beyond the ventral tip of the cannula. Each injection consisted of a 0.3 μl volume of either the mGluR group I and II agonist, (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD); mGluR1/5 agonist, (*S*)-3,5-dihydroxyphenylglycine (DHPG); the mGluR5 agonist, (RS)-2-chloro-5-hydroxyphenylglycine (CHPG); the mGluR5 antagonist, (3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine hydrochloride (MTEP)); the mGluR1 antagonist, 6-amino-n-cyclohexyl-n,3-dimethylthiazolo[3,2-a]benzimi dazole-2-carboxamide hydrochloride (YM-298198), the group I mGluR antagonist, n-Phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxamide (PHCCC) at varying doses or the vehicle (VEH), artificial cerebrospinal fluid (ACSF) where indicated. The ACSF consists of (in mM): Na^+ (147), Cl^- (154), K^+ (3.0), Ca^{2+} (1.2) and Mg^{2+} (0.9) with a pH of 7.4, except for ACPD administration, when 0.1 M NaOH was used to improve the solubility of ACPD. All drugs were purchased from Tocris (St. Louis, MO). Drug doses were extrapolated from previous findings. Drug dilutions were prepared one day prior to the first test day and aliquots stored at −80 °C. When tested under satiated conditions, fresh mash was given at least 1 h prior to testing, at the onset of the light cycle. For food deprived or nocturnal feeding conditions, fresh mash was given immediately after injection at the commencement of the dark cycle. For all experiments, doses were given in counterbalanced order across test days. No single rat was administered more than 16 individual injections. Food intake was typically measured at 15 min and 30 min as well as 1, 2 and 4 h post-injection. All Subjects were fed within their homecage and food bowls weighed on a daily calibrated scale. Between tests, rats had at least one undisturbed day with ad libitum access to food and water unless otherwise noted.

2.3. Experiment 1: bilateral activation of LH mGluR group I/II receptors elicits feeding

In order to determine whether broad activation of mGluRs elicits feeding, a group of 8 rats was given bilateral injections of ACPD, the mGluR group I/II agonist, at 100 nmol or VEH. Additionally, to determine if specific mGluR group I activation was sufficient to induce food intake, DHPG at 1 nmol or ACSF was administered bilaterally within the LH of 6 different rats. These injections were bilateral to maximize their effectiveness and food intakes were measured from 15 min to 2 h post-injection.

2.4. Experiment 2: unilateral activation of LH mGluR1/5 elicits feeding

To determine whether group I mGluR activation in the LH elicits eating, rats were given unilateral injections of DHPG at 0.1 nmol, 0.5 nmol, 1 nmol, 5 nmol, 10 nmol, 25 nmol, 50 nmol or ACSF. Due to the wide range of drug concentrations, half the doses were tested in one group of 11 rats and the other half were tested in another group of 11 rats. Unilateral injections were employed to test the effectiveness of single site administration and to limit tissue damage. The data sets were then combined post hoc.

2.5. Experiment 3: unilateral activation of mGluR elicits eating

To determine if specific activation of mGluR5 increased food intake, a group of 19 rats was given unilateral LH injections of CHPG at 0.1 nmol, 0.5 nmol, 1 nmol, 5 nmol, 10 nmol or ACSF. As these doses were ineffective, a second batch of 19 rats was given unilateral LH injections of CHPG at 25 nmol or 50 nmol.

2.6. Experiment 4: does blocking mGluR1, mGluR5 or both receptors prevent DHPG-induced feeding within the LH?

To confirm receptor selectivity, a group of 11 rats was given unilateral injections of MTEP, a mGluR5 antagonist, (0.1 nmol, 1 nmol, 10 nmol) or YM-298198, a mGluR1 antagonist, (0.1 nmol, 1 nmol, 10 nmol) 5 min before injection of 1 nmol DHPG. An additional group of 7 rats was given unilateral injections of PHCCC (10 nmol) 5 min before LH injection of 1.0 nmol DHPG.

2.7. Experiment 5: does antagonism of mGluR1/5 in the LH suppress spontaneous food intake?

Animals implanted with bilateral LH cannulas were tested during the dark phase ($n = 11$). Bilateral injection of antagonists was employed to block the effects of bilateral release of endogenous glutamate. Specifically, satiated animals were tested 0–30 min before the onset of the dark phase with bilateral LH injection of either MTEP (0.1 nmol, 1 nmol or 10 nmol), YM-298198 (0.1 nmol, 1 nmol or 10 nmol) or ACSF and given fresh mash post-injection. A second group of rats ($n = 9$) were similarly tested with PHCCC (1 nmol or 10 nmol). Food intakes were measured 15 min to 4 h post-injection and a rest day was given between injections.

The animals used for the nocturnal PHCCC tests were food deprived for 24 h and once again injected in counterbalanced order with PHCCC (1 nmol, 10 nmol, 50 nmol) or ACSF. Fresh mash was given post-injection. Food intakes were measured 15 min to 4 h post-injection with a day between injections.

2.8. Histology and statistics

After the final behavioral test, animals were deeply anesthetized with Nembutol and perfused transcardially with 4% formaldehyde. The brains were removed from the skull and placed in the same fixative for at least 24 h. Using dry ice, brains were frozen, 100 μm thick coronal sections were cut and mounted on polarized glass slides. Slides were Nissil stained and coverslipped for later examination. Accuracy of placements was determined by comparing the image obtained using a projection microscope and the rat brain atlas of Paxinos and Watson (2007). Data from any placements 1.0 mm or more outside the borders of the intended LH injection site were discarded.

Food intake data were analyzed using ANOVA followed by Fisher's LSD (Least Significant Difference) test. A standard significance level of $p < 0.05$ was used for all tests.

3. Results

3.1. Experiment 1: bilateral activation of LH mGluR group I/II receptors elicits feeding

Representative cannula placements are presented in Fig. 1A and B. Bilateral administration of ACPD, the broad mGluR group I and II agonist, within the LH of satiated animals elicited feeding at a dose of 100 nmol. As shown in Fig. 2A animals ate an average of 6.7 g within 15 min of the injection with a maximum intake of 9.5 g. Repeated measures two-way ANOVA revealed significant effects of bilateral ACPD treatment [$F_{1,63} = 6.6$, $p < 0.05$], time [$F_{3,63} = 3.7$, $p < 0.05$] and interaction of both [$F_{3,63} = 4.1$, $p < 0.05$]. In comparison, bilateral administration of 1 nmol DHPG produced significant food intake 1hr post-injection with a total of about 2.3 g at 2 h