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Research Report

Stimulation of lateral hypothalamic kainate receptors selectively elicits feeding behavior

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ABSTRACT

Glutamate and its receptor agonists, NMDA, AMPA, and KA, elicit feeding when microinjected into the lateral hypothalamus (LH) of satiated rats. However, determining the relative contributions of AMPA receptors (AMPARs) and KA receptors (KARs) to LH feeding mechanisms has been difficult due to a lack of receptor selective agonists and antagonists. Furthermore, LH injection of KA produces behavioral hyperactivity, questioning a role for KARs in feeding selective stimulation. In the present study, we used the KAR agonist, (*RS*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl) propanoic acid (ATPA), which selectively binds the GluR5 subunit of KARs, to stimulate feeding, presumably via KAR activation. Using ATPA, we tested whether: (1) LH injection of ATPA elicits feeding, (2) prior treatment with the non-selective AMPA/KAR antagonist, CNQX, suppresses ATPA-elicited feeding, and (3) LH injection of ATPA elicits behavioral patterns specific for feeding. We found that injection of ATPA (0.1 and 1 nmol) elicited an intense feeding response (e.g., 4.8±1.6 g) that was blocked by LH pretreatment with CNQX, but was unaffected by pretreatment with the AMPAR selective antagonist, GYKI 52466. Furthermore, minute-by-minute behavioral analysis revealed that LH injection of ATPA increased time spent feeding to 55% of the initial test period with little or no effects on other behaviors at any time. In contrast, LH injection of KA similarly increased feeding but also produced intense locomotor activity. These data suggest that selective activation of LH KARs containing GluR5 subunit(s) is sufficient to elicit feeding.

1. Introduction

One of our major goals has been to determine the role of LH glutamate in the stimulation of feeding behavior. Toward this end, we have employed agonists of *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxale propionate (AMPA), and kainate (KA) receptors to provide evidence that these ionotropic glutamate receptor (iGluR) subtypes may play a role in feeding stimulation (Duva et al., 2002; Hettes et al., 2003; Khan et al., 1999; Stanley et al., 1993a,b, 1996). Additionally, we have demonstrated that LH pretreatment with the NMDA receptor (NMDAR) antagonist, D(–)-2-amino-5-phosphonopentanoic acid (DAP-5), blocked food intake elicited by subsequent LH injection of NMDA without affecting

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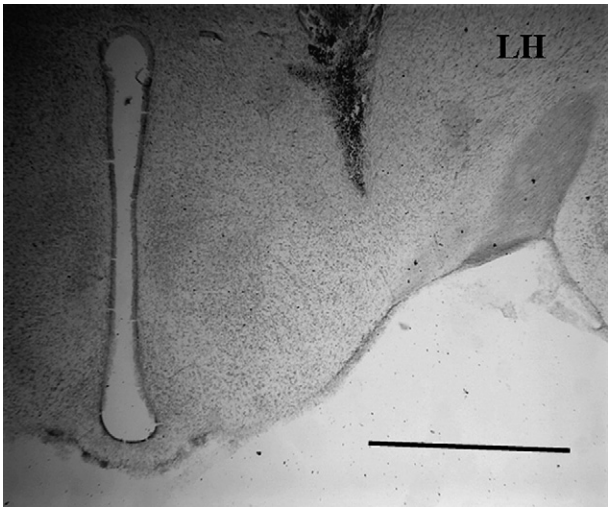

Fig. 1 – Cannula localized to the LH. Digital photomicrograph of coronal thionin stained section showing representative cannula placement in the LH. Scale bar equals 1 mm.

feeding elicited by LH injection of AMPA or KA (Stanley et al., 1996). While these data strongly suggest that activation of NMDARs in the LH elicits feeding, they also indicate that stimulation of AMPA receptors (AMPARs) and/or KA receptors (KARs) can elicit feeding in the absence of NMDAR activation.

Determining the relative contributions of the AMPA and KA iGluR subtypes to glutamatergic feeding mechanisms has been difficult due to the paucity of agonists and antagonists selective for each of these receptors (Lauridsen et al., 1985). However, (RS)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl) propanoic acid (ATPA) has been demonstrated to be a selective agonist for KARs containing the GluR5 subunit (Lauridsen et al., 1985; Pinheiro and Mulle, 2006), with over 100× greater potency for KARs than AMPARs (Clarke et al., 1997; Hoo et al., 1999). Here, we attempt to determine whether selective stimulation of KARs in the LH of satiated rats is sufficient to elicit feeding.

Several problems were associated with the presumed stimulation of KARs in our previous research (Stanley et al., 1993a,b). First, KA, the agonist we employed, is not specific for KARs versus AMPARs (Krogsgaard-Larsen et al., 1980). Second, KA is a potent neurotoxin and has been used to lesion brain tissue, including the LH (Lenard et al., 1988). Lastly, LH injection of KA produces intense and prolonged behavioral hyperctivity (Stanley et al., 1993a). This is troublesome as feeding behavior can occur as a component of general arousal (Antelman and Szechtman, 1975; Antelman et al., 1976; Morley et al., 1983) in the absence of direct activation of neural mechanisms specific to feeding stimulation. We suspected that using ATPA might resolve some of the complications associated with KA injection. Therefore, we tested the selective GluR5 agonist, ATPA, to determine whether: (1) LH injection of ATPA would elicit feeding, (2) this feeding could be suppressed by prior injection of an AMPA/KAR antagonist, and (3) behavioral changes elicited by its injection were specifically related to food intake. For comparison, we conducted similar tests using KA.

2. Results

2.1. *Locus of injection sites*

Histological analysis of the cannulation sites confirmed that the injections were typically centered in the middle region of the LH along its longitudinal axis, at least 0.3 mm lateral to the fornix. A representative example is provided in Fig. 1. The behavioral data from animals with placements outside of the LH were excluded from analysis.

2.1.1. *Experiment 1: LH injection of the KAR agonist, ATPA, elicits feeding in a dose-dependent manner*

As shown in Fig. 2, ATPA at 0.1 and 1.0 nmol elicited eating at intervals from 30 min to 4 h post injection. One-way ANOVAs revealed significant effects of dose at the 30 min, 1 h, and 4 h post injection times [$F_{3,44}$=6.76, 8.39, and 4.26 respectively (p<0.01)]. Compared to the dilute aCSF intake score of 0.1±0.4 g at 30 min post injection, the 0.1 and 1.0 nmol doses of ATPA increased consumption to 2.8±0.7 g and 4.8±1.6 g, respectively, with no statistically significant further increases at later intervals. These data suggest that the presumed stimulation of LH KARs by ATPA rapidly elicits feeding and that the effect is short-lived.

2.1.2. *Experiment 2: LH injection of the non-selective AMPA/KAR antagonist, CNQX, but not the AMPAR selective antagonist, GYKI 52466, blocks subsequent ATPA-elicited feeding*

To examine whether ATPA- or KA-elicited feeding might result primarily or exclusively from activation of KARs, as opposed to concurrent activation of AMPARs, we tested whether LH pretreatment with CNQX or GYKI 52466 would suppress ATPA- or KA-elicited feeding. As shown in Fig. 3A and consistent with the results of Experiment 1, LH injection of ATPA elicited eating (10.9±2.6 g at 30 min) and pretreatment with CNQX blocked that

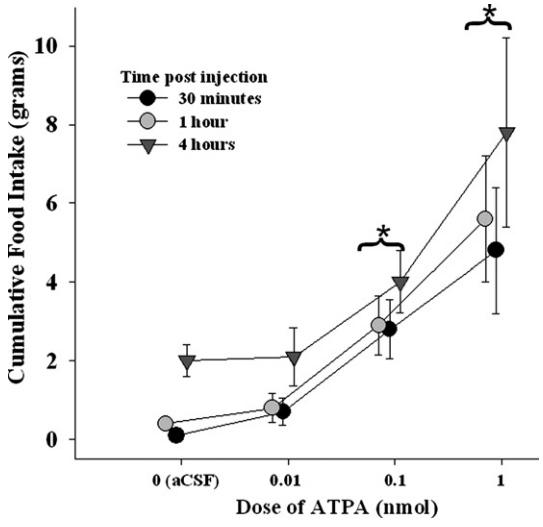

Fig. 2 – ATPA injected into the LH elicits feeding. Cumulative food intake (mean grams±SEM) 0.5, 1, and 4 h following injection of aCSF vehicle, or ATPA at 0.01, 0.1, and 1 nmol (N=12). *p<0.05, by one-way ANOVA and Student–Newman–Keuls test compared to vehicle (aCSF) control injection at matched times.