Reduction of food intake by cholecystokinin requires activation of hindbrain NMDA-type glutamate receptors

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Wright J, Campos C, Herzog T, Covasa M, Czaja K, Ritter RC. Reduction of food intake by cholecystokinin requires activation of hindbrain NMDA-type glutamate receptors. Am J Physiol Regul Integr Comp Physiol 301: R448–R455, 2011. First published May 11, 2011; doi:10.1152/ajpregu.00026.2011.—Intraperitoneal injection of CCK reduced food intake and triggered behavioral patterns similar to natural satiation. Reduction of food intake by CCK is mediated by vagal afferents that innervate the stomach and small intestine. These afferents synapse in the hindbrain nucleus of the solitary tract (NTS) where gastrointestinal satiation signals are processed. Previously, we demonstrated that intraperitoneal (IP) administration of either competitive or noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonists attenuates reduction of food intake by CCK. However, because vagal afferents themselves express NMDA receptors at both central and peripheral endings, our results did not speak to the question of whether NMDA receptors in the brain play an essential role in reduction of feeding by CCK. We hypothesized that activation of NMDA receptors in the NTS is necessary for reduction of food intake by CCK. To test this hypothesis, we measured food intake following IP CCK, subsequent to NMDA receptor antagonist injections into the fourth ventricle, directly into the NTS or subcutaneously. We found that either fourth-ventricle or NTS injection of the noncompetitive NMDA receptor antagonist MK-801 was sufficient to inhibit CCK-induced reduction of feeding, while the same antagonist doses injected subcutaneously did not. Similarly fourth ventricle injection of 3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphoric acid (D-CPPene), a competitive NMDA receptor antagonist, also blocked reduction of food intake following IP CCK. Finally, D-CPPene injected into the fourth ventricle attenuated CCK-induced expression of nuclear c-Fos immunoreactivity in the dorsal vagal complex. We conclude that activation of NMDA receptors in the hindbrain is necessary for the reduction of food intake by CCK. Hindbrain NMDA receptors could comprise a critical avenue for control and modulation of satiation signals to influence food intake and energy balance.

vagus; satiation; gut-brain peptides

SATIATION IS THE PROCESS by which food entering the gastrointestinal tract gradually reduces food intake, eventually resulting in termination of a meal. Previous reports from our group and others indicate that systemic administration of antagonists of N-methyl-D-aspartate-type glutamate receptors (NMDAr antagonists) delays satiation and increases meal size (see, for example, Refs. 8, 9, and 25). Additionally, we demonstrated that injecting NMDAr antagonists directly into the nucleus of the solitary tract (NTS), where vagal afferents from the gastointestinal tract synapse, increases meal size (19, 23, 46) and that lesions of the NTS abolish this effect (47). These observations suggest that NMDA receptors, perhaps in the NTS, participate in vagally mediated control of food intake.

The hypothesis that NMDA receptors participate in the process of satiation is supported by our prior reports that peripherally administered NMDA receptor antagonists attenuate reduction of food intake induced by CCK (11, 19), a gut peptide known to reduce food intake by activating abdominal vagal afferents (43, 44). However, functional evidence suggests that NMDA receptors on peripheral vagal afferent fibers modulate gastrointestinal sensory information (42). Consequently, it is not clear whether systemically administered NMDA receptor antagonists attenuate reduction of food intake by interfering with CCK-induced vagal activation in the periphery, or by altering vagal communication in the hindbrain. In the experiments reported here, we tested the hypothesis that antagonism of NMDA receptors in the hindbrain, near the site of vagal afferent termination, would be sufficient to prevent reduction of food intake and neuronal activation following intraperitoneal (IP) injection of CCK.

We found that competitive and noncompetitive NMDA receptor antagonists injected into the fourth ventricle or directly into the NTS, at doses that had no effect when injected subcutaneously or intraperitoneally, blocked reduction of food intake by IP CCK. We also found that fourth-ventricle administration of a competitive NMDA receptor antagonist attenuated CCK-induced increase in c-Fos expression in the hindbrain. Taken together, these results indicate that antagonism of NMDA receptors in the hindbrain is sufficient to attenuate reduction of food intake by CCK, and are consistent with the hypothesis that hindbrain NMDA receptors participate in control of food intake by modulating vagally mediated satiation signals in the hindbrain.

METHODS

Subjects. Male adult Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) were individually housed in hanging wire mesh cages in a vivarium under conditions of controlled illumination (12:12-h light-dark cycle; lights out 1800), humidity (70%), and temperature (22°C). Rats weighed 280–350 g at the beginning of experiments. Rats were handled daily and habituated to laboratory conditions before surgery or testing began, and they had ad libitum access to pelleted rodent diet (Teklad, Kent, WA) and water except during experiments and overnight fasts, as described below. All animal housing and experiments reported here were conducted in compliance with the National Institutes of Health’s Guide for Care and Use of Laboratory Animals under a protocol approved by the Washington State University Institutional Animal Care and Use Committee.
Drugs. MK-801 (Sigma, St. Louis, MO), d-3-(2-carboxypropionyl-4-yl)-1-propyl-1-phosphoric acid (D-CPPene, Tocris, Ellisville, MO), and sulfated cholesketokin octapeptide (CCK-8, Peptides International, Louisville, KY) were dissolved in sterile 0.9% NaCl. Peripheral injections of MK-801 and D-CPPene were made subcutaneously in a volume of 0.4 ml. CCK-8 was injected intraperitoneally in a volume of 1.0 ml/kg BW. Central injections of MK-801 and D-CPPene were made into the fourth ventricle (4V; 3 µl) or into the NTS (100 nl) were made via a 26-gauge guide cannula using a 33-gauge injector attached to a 5-µl Hamilton syringe. Sterile 0.9% NaCl injections served as the vehicle controls in all experiments.

Surgical procedures. Rats were anesthetized with 50 mg/kg ketamine, (Pfizer, New York, NY), 25 mg/kg xylazine (Vedco, St. Joseph, MO), and 2 mg/kg acepromazine (Boehringer Ingelheim Vetmedica, St. Joseph, MO), placed in a stereotaxic instrument, with head leveled between lambda and bregma, and implanted with 26-gauge stainless-steel guide cannulas (McMaster-Carr, Santa Fe Springs, CA) aimed for the rostral 4V (~10.5 mm caudal from bregma, −6.05 mm ventral from surface of the dura, and 0.0 mm from midline) or left medial NTS (0.1 mm caudal to the occipital crest 0.8 mm lateral to the midline, 7.8 mm ventral to the surface of the skull). Cannulas were secured to the skull using stainless-steel screws and mepacrynate (OrthoJet, Patterson Dental Supply, Spokane, WA). Immediately following surgery, the analgesic flunixin meglumine (2.5 mg/kg; MWI Veterinary Supply, Meridian, ID) and the antibiotic procaine penicillin G (300,000 units/kg; Norbrook, Lenexa, KS) were administered subcutaneously. Rats were allowed 2 wk postsurgical recovery, after which they all exceeded their presurgical body weights.

Effects of fourth-ventricular vs. peripheral administration of MK-801 on CCK-mediated reduction of chow intake. Rats (n = 15) were adapted to eat a meal of pelleted rodent diet over 30 min, following an overnight 15-h fast. Briefly, food, but not water, was removed 1 h prior to lights out, and a weighed amount was returned between 0900 and 1000 the following day. Food intake and spillage were measured over the ensuing 30 min, after which the rats were allowed ad libitum access to both food and water for at least the next 48 h, before another overnight fast was imposed. On experimental days, each rat received a 3-µl injection of either 0.9% NaCl or 6 µg MK-801 in 3 µl 0.9% NaCl via 4V cannula. This dose was chosen because it used for our behavioral studies, to induce a maximal amount of c-Fos expression in hindbrain nuclei, as previously described (15, 35). Briefly, sections were incubated in 0.1 M phosphate buffer (pH 7.4) for 18 h, followed by 4% paraformaldehyde (#19202; Electron Microscopy, Hatfield, PA) in 0.1 M phosphate buffer (pH 7.4). Immediately after perfusion, brains were collected, blocked, post-fixed in the same fixative for 2 h, and cryoprotected in 0.1 M phosphate buffer containing 25% sucrose overnight at 4°C. Thirty-micrometer coronal sections through the hindbrain were cut in a cryostat for immunohistochemical detection, and quantification of c-Fos-immunoreactive neuronal nuclei, as previously described (15, 35). Briefly, sections were incubated overnight 15-h fast. Food intake and spillage were measured, as described above. Each rat received the following testing injection combinations in the following order in both the NTS injection and subcutaneous injection tests: SC NaCl/IP NaCl, SC NaCl/IP CCK, SC MK-801/IP CCK, SC MK-801/IP NaCl, and SC NaCl/IP NaCl. For data analysis and presentation, intakes following the first and last NaCl/IP NaCl tests were averaged.

Effect of fourth ventricular vs. peripheral administration of D-CPPene on CCK-mediated reduction of chow intake. To examine the effect of hindbrain administration of a competitive NMDA receptor antagonist on reduction of food intake by CCK, D-CPPene (40 ng in 3 µl) was injected into the 4V (n = 11) following an overnight (16–20 h) fast, as described above. We chose the 40-ng dose of D-CPPene based on our prior dose-response studies with centrally administered competitive NMDA antagonists, including D-CPPene (18). Treatment combinations were as follows: 4V NaCl/IP NaCl, 4V NaCl/IP CCK, 4V D-CPPene/IP CCK, 4V NaCl/IP NaCl, and 4V NaCl/IP NaCl. D-CPPene was always administered 1 min before IP CCK (8–4 µg/kg). Immediately after CCK-8 injection, a preweighed amount of pelleted rodent diet was presented, and intake less waste was recorded at 30 min post-CCK-8 injection. Intakes for the first and last NaCl/IP NaCl tests were averaged for the purposes of data presentation and analysis.

Using rats from the same group tested for effects of 4V D-CPPene, we also examined the ability of IP D-CPPene to prevent CCK-induced reduction of food intake. Our prior published work indicated that an IP dose of 2 mg/kg, but not 0.5 or 1 mg/kg, of D-CPPene increases meal size in rats (19). Furthermore, we found that the 2 mg/kg dose was sufficient to attenuate reduction of food intake by IP CCK (19). Therefore, in the current experiment, we used a protocol identical to that used for testing the effects of 4V and subcutaneous NMDA antagonists on CCK-induced reduction of food intake, to examine the efficacy of D-CPPene (1 mg/kg; n = 5) and D-CPPene (2 mg/kg; n = 6) for the ability to attenuate reduction of food intake by CCK-8 (2 µg/kg ip). In so doing, we had the opportunity to replicate our prior results, using the 2 mg/kg ip dose, while using the 1 mg ip dose as a very conservative control for potential peripheral effects of our 4V-injected D-CPPene. The order of testing was the same as described in all previous experiments, except D-CPPene or 0.9% NaCl was injected intraperitoneally in a volume with 1 ml/kg. Thirty-minute food intake was measured as described above.

Effects of 4V administration of D-CPPene on CCK-induced expression of hindbrain c-Fos-immunoreactivity. To determine whether antagonism of hindbrain NMDA receptors would attenuate CCK-induced activation of neurons in the dorsal vagal complex, as indicated by increased expression of hindbrain c-Fos immunoreactivity, a group of naïve rats (n = 14) was divided in four subgroups of 3–5 rats each. After an overnight (15 h) fast, each subgroup received one of the following treatments: 4V NaCl/IP NaCl (n = 3), 4V NaCl/IP CCK (n = 3), 4V D-CPPene/IP CCK (n = 5), and 4V D-CPPene/IP NaCl (n = 3). Fourth-ventricle injections of D-CPPene (40 ng) or 0.9% NaCl were in volumes of 3 µl and were followed immediately by an IP injection of either CCK-8 (10 µg/kg) or 0.9% NaCl. We chose to use a CCK-8 dose of 10 µg/kg, rather than the 2 µg/kg dose that we used for our behavioral studies, to induce a maximal amount of c-Fos and reduce variability in expression between animals. Rats were not given food after injections. Rather, 90 min after IP injection, animals were rapidly and deeply anesthetized with isoflurane, exsanguinated, and perfused intracardially with 0.1 M phosphate buffer NaCl followed by 4% paraformaldehyde (#19202; Electron Microscopy, Hatfield, PA) in 0.1 M phosphate buffer (pH 7.4). Immediately after perfusion, brains were collected, blocked, post-fixed in the same fixative for 2 h, and cryoprotected in 0.1 M phosphate buffer containing 25% sucrose overnight at 4°C. Thirty-micrometer coronal sections through the hindbrain were cut in a cryostat for immunohistochemical detection, and quantification of c-Fos-immunoreactive neuronal nuclei, as previously described (15, 35). Briefly, sections were incubated...
Significantly different intakes (P < 0.05). However, wherever actual confidence limits were substantially less than 0.05, those P values are provided.

Figure 1 illustrates the effect of IP injection of CCK-8 (2 μg/kg) on reduction of 30-min food intake by 4V MK-801/IP CCK-8. a,b Different letters above bars indicate significantly different intakes (P < 0.01).

Prevention of CCK-8-induced reduction of food intake by NMDA channel blocker, MK-801. Figure 1 illustrates the effect of injecting MK-801 (500 ng/rat) directly into the NTS or subcutaneously on reduction of 30-min food intake following IP injection of CCK-8 (2 μg/kg). Histological examination revealed that 9 of 14 rats with cannulas aimed for the dorsal vagal complex had cannula tips located in the medial NTS or in the dorsal aspect of the DMV. The remaining 5 cannula placements were located in the cerebellum and were not included in statistical analysis for effects of NTS injections on reduction of food intake by IP CCK-8. Administration of MK-801 (500 ng) directly into the nucleus of the solitary tract prior to IP injection of CCK-8 prevented CCK-induced reduction of food intake [F(3,8) = 5.199, P = 0.007] (Fig. 2). Food intake following NTS MK-801/IP CCK-8 injection did not differ significantly from the NTS NaCl/IP NaCl condition, but it was significantly greater than following NTS NaCl/IP CCK (P < 0.01). When we injected the same

RESULTS

Prevention of CCK-8-induced reduction of food intake by 4V or NTS injection of MK-801. Figure 1 illustrates the effect of 4V or subcutaneous injection of MK-801 (6 μg/rat) on reduction of 30-min food intake following IP injection of CCK-8 (2 μg/kg). There were significant differences between treatment conditions in the 4V injection experiment [F(3,14) = 44.301, P < 0.001]. Thirty-minute food intake following 4V NaCl and CCK-8 (2 μg/kg ip) was reduced compared with intake following 4V NaCl and IP NaCl (P < 0.01). Intake after 4V MK-801/IP CCK-8 did not differ from intake after 4V NaCl/IP NaCl, but it was significantly greater than intake after 4V NaCl/IP CCK (P < 0.001). There also were significant differences between treatment conditions following subcutaneous injections of MK-801 or NaCl in combination with IP CCK or NaCl [F(3,7) = 79.5, P < 0.001]. Specifically, compared with SC NaCl/IP NaCl, 30-min food intake was significantly reduced following SC NaCl/IP CCK (P < 0.001). However, subcutaneous injection of MK-801 (6 μg) did not attenuate reduction of food intake by IP CCK. Intake after SC MK-801/IP CCK was significantly reduced compared with intake after SC NaCl/IP NaCl (P < 0.001) and was not significantly different from intake after SC NaCl/IP CCK.

Figure 2 illustrates the effects of injecting MK-801 (500 ng/rat) directly into the NTS or subcutaneously on reduction of 30-min food intake following IP injection of CCK-8 (2 μg/kg). Histological examination revealed that 9 of 14 rats with cannulas aimed for the dorsal vagal complex had cannula tips located in the medial NTS or in the dorsal aspect of the DMV. The remaining 5 cannula placements were located in the cerebellum and were not included in statistical analysis for effects of NTS injections on reduction of food intake by IP CCK-8. Administration of MK-801 (500 ng) directly into the nucleus of the solitary tract prior to IP CCK-8 prevented CCK-induced reduction of food intake [F(3,8) = 5.199, P = 0.007] (Fig. 2). Food intake following NTS MK-801/IP CCK-8 injection did not differ significantly from the NTS NaCl/IP NaCl condition, but it was significantly greater than following NTS NaCl/IP CCK (P < 0.01). When we injected the same...
500-ng dose of MK-801 subcutaneously prior to CCK administration, reduction of food intake by IP CCK was not attenuated. Results from 4V and NTS injections of MK-801 indicate that noncompetitive blockade of hindbrain NMDA channels prevents CCK-induced reduction of food intake at doses that are not effective when injected subcutaneously.

Prevention of CCK-8-induced reduction of food intake by 4V injection of d-CPPene. Unlike MK-801, a noncompetitive blocker of the NMDA receptor ion channel, d-CPPene competes with glutamate for its binding site on the NR2A and NR2B channel subunits. Figure 3C illustrates the effects of 4V d-CPPene (40 ng/rat) on reduction of 30-min food intake following IP injection of CCK-8 (2 μg/kg). There were significant differences in food intake between treatment conditions when d-CPPene or NaCl was administered via the 4V followed by either intraperitoneally administered CCK-8 or NaCl \( F(3,9) = 41.851, P < 0.001 \). Thirty-minute food intake following 4V NaCl/IP CCK was significantly reduced compared with 4V NaCl/IP NaCl. Fourth-ventricle injection of d-CPPene (40 ng) prevented reduction of food intake by IP CCK. Specifically, intake following 4V d-CPPene/IP CCK did not differ from intake after 4V NaCl/IP NaCl. In experiments combining IP injection of d-CPPene at a dose of 1 mg/kg with IP CCK-8 (2 μg/kg), CCK-8 significantly reduced 30-min food intake \( F(3,4) = 29.214, P < 0.001 \). However, IP d-CPPene 1 mg/kg did not significantly attenuate reduction of food intake by IP CCK-8 (Fig. 3A). IP CCK-8 (2 μg/kg) reduced food intake \( F(3,5) = 23.139, P < 0.001 \), in another experiment in which the IP d-CPPene dose was increased to 2 mg/kg. However, reduction of 30-min food intake by IP CCK was prevented by 2 mg/kg d-CPPene. Intake after IP d-CPPene/IP CCK-8 was significantly greater than after IP NaCl/IP CCK-8 \( P < 0.001 \) and did not differ from IP NaCl/IP NaCl. Thus, 4V administration of a competitive NMDA receptor antagonist prevented reduction of food intake by IP CCK-8 at a dose two orders of magnitude lower than a dose that was effective when injected intraperitoneally.

Attenuation of CCK-8-induced increase in hindbrain c-Fos immunoreactivity by 4V injection of d-CPPene. In an experiment to determine whether 4V d-CPPene (40 ng) could attenuate increased expression of hindbrain c-Fos immunoreactivity following IP CCK-8 (10 μg/kg), there were significant effects of treatment on hindbrain c-Fos immunoreactivity \( F(3,10) = 80.430, P < 0.01 \). Moreover, there was significant interaction between treatment and brain area \( F(3,9) = 53.090, P < 0.001 \). Compared with IP NaCl, IP CCK-8 increased c-Fos immunoreactivity in the mNTS and AP \( P < 0.01 \), but not in the cNTS or DMV. Following 4V d-CPPene, c-Fos immunoreactivity in both the AP and NTS was significantly reduced compared with 4V NaCl/IP CCK-8 \( P < 0.01 \), but it remained significantly elevated compared with 4V NaCl/IP NaCl (Figs. 4 and 5).

**DISCUSSION**

Previously published findings from our group reveal that systemic administration of NMDA antagonists attenuates reduction of food intake by IP CCK (11, 19) and reduces CCK-induced c-Fos expression in the mNTS (19). These observations indicate that NMDA-type glutamate receptors participate in vagally mediated reduction of food intake. However, they do not provide any information regarding the localization of NMDA receptors that participate in CCK-induced reduction of food intake. This issue is not trivial because NMDA receptors are expressed by vagal afferent neurons in the nodose ganglia (12, 13), as well as by central vagal afferent terminals and postsynaptic neurons in the NTS (1). Moreover, electrophysiological experiments suggest that peripheral NMDA receptors may modulate gastrointestinal vagal afferent activity (42). Therefore, it is plausible that NMDA receptor antagonists could alter the behavioral responses to CCK through actions at peripheral vagal afferent sites. Our results reported here show that reduction of 30-min food intake by IP CCK is prevented by hindbrain administration of NMDA receptor antagonists. Moreover, we demonstrate that the same antagonist doses that prevent CCK-induced reduction of food intake when injected into the hindbrain fail to attenuate CCK’s effect when injected subcutaneously or intraperitoneally.
Therefore, our results indicate that central NMDA receptors play an important, and perhaps essential, role in control of food intake by CCK.

Our current results strongly support the hypothesis that hindbrain NMDA receptors are necessary participants in CCK-induced activation of mNTS neurons and reduction of food intake. Nevertheless, our results do not rule out additional participation from peripherally located NMDA receptors. Circumstantial evidence continues to suggest a possible contribution to CCK-induced reduction of feeding by peripheral NMDA receptors. As mentioned above, virtually all vagal afferent neurons, including those that innervate the upper GI tract, express NMDA receptor subunit immunoreactivity (12, 13). Furthermore, electrical activity recorded from the distal ends of cut vagal afferents is modulated by NMDA receptors (42), suggesting that receptors in peripheral vagal afferent membranes may participate in relaying sensory information to the brain. Immunoreactivity for glutamate and vesicular glutamate transporter (VGLUT) has been reported in neurons of enteric plexus of the guinea pig and rat small intestines (45, 51). Likewise, immunoreactivity to glutamate has been reported in the intrinsic and extrinsic innervations of the human digestive tract (15), and there is compelling evidence that at least some gastrointestinal vagal afferent terminals are immunoreactive for VGLUTs (32). Finally, calcium-dependent glutamate release has been demonstrated in the enteric nervous system (35). In summary, vagal afferent processes innervating the gastrointestinal tract express NMDA receptor subunits, and there appear to be multiple sources of glutamate that could act on peripheral vagal afferent processes. Therefore, our results do not obviate the possibility that, as well as central, NMDA receptors could participate in control of food intake by vagal signals from the GI tract, such as CCK.

At present, the cellular location of hindbrain NMDA receptors that are necessary for CCK-induced reduction of food intake is uncertain. NMDA receptor subunit immunoreactivity and mRNA have been observed in hindbrain neurons (5, 7, 21, 31, 49, 50), including those in the NTS. Therefore, it is plausible that NMDA antagonists attenuate reduction of food intake by vagal signals, including CCK, are located presynaptically on vagal afferent processes in the NTS. In support of this hypothesis, we have previously reported that administration of NMDA receptor antagonists into the NTS increases food intake (18, 23, 46). Most interesting, however, is the fact that after degeneration of central vagal afferent terminals, NTS injection of the antagonist does not increase food intake (17). These results are consistent with the working hypothesis that at least some hindbrain NMDA receptors participating in reduction of food intake by vagal signals, including CCK, are located presynaptically on vagal afferent processes in the NTS.

The cellular mechanisms by which antagonism of hindbrain NMDA receptors could attenuate reduction of CCK-induced reduction of food intake remain speculative. However, one possibility is that antagonism of presynaptic or extrasynaptic NMDA receptors reduces transmitter release from vagal afferent terminals in the NTS. This sort of modulation of neurotransmitter release by activation of presynaptic or extrasynaptic NMDA receptors is well documented in the central nervous system, including in the visual cortex (41), entorhinal cortex (6), and glutamatergic somatosensory afferent terminals in the spinal dorsal horn (3, 22, 39). Furthermore, at least one synapse has been described in which NMDA channels provide obligatory calcium influx to trigger glutamate release (10). In this instance, a glutamatergic axoaxonal input, with axonal

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**Fig. 4.** Effect of fourth-ventricle injection of NMDA receptor antagonist, d-CPPene, on number of c-Fos-immunoreactive nuclei in the hindbrain dorsal vagal complex 90 min after IP injection of CCK-8. c-Fos-immunoreactive nuclei were counted in coronal sections from 3 to 5 rats per treatment condition, at four rostrocaudal levels of the hindbrain, as established using the stereotaxic atlas of Paxinos and Watson (30). Counts were made for the following areas of interest at all rostrocaudal levels of the hindbrain, as established using the stereotaxic atlas of Paxinos and Watson (30): area postrema (AP), the commissural NTS (cNTS), the medial NTS (mNTS), and the dorsal motor nucleus of the vagus (DMV). The counts for areas of interest at all levels were summed for each animal, and averages and standard errors were calculated for each area under each treatment condition. Dissimilar letters above bars indicate significantly different numbers of c-Fos-immunoreactive nuclei (P < 0.01).
postsynaptic NMDA receptors, is required in order for an invading action potential to trigger glutamate release from the innervated terminal. Conceivably, descending projections from brain areas, such as the paraventricular hypothalamic nucleus (52) or the amygdala (40), might provide glutamatergic axoaxonal synapses on preterminal vagal afferent endings, which could modulate transmitter release from vagal afferent terminals. Alternatively, extrasynaptic vagal afferent NMDA receptors could be activated by glial glutamate release (16, 38), thereby enhancing their excitability and increasing transmitter release from vagal afferent terminals when invading action potentials depolarize them.

One might think that positive modulation of glutamate release from vagal afferent terminals by NMDA receptors located on the afferents themselves is a recipe for runaway positive feedback. However, it should not be assumed that vagal afferent NMDA receptors are auto-receptors for vagally released glutamate. If NMDA receptors that modulate transmitter release from primary vagal afferents were located on portions of the preterminal axon, apart from the site where glutamate is supplied to NTS postsynaptic neurons, then uncontrolled positive feedback would be obviated. Unfortunately, ultrastructural and electrophysiological examinations of NMDA receptor distributions and function on vagal afferents have not reached the necessary level of resolution to evaluate this hypothesis.

Vagal afferents that are activated by CCK synapse on neurons in the NTS of the dorsal vagal complex (34, 37) and increased c-Fos immunoreactivity following CCK injection are interpreted to reflect the increase in vagal afferent activation that is evoked by CCK (29). We found that 4V administration of a competitive NMDA receptor antagonist, d-CPPene, attenuated the increase in nuclear c-Fos immunoreactivity in the dorsal vagal complex following CCK injection, supporting the interpretation that NMDA antagonists attenuate reduction of feeding by CCK by reducing vagal afferent activation of postsynaptic neurons in the NTS. It is worth noting, however, that while d-CPPene reduced CCK-induced NTS c-Fos expression, it did not return it to levels expressed by vehicle-treated control rats or by rats that received d-CPPene, but not CCK. There are several potential explanations for the fact that attenuation of CCK-induced c-Fos expression is significant, but incomplete. First, most electrophysiological experiments indicate that vagal afferent activation of fast excitatory conduances in NTS neurons is mediated primarily by AMPA/kainate-type glutamate receptors (2). However, NMDA receptors may play a modulatory role for some, but perhaps not all, vagal afferent inputs. For example, NMDA receptor currents in some neurons are responsible for extending the time period during which postsynaptic neurons are depolarized following primary activation of AMPA/kainate-type glutamate receptors (2). If this mechanism pertains to CCK-induced activation of

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**Fig. 5.** Representative images of dorsal hindbrain sections stained to reveal c-Fos-immunoreactivity following fourth-ventricle d-CPPene and IP CCK. Column labels indicate treatment conditions, and row labels indicate the distance caudal to bregma for sections in that row. 4th V, fourth ventricle; NTS, nucleus of the solitary tract, including the medial and commissural subnuclei; AP, area postrema.
vagal afferents, then antagonism of hindbrain NMDA receptors might reduce, but not abolish, increased excitation of NTS neurons excited by CCK. An alternative possibility is that NMDA receptors are necessary for excitation of some, but not all, CCK-responsive vagal circuits. In this case, one would predict that NMDA receptors, while critical for CCK-induced c-Fos expression in neurons that participate in control of food intake, might not be essential for activation of neurons that mediate other responses to CCK. In support of this hypothesis, it is pertinent to note that reduction of food intake and inhibition of gastric emptying by CCK both are mediated by vagal afferent neurons (33, 34, 36, 43, 44). However, as we previously reported, NMDA receptor antagonism attenuates only CCK-induced reduction of food intake, but not CCK-induced inhibition of gastric emptying (11). A third possibility is that d-CPPene doses that were able to block the behavioral consequences of exogenous CCK administration did not eliminate release of afferent transmitter entirely, but rather reduced release of a peptide cotransmitter necessary for the behavioral effects of CCK. A fourth alternative explanation for incomplete reduction of CCK-induced c-Fos immunoreactivity relates to the fact that d-CPPene competes with glutamate for binding to the NR2(A/B) subunit of the NMDA receptor. Because we administered the antagonist as a bolus prior to injection of CCK, it is possible that effective competitive concentrations of d-CPPene were present at the receptors for only a portion of the time during which CCK was stimulating glutamate release and, therefore, only reduced NMDA receptor activation during part of the period of vagal activation. A test of this last hypothesis theoretically is possible using a noncompetitive, open-channel, antagonist, like MK-801. However, we have observed that MK-801, by itself, induces very high levels of c-Fos expression throughout the brain, including in the DVC. Therefore, we have not found it useful to substitute this antagonist in experiments where c-Fos expression is the dependent variable.

Perspectives and Significance

The results of our experiments indicate that injections of NMDA receptor antagonists into the NTS, where vagal afferents terminate, reverse reduction of food intake by systemically administered CCK. Consistent with reduction of CCK-evoked vagally mediated NTS excitation, 4V NMDA receptor antagonist injection also reduce CCK-induced c-Fos immunoreactivity in the NTS. These results indicate that NMDA receptors in the dorsal vagal complex of the hindbrain play an essential role in vagally mediated control of food intake by CCK. The cellular mechanisms that account for such dramatic attenuation of CCK’s effects are not yet clear. However, NMDA receptors located on vagal afferents and/or on NTS neurons, could provide a means for glutamatergic inputs from other brain regions to adjust the strength of gastrointestinal satiation signals at the point where they enter the brain. Moreover, it is possible that activation of hindbrain NMDA receptors may trigger plastic changes in vagal afferent/NTS synapses, thereby altering vagal satiation signals. NMDA receptor channels are calcium channels (14). Hence, their activation not only affects membrane charge transfer, but also provides increased intracellular calcium that initiates signaling cascades, protein phosphorylations, and transcriptional changes, leading to alterations in neuronal function that may occur over seconds, minutes, and hours (20, 26, 48). Consequently, NMDA receptor activation can translate brief neuronal volleys into sustained changes in neuronal and network activity that influence behaviors. The classical example of this sort of NMDA receptor-mediated plasticity is, of course, long-term potentiation (4, 27, 28), which now is recognized in many CNS structures beyond the hippocampus (24), perhaps including the NTS. Consequently, hindbrain NMDA receptors on or near vagal afferent endings comprise an exciting avenue of investigation into the neural substrates of satiation and could provide an opportunity for therapeutic intervention in satiation mechanisms.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

References

HINDBRAIN NMDA RECEPTORS MEDIATE CCK FEEDING EFFECT


