NMDA and non-NMDA Receptors Mediate Responses in the Primary Gustatory Nucleus in Goldfish

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Abstract

Primary gustatory afferents from the oropharynx of the goldfish, *Carassius auratus*, terminate in the vagal lobe, a laminated structure in the dorsal medulla comparable to the gustatory portion of the nucleus of the solitary tract in mammals. We utilized an *in vitro* brain slice preparation to test the role of different ionotropic glutamate receptor subtypes in synaptic transmission of gustatory information by recording changes in field potentials after application of various glutamate receptor antagonists. Electrical stimulation of the vagus nerve (NX) evokes two short-latency postsynaptic field potentials from sensory layers of the vagal lobe. 6,7-Dinitroquinoxaline-2,3-dione and 6-nitro-7-sulphamoylbenzo[*f*]quinoxaline-2,3-dione, two non-*N*-methyl-D-aspartate (NMDA) ionotropic receptor antagonists, blocked these short-latency potentials. Slower potentials that were revealed under Mg^{2+}-free conditions, were abolished by the NMDA receptor antagonist, D(–)-2-amino-5-phosphonovaleric acid (APV). Repetitive stimulation produced short-term facilitation, which was attenuated by application of APV. These results indicate that the synaptic responses in the vagal lobe produced by stimulation of the gustatory roots of the NX involve both NMDA and non-NMDA receptors. An NMDA receptor-mediated facilitation may serve to amplify incoming bursts of primary afferent activity.

Introduction

Excitatory amino acids (EAAs) are the neurotransmitters of primary afferent fibers in many sensory systems. Recent evidence from immunocytochemical and electrophysiological studies indicate that glutamate or glutamate receptors mediate neurotransmission at the primary central targets of visual (Li *et al.*, 1996), auditory (Hackney *et al.*, 1996), somatosensory (Clements and Beitz, 1991), olfactory (Berkowitz *et al.*, 1994) and vomeronasal (Dudley and Moss, 1995) systems. Recent studies of the gustatory system in mammals and fish indicate that glutamate receptors are involved in gustatory transmission as well (Finger, 1994; Wang and Bradley, 1995; Li and Smith, 1997; Smeraski *et al.*, 1998).

Study of neurotransmitters involved at the primary gustatory nucleus in mammals, the nucleus of the solitary tract (nTS), is complicated by the fact that the nTS not only receives taste information from the oropharynx via facial, glossopharyngeal and vagus nerves, but also receives general visceral sensory information from cardiovascular, respiratory and gastrointestinal systems via the glossopharyngeal and vagus nerves (Hamilton and Norgren, 1984; Loewy, 1990). Although the nTS in mammals is organized into subnuclei according to a rough somatotopic/viscerotopic mapping of afferents from oropharyngeal and visceral regions, there is extensive rostrocaudal overlap of functional domains (Whitehead and Frank, 1983; Hamilton and Norgren, 1984; Altschuler *et al.*, 1989; Loewy, 1990; Takagi *et al.*, 1995). This distribution of diverse projections along the rostrocaudal extent of the nTS makes it difficult to study *in vitro* the synaptic transmission intrinsic to only the gustatory system. Further, electrical stimulation of the solitary tract in *in vitro* preparations of the mammalian nTS not only activates gustatory fibers, but is likely to activate intranuclear, interneuronal systems as well (Brooks *et al.*, 1992; Kawai and Senba, 1996). Conversely, determining the relative contributions of glutamate receptor subtypes *in vivo* is difficult without control over the extracellular medium and antagonist concentrations. Nonetheless, both *in vitro* and *in vivo* studies of the rostral nTS suggest that both non-NMDA and NMDA glutamate receptor subtypes contribute to gustatory transmission (Wang and Bradley, 1995; Li and Smith, 1997). Likewise, in caudal, non-gustatory nTS, although fast synaptic transmission involves primarily non-NMDA receptors (Andresen and Yang, 1990; Andresen and Kunze, 1994), a recent intracellular study has found that NMDA receptor-mediated currents also contribute to synaptic transmission between

visceral afferent fibers and nTS neurons (Aylwin *et al.*, 1997).

Studies of the mammalian gustatory system have focused on rostral regions of the nTS that receive input from facial and glossopharyngeal nerves. In contrast, studies of synaptic transmission that focus on more caudal regions of the nTS involve vagal input associated with general visceral systems (e.g. cardiovascular). Thus, the relative contributions of glutamate receptor subtypes to gustatory transmission of the vagus nerve remains unclear.

The gustatory system in goldfish, unlike mammalian systems, has a clear segregation of gustatory nuclei from general visceral sensory nuclei, as well as a clear separation of vagal from facial or glossopharyngeal gustatory nuclei. Vagal gustatory afferents project topographically (Morita and Finger, 1985) to a specialized vagal lobe used in food selection and retention (Sibbing *et al.*, 1986). The separation of gustatory from general visceral sensory input and the overall organization of the vagal lobe (Morita and Finger, 1985) make it an ideal structure in which to examine the physiological and pharmacological properties of a primary vagal gustatory nucleus.

Extracellular recordings from an *in vitro* slice preparation (Finger and Dunwiddie, 1992) revealed two short-latency negative-going postsynaptic potentials evoked from sensory layers of the vagal lobe following electrical stimulation of incoming vagal gustatory root fibers. The elimination of these responses by kynurenic acid, a non-specific glutamate antagonist, suggests that EAA receptors may mediate synaptic transmission in this primary vagal gustatory nucleus (Finger, 1994; Smeraski *et al.*, 1998). Here we report the respective contributions of NMDA and non-NMDA receptors on responses evoked by stimulation of the vagal primary gustatory fibers.

Portions of the data reported below have been presented in abstract form (Smeraski *et al.*, 1996a,b; Finger *et al.*, 1997).

Materials and methods

Slice preparation

For the initial experiments we prepared goldfish (10–20 cm, *Carassius auratus*) for *in vitro* physiological recordings as described previously (Finger and Dunwiddie, 1992). The brains were removed and blocked in the transverse plane following transcardial perfusion with cold, oxygenated artificial cerebrospinal fluid (aCSF, see below). Subsequent slicing (300–800 μm thick) on a Vibratome involved mounting the vagal lobe on a platform with acrylate tissue glue (Vetbound, 3M, St Paul, MN) and embedding the lobe in 2% agar. Latter preparations of the vagal lobe eliminated prior perfusion with aCSF and the use of a Vibratome. Instead, after rapid removal of the brain from the skull, the brain was placed on an operating platform made of Sylgard (Dow Corning, Midland, MI).The vagal lobe then was cut in the transverse plane with a scalpel and the slices (500 μm–1 mm thick) covered with oxygenated aCSF.

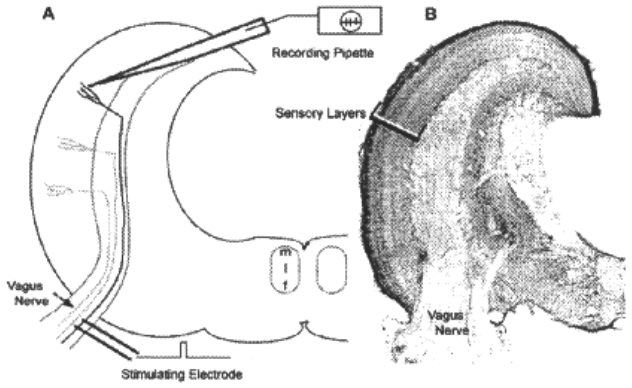

Figure 1 **(A)** Schematic representation illustrating stimulating electrode on incoming vagal fibers in a transverse slice of the vagal lobe. The recording electrode is placed at a depth within sensory layers that show maximal responses of both N2 and N3 peaks at a given stimulus position within the vagal root (see Finger and Dunwiddie, 1992). As the recording electrode is placed at different sites along a course parallel to the dorsal surface of the lobe, evoked responses following electrical stimulation are observed only within a limited area, indicating that only a relative small number of afferent fibers are effectively stimulated. **(B)** Photomicrograph of a vagal lobe section illustrating sensory layers (bracket). Figure adapted from Finger and Dunwiddie (1992).

Extracellular recordings

Vagal lobe slices were placed in a recording chamber and superfused continuously with fresh oxygenated aCSF (20–25°C) at a flow rate of 2 ml/min. Teflon-coated nichrome bipolar electrodes connected to a Medical Systems stimulus isolation unit were used to stimulate the afferent gustatory fibers (NX, vagus nerve, Figure 1). Generally, single pulses (0.2 ms in duration at 20 V) or a pair of pulses (60 ms apart) were applied every 5–15 s while searching for responses from local regions (within layers VI–VIII) of the sensory layers of the vagal lobe. The paired-pulse stimulation paradigm has the advantage of permitting assessment of both the initial response (following the first stimulus of a pair), as well as any short-term facilitation/inhibition of the second response (Finger and Dunwiddie, 1992). The recording electrode (2M NaCl-filled glass micropipette) was placed at a site along the cut surface of the vagal lobe that exhibited the maximum amplitude of the evoked postsynaptic population responses at a given stimulus strength (see Results; Finger and Dunwiddie, 1992). After the optimal recording site was determined, stimulus strengths were reduced to evoke approximately one half to two thirds the maximal amplitude response. Stimulation rates for paired-pulse paradigms (with inter-pulse-intervals [IPIs] of 60 ms) were every 15, 30 or 60 s. For repeated stimulation, trains of five pulses with IPIs of 15 ms were applied once every 60 or 90 s for 5–6 min, followed by a period of no stimulation for 5–10 min.

Pharmacology

Known concentrations of antagonists were tested by using calibrated syringe pumps to inject antagonists into the flow of aCSF (2 ml/min) that entered the recording chamber. Different concentrations of antagonists were obtained by adjusting the speed of the syringe pump. The stock solutions of antagonists were 100–1000 times the final chamber concentration, so injection of drugs did not significantly affect the flow rate or pH. All drug concentrations listed below refer to final bath concentrations. Antagonists were applied for 15–30 min, to allow for bath equilibration and until the evoked waveform stabilized. The interval between application of different drugs was at least 20–30 min, to allow the evoked responses to approach initial control levels or stabilize after washout of the drug. However, in cases when a combination of drugs was used, each drug was added to the bathing medium sequentially, so that effects of the first drug alone were observed. In some cases the order of application of each drug in subsequent trials was then reversed. Antagonists at concentrations selective for non-NMDA receptors were 6,7-dinitroquinoxaline-2,3-dione (DNQX; 5 or 10 μM) and 6-nitro-7-sulphamoyl-benzo[*f*]quinoxaline-2,3-dione (NBQX or its disodium salt; 1 or 5 μM). D(–)-2-amino-5-phosphonovaleric acid (APV; 50 μM, dissolved in water) was applied to block NMDA receptors. The antagonists DNQX, NBQX and NBQX disodium salt were obtained from Tocris Cookson, Inc. (Ballwin, MO), APV from Sigma (St Louis, MO). DNQX and NBQX were first dissolved in a small quantity (~200 μl) of dimethyl sulfoxide (DMSO; Sigma), then diluted with water so that the final concentration of DMSO in the bathing medium was not greater than 0.05%. The lipophilic antagonists DNQX and NBQX do not typically wash out quickly from tissue. In our goldfish slices, both of the drugs required long (~2 h) washout times for recovery, in those cases when recovery occurred. These long times may be due to a variety of technical factors, but are most likely related to the thicker slices used in the present studies.

aCSF medium

Standard aCSF contained (in mM) 131 NaCl, 20 $NaHCO_3$, 2 KCl, 1.25 KH_2PO_4 monobasic, 2 $MgSO_4$, 2.5 $CaCl_2$, 10 dextrose, pH 7.0–7.2 after oxygenation (Mathieson and Maler, 1988). In addition to standard aCSF, Mg^{2+}-free aCSF ($MgSO_4$ omitted and $CaCl_2$ increased to 4.5 mM) was also used as a bathing medium to reveal any responses blocked by the presence of Mg^{2+}. Also, Ca^{2+}-free aCSF bathing medium was made similar to the standard aCSF, but contained 2.5 mM $MgCl_2$ and no $CaCl_2$.

Data analysis

Electrophysiological data were amplified using a high gain differential AC amplifier, digitized with an A/D converter (RC Electronics) and subsequently analyzed by a PC based software developed in the laboratory (of T.V.D.). Evoked field potentials from single slices were averaged over several minutes (5–36 sweeps) of stable responding before, during and after application of antagonists or change in bathing medium (aCSF). Changes in amplitude of the postsynaptic responses under antagonistic conditions were characterized as a percent of the control (without antagonists) and exemplified changes in synaptic efficacy. A change of 20% from control was used as a conservative cutoff to indicate a substantial change in response amplitude was due to antagonist application.

Results

Non-NMDA receptor antagonists: DNQX and NBQX

As has been previously reported, electrical stimulation of vagal afferents evokes a complex response; the two principal negative-going components (N2 and N3, Figure 2A) are synaptically mediated (Finger and Dunwiddie, 1992). Both N2 and N3 peak responses were abolished following application of a selective non-NMDA receptor antagonist, DNQX (10 μM, n = 7 slices). In four of the seven slices, the amplitudes of both N2 and N3 population responses approached control levels within 30–100 min of washout of the antagonist (Figure 2). In the remaining three slices, only the N2 peak showed minor recovery to control levels (≤40% of control amplitude) even after 40–75 min of washout of the antagonist.

Another non-NMDA competitive antagonist, NBQX, has been reported to be more effective than DNQX in blocking AMPA responses (Yu and Miller, 1995). At 5 μM concentrations, NBQX blocked both N2 and N3 responses (n = 4 slices, not shown). Recovery of the evoked responses following removal of NBQX (60–140 min) from the bathing medium was minimal in three slices; neither response (N2 or N3) recovered in the fourth slice. Thus the two non-NMDA antagonists DNQX and NBQX are effective at blocking the fast synaptic responses to gustatory nerve stimulation.

Mg^{2+}-free aCSF and NMDA receptor antagonist: APV

To test whether NMDA receptors are involved in primary afferent activation of sensory neurons in the vagal lobe, the brain slices were superfused with Mg^{2+}-free aCSF. The removal of Mg^{2+} from the medium eliminates the voltage-dependent Mg^{2+}-block of NMDA receptors and can reveal slower wave components characteristic of NMDA receptor-mediated responses (McBain and Mayer, 1994). Responses evoked in Mg^{2+}-free medium are illustrated in Figures 3 and 4. Following vagal nerve stimulation in Mg^{2+}-free medium, an additional negative-going potential with longer peak latency (~10–15 ms) and duration (20–40 ms) typically was uncovered. The amplitudes of N2 and N3 population responses to the first stimulus pulse of a pair also were generally enhanced, which is likely attributable to increased transmitter release in the Mg^{2+}-free high-Ca^{2+} buffer. These

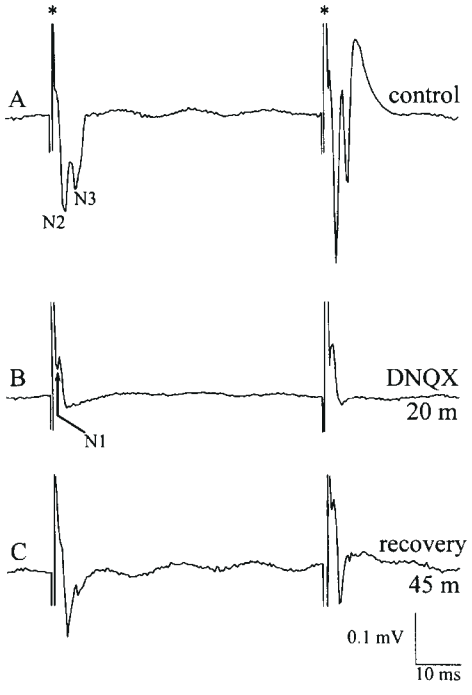

Figure 2 Signal averages of field potentials evoked by paired-pulse stimulation from a single slice under **(A)** control conditions in standard aCSF bathing medium prior to antagonist application, **(B)** in the presence of DNQX (10 μM) for 20 min and **(C)** during recovery from antagonist following 45 min in standard aCSF. N2 and N3, postsynaptic responses; N1, presynaptic fiber spike (see Finger and Dunwiddie, 1992), which at times can be observed in averaged waveforms as a shoulder on the negative-going phase of the N2 peak; * denotes position of stimulus artifact that was truncated for each trace in this and subsequent figures. Note the facilitated response following the second stimulus in this slice under control conditions. In another slice (Figure 3A), the second response did not exhibit paired-pulse facilitation.

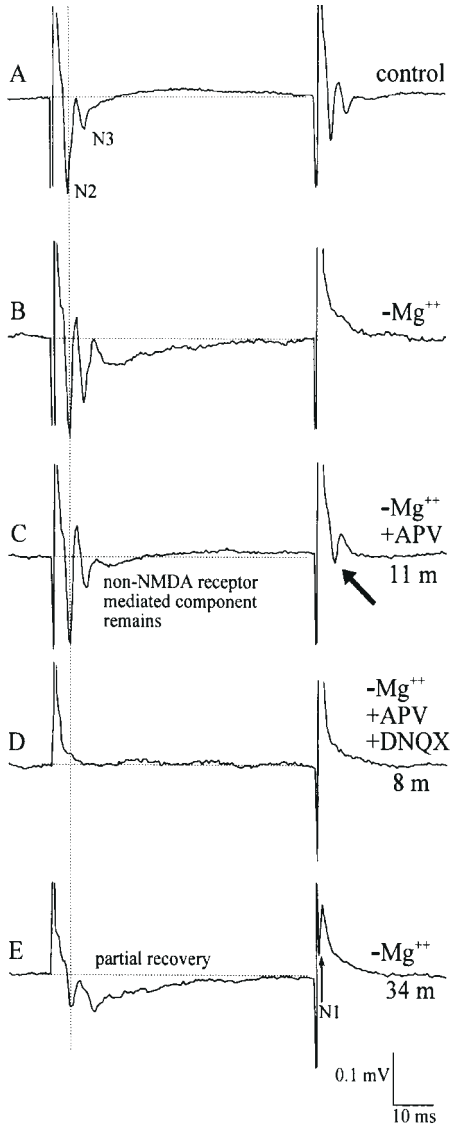

Figure 3 Evoked responses following paired-pulse stimulation of vagal fibers **(A)** under control conditions in standard aCSF, **(B)** in Mg^{2+}-free aCSF ($-Mg^{2+}$); note the absence of response following the second stimulus pulse, **(C)** in the presence of APV (50 μM) in Mg^{2+}-free aCSF for 11 min; the 'arrow' notes that second response partially returned, **(D)** in the presence of both NMDA and non-NMDA antagonists (APV and DNQX, 50 and 10 μM respectively) in Mg^{2+}-free aCSF for 8 min and **(E)** during washout of the antagonists, illustrating partial recovery of responses in Mg^{2+}-free aCSF for 34 min; note the absence of a second response again. The vertical dotted line aligns N2 peak across traces in this and subsequent traces.

changes in the response waveform under Mg^{2+}-free conditions suggest that NMDA receptors are present but make little contribution to the synaptic currents evoked by single pulse stimulation in standard aCSF conditions.

Addition of APV, a potent competitive NMDA receptor antagonist, blocked the slower component evoked under Mg^{2+}-free conditions. The recovery phase of the evoked responses (following the first stimulus pulse) returned to baseline sooner (horizontal dotted line, Figures 3C and 4C), as in control conditions. Application of APV in Mg^{2+}-free medium also reversibly decreased the amplitude of the N2 response by >20% in 2/6 slices. The amplitude of the N3 component decreased ≥20% in 5/6 slices in the presence of APV. Recovery of N3 following washout of APV occurred in five of the six slices. These results indicate that the late potential that appears under Mg^{2+}-free conditions is mediated by NMDA receptors, and that an NMDA receptor-mediated component (i.e. APV-sensitive) may contribute to the field potential that underlies the N2 and N3 peaks.

In standard aCSF, a second stimulus pulse at a 60 ms interpulse interval, reliably evoked responses with both the N2 and N3 components. In contrast, in Mg^{2+}-free medium, the responses (both fast N2 and N3 peaks and slow components) following the second stimulus pulse were abolished or greatly diminished (Figures 3B,E and 4B,D). In the presence of APV, the evoked responses following the second stimulus pulse (arrow in Figures 3C and 4C) partially return. Generally, APV added to Mg^{2+}-free bathing

medium converted the evoked response to waveforms that resemble those under control conditions (in aCSF containing Mg^{2+}).

Effects of NMDA and non-NMDA antagonist 'cocktails' in Mg^{2+}-free aCSF

To gain a better understanding of the relative contributions of NMDA and non-NMDA receptors in evoked responses, we sequentially added antagonists to the Mg^{2+}-free medium. Figures 3–5 present postsynaptic responses in Mg^{2+}-free medium with combinations of NMDA and non-NMDA receptor antagonists (APV, DNQX and NBQX). Following the elimination of the NMDA receptor-mediated components by application of APV, addition of a second antagonist, DNQX (Figure 3D), to the bathing medium eliminated the remaining N2 and N3 fast components of the evoked waveform in one slice. Three additional slices were tested with the order of the antagonists reversed: first DNQX, then DNQX + APV (Figure 4). Application of the non-NMDA antagonist, DNQX alone (5 or 10 μM) to the Mg^{2+}-free medium abolished or greatly attenuated the fast components (i.e. the N2 and N3 potentials) in each of three slices. Application of DNQX did not affect the slower, long lasting component of the waveform (Figure 4E). A complete blockade of the evoked responses (N2, N3 and the late component) was achieved in each of the three slices by the combination of DNQX and the NMDA antagonist, APV (Figure 4F). Although slower components recovered following washout of the antagonists, only a partial recovery of the N2 and N3 peaks were observed in two of the three slices initially treated with DNQX. Similar results (elimination of only the fast peak components of the waveform) were obtained using 1 μM NBQX (Figure 5), and with 5 μM NBQX in three other slices. Application of both NBQX and APV eliminated fast and late components of the response (Figure 5, bottom trace). The N2 and N3 peak responses partially recovered in one slice but did not recover in two slices during NBQX and APV washout. NMDA receptor-mediated slower components did return upon washout of antagonists (not shown).

NMDA receptors, APV and short-term synaptic plasticity

The application of APV had only slight effects on the response amplitudes of N2 and N3 in slices superfused with standard aCSF medium: N2 decreased by ≥20% in only 1/4 slices, and N3 decreased by ≥20% in 3/4 slices tested in APV. Figure 6 illustrates the effects of APV on responses evoked following the first stimulus pulse in the slice that exhibited an APV-sensitive component at the N2 peak. The amplitudes of N2 and N3 in Figure 6 were reduced by ~27 and 40% respectively. The apparent effect of APV on the amplitude of N3 was proportionally more than its effect on N2 peaks in each of the slices tested. Following washout of APV, the amplitudes returned to control levels.

Under control conditions, the amplitudes of the synaptic potentials following the second pulse of a pair were sometimes facilitated relative to the responses to the first pulse, although in other cases, both N2 and N3 were depressed, or

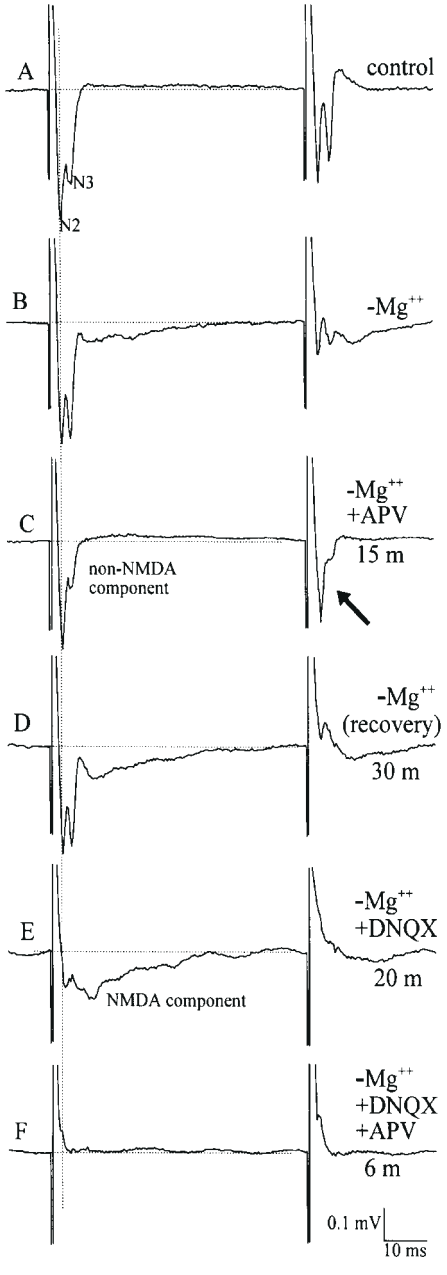

Figure 4 As in Figure 3, the first three traces illustrate components of the evoked response under **(A)** control conditions (standard aCSF), **(B)** in Mg^{2+}-free aCSF (-Mg^{2+}) and **(C)** in the presence of APV (50 μM) for 15 min. Following washout of APV from the bathing medium for 30 min (**D**, recovery), application of DNQX **(E)** for 20 min abolished the N2 and N3 peaks, isolating the NMDA receptor-mediated component ('NMDA component') of the evoked response. Note that in DNQX (E), the second response remained absent, as in Mg^{2+}-free aCSF (D). This differs from (C) (arrow) in that the second response partially returns in APV. Responses are completely abolished in the presence of DNQX and APV **(F)** for 6 min.

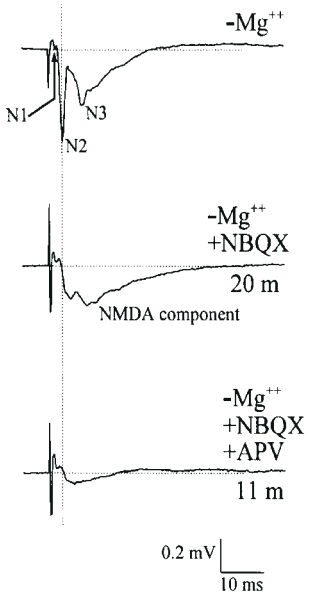

Figure 5 NBQX (1 μM, applied for 20 min) blocks fast synaptic transmission (i.e. the N2 and N3 peaks), revealing the NMDA receptor-mediated component as in Figure 4. Note that the latency to onset of the NMDA receptor-mediated component ('NMDA component') in this slice and the one illustrated in Figure 4 corresponds to the peak latency for the N2 component (vertical dotted line). Subsequent addition of APV for 11 min eliminates this response. Only one stimulus pulse is illustrated, since under Mg^{2+}-free aCSF conditions the second response typically is absent.

one was attenuated and the other facilitated (see Figures 2A and 3A). Synaptic facilitation and fatigue in the vagal lobe have been described previously by Finger and Dunwiddie (1992), and were found to be influenced by repetition rate and number of pulses in the stimulus train. To further characterize this short-term synaptic plasticity, stimulus trains of five pulses with IPIs of 15 ms were applied to the vagal afferents to induce synaptic facilitation. Typically under repetitive stimulation, N2 responses were either unaffected or fatigued, while N3 responses were facilitated following the second, third and fourth stimulus pulse (i.e. within 45–60 ms following the first stimulus). The response following the fifth pulse, occurring >60 ms after the first stimulus pulse, was markedly reduced.

Figure 7 illustrates the effects of APV on facilitation evoked by repetitive stimulation. Comparable results were obtained from four additional slices that exhibited facilitation. APV has its greatest effect on the magnitude of N3 following the second, third or fourth pulse of a stimulus train. APV attenuated the facilitation of the N3 responses and also slightly diminished the amplitude of each of the N2 responses. As in paired-pulse paradigms, APV had lesser effects on N2 than N3. These results implicate an NMDA receptor-mediated mechanism in the short-term facilitation associated with gustatory nerve stimulation.

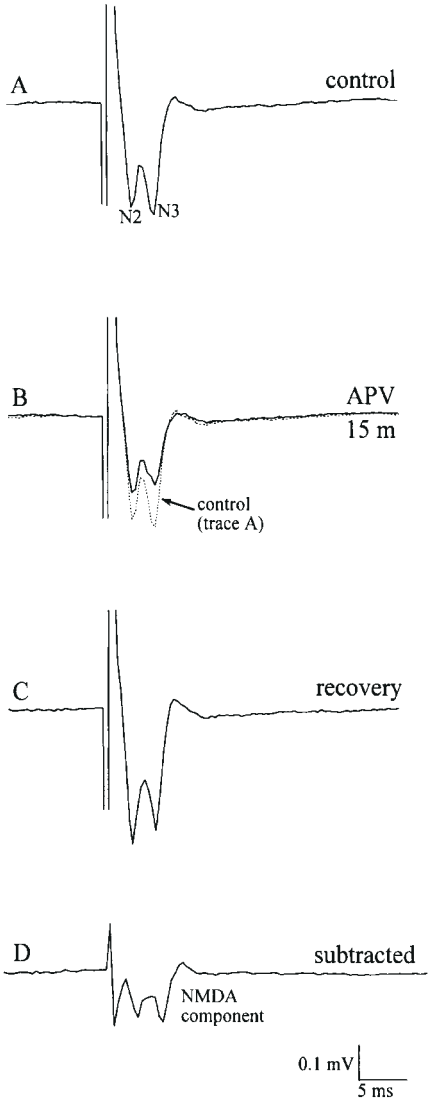

Figure 6 Evoked response following the first stimulus pulse under **(A)** control conditions (standard aCSF), **(B)** in the presence of APV (50 μM) for 15 min and **(C)** during washout of APV (recovery). Control trace (dotted waveform in B) is superimposed on evoked response under APV conditions (lined trace). **D** illustrates the NMDA receptor-mediated component ('NMDA component') of the response by subtracting the trace shown in B from the averaged traces of A and C.

Discussion

The goal of this study was to characterize the neurotransmitters and receptors utilized by primary gustatory afferents of the vagus nerve. We find that both non-NMDA (kainate/AMPA) and NMDA receptor subtypes of the glutamate family of receptors are involved in this system.

Non-NMDA and NMDA antagonists mediate fast synaptic transmission

Fast glutamatergic synaptic transmission is generally mediated by non-NMDA receptors whereas potentials that have slow onset (~10–20 ms to peak) with long durations

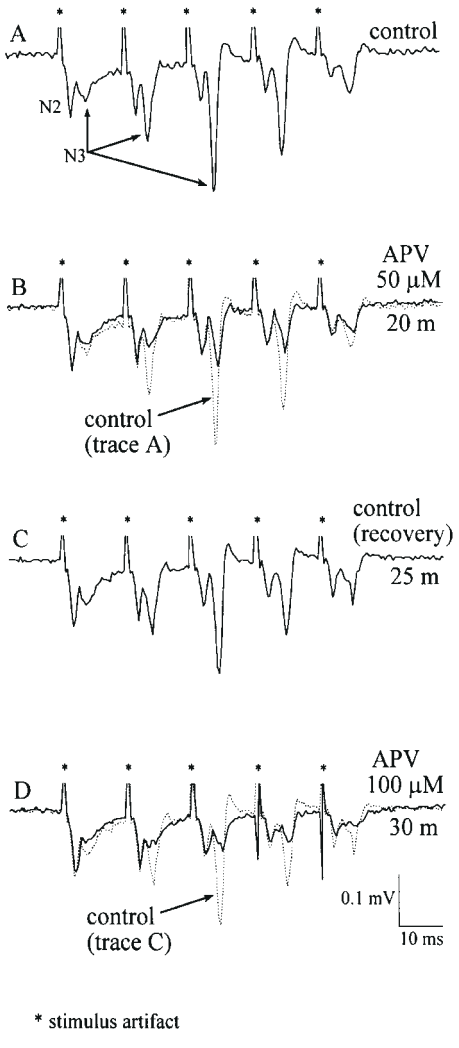

Figure 7 Evoked potentials following a train of five pulses (15 ms apart). Responses evoked **(A)** under standard aCSF conditions (control), **(B)** in the presence of APV (50 μM) for 20 min, **(C)** following 25 min of washout of the antagonist (recovery/control) and **(D)** in the presence of a higher concentration of APV (100 μM) for 30 min. Control traces (dotted traces) are superimposed on evoked responses under APV conditions (B and D, lined traces) to illustrate attenuation of facilitation by the NMDA antagonist. Note the reduction of the facilitated N3 peak following the fourth and fifth stimuli under control conditions.

are typically mediated by NMDA receptors (McBain and Mayer, 1994). The elimination of the fast postsynaptic response peaks N2 and N3 following the administration of either DNQX and NBQX establish that AMPA/kainate receptors with properties similar to their mammalian counterparts play an essential role in mediating gustatory transmission. The slight decrease in the amplitudes of N2 and N3 responses in some slices following the addition of APV to the standard aCSF bathing medium (Figure 6) suggests that NMDA-type receptors also may play a role in fast synaptic transmission (within 2–5 ms). Further, under Mg^{2+}-free conditions, in the presence of DNQX or NBQX, some short-latency APV-sensitive activity remains. NMDA receptors, although typically attributed with slow kinetic properties, also mediate fast potentials at auditory nerve synapses in goldfish (Wolszon *et al.*, 1997) and vestibular synapses in frogs (Straka and Dieringer, 1996) and rats (Kinney *et al.*, 1994).

The voltage-sensitive Mg^{2+}-block, characteristic of NMDA receptors, can be removed following depolarization of the neurons (presumably) by glutamate activating AMPA/kainate receptors. This suggests that those slices that had responses with an APV-sensitive component may have been depolarized sufficiently through non-NMDA receptors to briefly relieve the Mg^{2+}-block of the NMDA receptor. Since glutamate or another EAA is likely released at this synapse, NMDA receptor-mediated responses then could be evoked. Because non-NMDA receptors exhibit rapid decay times (Edmonds *et al.*, 1995), the release from Mg^{2+}-block would be only transient. That APV only affected the responses in some slices may reflect differences in the relative amount of depolarization of the postsynaptic neurons from slice to slice (due to differences in stimulus strengths), or possibly a heterogeneous distribution of NMDA receptors.

The complete elimination of the peaks under AMPA/kainate antagonist conditions is not inconsistent with the observation that NMDA receptor-mediated (APV-sensitive) components partially underlie the N2 and N3 peaks. The Mg^{2+}-block in standard aCSF would have prevented activation of NMDA receptors, and because non-NMDA receptors were blocked by selective antagonists, sufficient depolarization of the cells to remove the Mg^{2+}-block would not occur. This is also consistent with the hypothesis that NMDA and non-NMDA receptors can be colocalized on the same target cells.

Preliminary results from radioligand binding studies for NMDA and AMPA in tissue sections of the vagal lobe impart further evidence of the presence of these glutamate receptor subtypes (Smeraski *et al.*, 1996b). Recent studies showing uptake of cobalt in cells activated by EAAs also indicate the presence of glutamate receptors in the vagal lobe (Smeraski *et al.*, 1997). Our results extend to the vagal taste system the findings observed in the facial and glossopharyngeal taste systems in the rostral nTS of mammals (Wang and Bradley, 1995; Li and Smith, 1997): that non-NMDA and NMDA receptor subtypes are intrinsic to synaptic transmission and that an EAA, like glutamate, is the neurotransmitter of the primary gustatory afferents. Furthermore, caudal portions of the mammalian nTS that receive vagal input from viscera (cardiovascular, pulmonary, gastrointestinal) also were found to contain non-NMDA and NMDA receptors (Reis *et al.*, 1981; Miller and Felder, 1988; Andresen and Yang, 1990; Andresen and Kunze, 1994; Zhang and Mifflin, 1995; Andresen and Mendelowitz, 1996; Aylwin *et al.*, 1997). In both rostral (gustatory) and more caudal (general visceral) portions of the nTS, non-NMDA receptors contribute significantly to the fast component

of primary afferent transmission, as in our vagal lobe preparations.

Slower NMDA receptor-mediated potential uncovered in Mg^{2+}-free aCSF

Removing the Mg^{2+} from the bathing medium of the slice revealed an NMDA receptor-mediated (APV-sensitive) synaptic potential with a slow rise time and prolonged duration. In addition, the enhancement of the amplitudes of N2 and N3 responses under Mg^{2+}-free conditions was attenuated by APV.

As in standard aCSF, addition of non-NMDA antagonists to the Mg^{2+}-free aCSF eliminates the fast components of the evoked response, but does not affect the slower potential observed in Mg^{2+}-free aCSF. This slower potential was APV-sensitive and thus mediated by NMDA receptors. In Mg^{2+}-free aCSF with DNQX/NBQX, the onset of the NMDA receptor-mediated field potential occurs with the same latency as the peak of the N2 response. However, because the kinetics of NMDA receptor activation are relatively slow, the earliest peak of the NMDA receptor-mediated component lags the N2 peak by ~1 ms. This explains why APV had little effect on the N2 peak compared with N3. Because of the longer latency of onset and slower kinetics of the NMDA receptor-mediated component, its contribution is greater to the N3 response than to the N2 peak (both in standard physiological concentrations of Mg^{2+} and Mg^{2+}-free conditions).

An unexpected effect of Mg^{2+}-free bathing medium was the elimination of both the rapid and slow components of the response following the second stimulus pulse in the paired-pulse paradigm. The finding that APV partially rescues the response following the second pulse indicates that NMDA receptors are involved in the loss of the second evoked response. Activation of NMDA receptors, and the increase in intracellular Ca^{2+}, may play a role in the subsequent inactivation of the synaptic response. One characteristic of NMDA receptors is that they 'run down' or inactivate with increases in intracellular Ca^{2+} (Rosenmund and Westbrook, 1993; McBain and Mayer, 1994; Rosenmund *et al.*, 1995). If this occurs in the receptors underlying the N2 and N3 responses, it could explain the interaction between the NMDA responses and the amplitude of the synaptic response to a second stimulus pulse. An intracellular rise of Ca^{2+} ions might indirectly inactivate the non-NMDA receptors (e.g. via dephosphorylation) and block the fast components of the response (Lisman, 1989). An alternative possibility is that the site of interaction is presynaptic, i.e. that activation of presynaptic NMDA receptors can inhibit the subsequent release of transmitter to a second stimulus. The inactivation of the second response is temporary: both slow and fast components return within 15 s. It is possible that the inactivation of the second response by increases in intracellular Ca^{2+} may be a protective mechanism to limit activation and Ca^{2+} influx into the postsynaptic cell.

Short-term synaptic plasticity involves NMDA receptors

The facilitation of the N3 potential following repetitive stimulation is APV-sensitive and therefore mediated by NMDA receptors. The repeated stimuli likely removed the voltage sensitive Mg^{2+}-block, allowing activation of the NMDA receptor as described in the experiments above. One possible mechanism for this interaction would be postsynaptic, i.e. that the Ca^{2+} influx associated with the activation of the NMDA receptor induces a transient facilitation of the AMPA/kainate component of the response. Alternatively, it is certainly possible that an NMDA receptor-mediated Ca^{2+} influx into the presynaptic nerve terminal enhances transmitter release during successive responses in a stimulus train. However, note that the amplitude of the N2 response during repetitive stimulation did not increase.

The rapid attenuation of the N3 facilitation following 60 ms of repetitive stimulation under control conditions (Figure 7, fifth pulse) is reminiscent of the inactivation of the responses following the second stimulus in the paired-pulse experiments with IPIs of 60 ms. The time frames of either attenuated facilitation or inactivation overlap, possibly indicating similar control mechanisms. The mechanism underlying this effect could be either presynaptic (Zucker, 1989) or postsynaptic (Lisman, 1989; Rosenmund and Westbrook, 1993; Rosenmund *et al.*, 1995; Wang and Kelley, 1996), as proposed earlier.

In any case, the NMDA receptor-mediated short-term plasticity of the response implies that the temporal characteristics of incoming gustatory signals are crucial in determining the efficacy of transmission of gustatory information. Thus, a burst of initial activity from primary afferents should be transmitted much more effectively than a like number of temporally spaced impulses. Primary gustatory afferents typically show a transient rise in activity followed by a slower declining plateau of activity (Konishi and Zotterman, 1961; Smith and Bealer, 1975). The NMDA receptor-mediated short-term facilitation would serve to amplify the second-order response to the incoming burst. Further, the subsequent inhibition of responses after the first 50–60 ms may provide a clear time marker for stimulus onset.

Alternatively, short-term plasticity may function to integrate inputs to different portions of the dendritic tree of second-order neurons. In the vagal lobe, most cells have radially oriented dendrites which span layers (Morita *et al.*, 1983) receiving inputs from different oral surfaces: branchial inputs end more superficially in the lobe than do palatal gustatory inputs (Morita and Finger, 1985). The coordinated activation of the two vagal lobe layers would be expected to produce facilitated responses relative to the

activity in only one of the afferent systems. Thus, a food particle trapped between the palatal organ and branchial surface, as occurs during feeding (Sibbing *et al.*, 1986), would produce an enhanced second order response in the vagal lobe.

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