Metabotropic Glutamate Receptor Activation Modulates Sound Level Processing in the Cochlear Nucleus

DAN H. SANES,¹ JOANN McGEE,² AND EDWARD J. WALSH²
¹Center for Neural Science and Department of Biology, New York University, New York, New York 10003; and
²Developmental Auditory Physiology Laboratory, Boys Town National Research Hospital, Omaha, Nebraska 68131

Sanes, Dan H., JoAnn McGee, and Edward J. Walsh. Metabotropic glutamate receptor activation modulates sound level processing in the cochlear nucleus. J. Neurophysiol. 80: 209–217, 1998. The principal role of ionotropic glutamate receptors in the transmission and processing of information in the auditory pathway has been investigated extensively. In contrast, little is known about the functional contribution of the G-protein–coupled metabotropic glutamate receptors (mGluRs), although their anatomic location suggests that they exercise a significant influence on auditory processing. To investigate this issue, sound-evoked responses were obtained from single auditory neurons in the cochlear nucleus complex of anesthetized cats and gerbils, and metabotropic ligands were administered locally through microionophoretic pipettes. In general, microionophoresis of the mGluR agonists, (1S,3R)-1-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, initially produced a gradual increase in spontaneous and sound-evoked discharge rates. However, activation and recovery times were significantly longer than those observed for ionotropic agonists, such as N-methyl-D-aspartate or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, consistent with the recruitment of a second-messenger system. The efficacy of mGluR agonists was diminished after administration of the mGluR antagonist, (+)-α-methyl-4-carboxyphenylglycine, consistent with a selective action at metabotropic recognition sites. In contrast, two distinct changes were observed after the mGluR agonist had been discontinued for several minutes. Approximately 50% of neurons exhibited a chronic depression of sound-evoked discharge rate reminiscent of long-term depression, a cellular property observed in other systems. Approximately 30% of neurons exhibited a long-lasting enhancement of the sound-evoked response similar to the cellular phenomenon of long-term potentiation. These findings suggest that mGluR activation has a profound influence on the gain of primary afferent driven activity in the caudal cochlear nucleus.

INTRODUCTION

The functional role of glutamate-gated cation channels has been investigated extensively in the auditory brain stem (Caspary 1986; Faingold et al. 1991; Hunter et al. 1992; Walsh et al. 1993; Wu and Kelly 1992; Zhou and Parks 1991), and it is clear that ionotropic glutamate receptors (iGluRs) mediate the rapid response of central auditory neurons to sound. However, the presence of second messenger-coupled glutamate receptors within the auditory brain stem suggests that ionotropic mechanisms may be modulated over the long term (Fotuhi et al. 1993; Shigemoto et al. 1992; Zirpel et al. 1994). Moreover, the discovery of a subpopulation of neurons within the cochlear nucleus of developing cats that display unusually long activation times in response to the ionophoresis of glutamate (Walsh et al. 1993, 1995), supports the idea that auditory processing is altered as a consequence of glutamate-induced second-messenger systems.

Metabotropic glutamate receptors (mGluRs) originally were characterized as glutamate recognition sites coupled to second-messenger–dependent inositol phospholipid metabolism (Nicoletti et al. 1986; Sladeczek et al. 1985), but the results of extended studies led to the recognition that a subgroup is negatively coupled to adenylyl cyclase (Nakanishi 1994; Pin and Bockstahn 1994). It is known now that the mGluR family consists of at least eight subtypes based on sequence homology, second-messenger system affiliation, and pharmacology (Nakanishi 1994; Pin and Bockstahn 1994). Recent anatomic studies have localized mGluRs to dorsal cochlear nucleus (DCN) neurons (Petraila et al. 1996; Shigemoto et al. 1992; Wright et al. 1996), along with the intracellular receptors that would be expected to mediate a response via the phospholipase C cascade (Rodrigo et al. 1994; Ryugo et al. 1995). DCN neurons integrate a relatively broad array of afferent inputs (Cant 1992). Stimulation of the auditory nerve elicits short latency excitatory potentials, followed by glycine-mediated inhibitory potentials among neurons in a brain slice preparation of the mouse DCN (Hirsch and Oertel 1988).

Given the heterogeneity and distribution of mGluR receptors, their activation may lead to several outcomes. First, because the dynamic operating range of brain stem auditory neurons is small relative to the range of hearing, mGluR activation may regulate neuronal sensitivity, compensating for rate saturation conditions encountered in various acoustic environments. In principle, such mGluR regulation of sensitivity would be related to the activation of a Ca²⁺ current that affects the release of neurotransmitter or some other aspect of near saturation level stimulation (Lachica et al. 1995). Alternatively, the selective activation of mGluRs within an afferent array could produce a qualitative change in a neuron’s processing characteristics. In the present study, we addressed these questions by recording from second-
METHODS

Surgical preparation and auditory stimulation

Adult cats (*Felis domesticus*) and gerbils (*Meriones unguiculatus*) were anesthetized such that responses to nociceptive stimuli (i.e., withdrawal from digit compression) were absent. At the start of the experiment, gerbils received 40 mg/kg ketamine and 0.2 mg/kg atropine intramuscularly, and cats received 0.25 mg/kg atropine im. Anesthesia was induced by administering pentobarbital sodium (*Nembutal*) intraperitoneally at a dose of 40 mg/kg for cats and 55 mg/kg for gerbils. Supplemental doses of pentobarbital were administered throughout the experiment as needed. At the end of an experiment, the animal was killed with an overdose of pentobarbital. The care and use of all animals were in accordance with the guidelines of the animal welfare committee of Creighton University and Boys Town National Research Hospital.

Standard surgical procedures were employed to expose the DCN. Details of the methods and data acquisition procedures employed in this investigation are available in Walsh et al. (1990, 1993). Briefly, a tracheotomy was performed, and the animal was ventilated artificially if necessary. The pinna and cartilaginous external ear canal were resected, a ventilation hole was placed in the bulla, and the posterior fossa was opened. Exposure of the cochlear nucleus was achieved by aspirating cerebellum. Earphones (Beyer DT48 transducers) were sealed in place, and the closed acoustic system was calibrated with a Bruel and Kjaer \( \frac{1}{2} \)-in microphone.

Acoustic stimuli were generated digitally by a custom designed PC-based system.

FIG. 1. Response of a cat cochlear nucleus neuron to ionophoresis of \((1S,3R)\)-1-aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD]. A: raster display shows the trial-by-trial response of the neuron to 20 dB SPL tone bursts at characteristic frequency (CF; 2.432 kHz) surrounding the time when \((1S,3R)\)-ACPD was ionophoresed (vertical line on right side of raster). In the short term, both spontaneous and sound-evoked discharge rates increased after ionophoresis. B: neuron had a pauser/buildup peristimulus time histogram in response to 55 dB SPL tone bursts at its CF. Stimulus period is shown beneath the histogram (sound on during thick envelope). C: time course of \((1S,3R)\)-ACPD actions, corresponding to the raster display in A, is plotted for spontaneous and driven discharge rates. “Spontaneous rates” were computed during the last 200 ms of the repetition interval. Period of drug application is shown as a line above the plot.

order neurons in the cochlear nuclear complex of adult cats and gerbils while ionophoresing mGluR agonists. The findings indicate that prolonged rate-intensity code modifications accompany mGluR activation.

FIG. 2. Acute effects of \((1S,3R)\)-ACPD on sound level coding in a cat neuron. Left: rate-level functions show the response of a cat neuron before (■) and during (○) the ionophoresis of \((1S,3R)\)-ACPD. Both spontaneous and driven responses increased, but threshold and dynamic range did not change. Right: individual raster plots are shown for each sound level before and during exposure to \((1S,3R)\)-ACPD. Stimulus period is shown beneath the rasters. Fifty stimuli per data point. Error bars indicate SD.

Electrophysiology and ionophoresis

Multibarrel glass micropipettes were positioned visually over the cochlear nucleus. The pipette used to record spike activity and the barrel used to balance current were filled with 1 M NaCl, and the remaining four barrels were filled with glutamate receptor agonists or antagonists of interest. The pharmacological agents used in the study included \((1S,3R)\)-1-aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD], \((2S,1'S,2'S)\)-2-(carboxycyclopropyl)glycine (\(-\text{CCG-I}\)), \((S)\)-\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazoloproprionic acid (AMPA), \((N)\)-methyl-\(\alpha\)-aspartate (NMDA), and \((+\)-\(\alpha\)-methyl-4-carboxyphenylglycine (MCPG). All agents were prepared at a concentration of 50 mM in H\(_2\)O except for AMPA (10 mM in 200 mM NaCl) and Glu (0.2 M in H\(_2\)O), titrated to a pH of 8–8.5 (using 1 N NaOH), and stored at 4°C until they were loaded into a barrel of the microionophoresis delivery system.
Action potentials from single neurons were isolated, and the time of occurrence of each discharge was digitized. Custom-designed software was used for the on-line display of various derived response measures. Discharge times and relevant experimental and stimulus-related information were stored in computer files for later analysis.

RESULTS

Effect of mGluR activation: qualitative observations

A total of 37 neurons were studied in four cats and 15 neurons were studied in three gerbils. All neurons reported in this study were encountered in the portion of the cochlear nuclear complex dorsal to the auditory nerve zone of bifurcation (i.e., the caudal cochlear nuclear complex, CCN), and only those neurons exhibiting pauser, buildup, or chopper temporal response patterns were included in this report. In the cat, 14 of 15 neurons that exhibited a chopper peristimulus time histogram (PSTH) were responsive to exogenous (1S,3R)-ACPD, as were the 4 pauser-buildup type neurons encountered. All chopper and pauser-buildup neurons recorded in gerbils also responded to (1S,3R)-ACPD.

In general, the acute effects of microionophoresing the mGluR agonists, (1S,3R)-ACPD or L-CCG-I, into the vicinity of a CCN neuron was to increase spontaneous and sound-evoked discharge rates, and to decrease response latency. A representative response from a cat neuron is illustrated in Fig. 1. The dot raster display of discharge activity (Fig. 1A) is shown for a pauser buildup neuron (Fig. 1B), and the discharge rate was computed and displayed on a trial-by-trial basis (Fig. 1C). Although the neuron’s driven and spontaneous rates (SR) were both enhanced by (1S,3R)-ACPD exposure, the alteration of SR lagged behind changes in drive rate. Neurons typically displayed a delay in activation after the onset of mGluR agonist microionophoresis (vertical line in the right margin of Fig. 1A). Comparable observations were made on >90% of neurons from the gerbil and cat cochlear nucleus during mGluR agonist exposure.

For the cat CCN neuron shown in Fig. 2, mGluR agonists boosted discharge rates in an intensity-independent manner. However, the response of other neurons included in this study to agonist application was potentiated selectively at higher stimulus levels, as shown in Fig. 3 for a gerbil CCN neuron. The example shown in Fig. 2 exhibited a chopper response to characteristic frequency (CF) tone bursts, whereas the one shown in Fig. 3 displayed a buildup pattern. The neuron shown in Fig. 3 was transformed from a buildup response to a pauser-buildup response pattern as a consequence of exogenous (1S,3R)-ACPD exposure. For intermediate stimulus levels, the neuron in Fig. 2 was transformed into a chopper, whereas the neuron in Fig. 3 was transformed into a pauser-buildup pattern.

FIG. 3. Acute effects of (15,3R)-ACPD on sound level coding in a gerbil neuron. Left: rate-level functions show the response of a gerbil neuron before (•) and during (○) the ionophoresis of (15,3R)-ACPD. In this case, there was a specific increase of driven activity at higher sound levels. Right: raster plots illustrate that temporal responses changed from a buildup pattern to a pauser/buildup pattern. Stimulus period is shown beneath the rasters. Fifty stimuli per data point. Error bars indicate SD.
from a chopper response to a primary-like response pattern as a consequence of exogenous (1S,3R)-ACPD exposure.

While recording from individual CCN neurons, the non-selective mGluR antagonist, MCPG, was microionophoresed for several minutes prior to the microionophoresis of (1S,3R)-ACPD or L-CCG-I to verify that the agonist effects were mediated by mGluRs. As shown in Fig. 4, MCPG significantly attenuated the response evoked by both L-CCG-I and (1S,3R)-ACPD. However, MCPG did not have a direct effect on sound-evoked discharge activity as assayed in this investigation.

**Time course for mGluR activation and recovery**

Activation times for mGluR-agonist-mediated changes were determined by estimating the time required to achieve 90% of the maximum sound-evoked discharge rate relative to the onset of microionophoresis. CF tone bursts were presented near threshold for several trials to generate control response levels followed by the administration of mGluR agonists. In agreement with a previous study (Walsh et al. 1993), the microionophoresis of the iGluR agonists, AMPA or NMDA, led to a rapid increase in sound-evoked discharge rate (Fig. 5), with a mean activation time of 10 s (Fig. 6). In contrast, microionophoretically administered mGluR agonists, (1S,3R)-ACPD or L-CCG-I, led to a longer latency change in sound-evoked discharge rates (Fig. 5) with a mean activation time of 42 s (Fig. 6).

Recovery times were not evaluated quantitatively in the present investigation, but discharge rates commonly recovered to premicroionophoresis levels within 1–2 min after the cessation of agonist ejection (Figs. 1 and 4). The prolonged nature of acute mGluR recovery dynamics is apparent when compared with the rapid recovery from ionotropic mGluR-mediated actions (Fig. 5).

**Long-term effects of mGluR activation: altered rate-level characteristics**

Beyond the short-term modification of responses, mGluR agonists also were found to alter the long-term response properties in ~80% of the 27 CCN neurons studied, particularly their sound level coding properties. Affected neurons fell into one of two categories: control rates were either enhanced or depressed. To assess the long-term alteration of affected neurons, changes in threshold at CF, maximum driven discharge rates, and the slope of rate versus level
curves induced by exposure to mGluR agonists were determined. A neuron was assigned to the long-term potentiation category if maximum driven rates or the slopes of rate versus level curves after exposure to (1S,3R)-ACPD or L-CCG-I were 20% greater (arbitrarily selected) than control values or if threshold improved by ≥5 dB. Assignment to the long-term depression category was made if maximum driven rates or rate versus level slopes were <20% of control values or if thresholds increased by ≥5 dB.

As shown for two cat CCN neurons in Figs. 7 and 8, sound-evoked discharge rates were depressed after exogenous (1S,3R)-ACPD, and the period of depression lasted ≥45 min after the offset of agonist exposure. The maximum discharge rate was most affected for the neuron in Fig. 7, whereas the absolute threshold was most affected for the neuron shown in Fig. 8. Neurons also responded to mGluR stimulation with a long-lasting enhancement of the sound-evoked discharge rate, as shown for a gerbil CCN neuron in Fig. 9. In this case, the maximum discharge rate was achieved at 50 dB SPL when the neuron was first isolated, but the cell exhibited a 5- to 10-dB increase in dynamic range and a doubling of the maximum discharge rate after exposure to (1S,3R)-ACPD.

While individual examples are shown for neurons recorded in both species, we could not detect significant differences in the population of recorded neurons. Therefore we have collapsed the findings across cat and gerbil and separated the effects according to temporal response categories. Neurons belonging to either chopper or buildup categories primarily were depressed (i.e., 50 and 55% respectively), although long-term enhancement was observed in ~30% of the cases thus far studied (Fig. 10).

**DISCUSSION**

The primary finding of this study was that specific mGluR agonists, L-CCG-I or (1S,3R)-ACPD (Ishida et al. 1990; Palmer et al. 1989), have the capacity to alter response characteristics of neurons in the cochlear nuclear complex for extended periods of time. This effect is mediated through mGluRs directly, as indicated by the blocking actions of the selective mGluR antagonist, MCPG (Jane et al. 1993; Lingenhoehl et al. 1993b). The finding that mGluR agonists increased discharge rates acutely was obtained in both species examined, and the activity levels of ~50% of neurons tested were depressed, as defined by either an increase in threshold or a decrease in dynamic range.

The present results are consistent with several studies in which mGluRs have been located on, or within, neurons of the cochlear nuclear complex. Transcripts of the mGluR1 subunit, which encode a glutamate receptor that is linked to phosphoinositide (PI) hydrolysis, are specifically localized in rat DCN neurons (Shigemoto et al. 1992). The mGluR1α protein has been localized immunocytochemically in the DCN (Fotuhi et al. 1993) at postsynaptic densities in cartwheel dendritic spines, in unipolar brush cell dendrites, and in fusiform (pyramidal) cells (Petraila et al. 1996; Wright et al. 1996). The mGluR2/3 protein is found at pre- and postsynaptic structures in the DCN neuropil and in small/medium cells (Petraila et al. 1996). Moreover, IP3 receptors have been localized to cartwheel neurons in the dorsal cochlear nucleus of a wide variety of species, including cats (Ryugo et al. 1995; Wright et al. 1996). The mGluR1α protein has the capacity to alter response characteristics, as indicated by the blocking actions of the selective mGluR antagonist, MCPG (Jane et al. 1993; Lingenhoehl et al. 1993b). The finding that mGluR agonists increased discharge rates acutely was obtained in both species examined, and the activity levels of ~50% of neurons tested were depressed, as defined by either an increase in threshold or a decrease in dynamic range.

The present results are consistent with several studies in which mGluRs have been located on, or within, neurons of the cochlear nuclear complex. Transcripts of the mGluR1 subunit, which encode a glutamate receptor that is linked to phosphoinositide (PI) hydrolysis, are specifically localized in rat DCN neurons (Shigemoto et al. 1992). The mGluR1α protein has been localized immunocytochemically in the DCN (Fotuhi et al. 1993) at postsynaptic densities in cartwheel dendritic spines, in unipolar brush cell dendrites, and in fusiform (pyramidal) cells (Petraila et al. 1996; Wright et al. 1996). The mGluR2/3 protein is found at pre- and postsynaptic structures in the DCN neuropil and in small/medium cells (Petraila et al. 1996). Moreover, IP3 receptors have been localized to cartwheel neurons in the dorsal cochlear nucleus of a wide variety of species, including cats (Ryugo et al. 1995; Wright et al. 1996). The mGluR1α protein has the capacity to alter response characteristics, as indicated by the blocking actions of the selective mGluR antagonist, MCPG (Jane et al. 1993; Lingenhoehl et al. 1993b). The finding that mGluR agonists increased discharge rates acutely was obtained in both species examined, and the activity levels of ~50% of neurons tested were depressed, as defined by either an increase in threshold or a decrease in dynamic range.

**FIG. 6.** Summary of the activation times for ionotropic and metabotropic agonists for neurons recorded in the cat caudal cochlear nucleus. Each distribution displays the time required to reach peak sound-evoked discharge rates from the onset of ionophoresis. Activation times were ~4 times longer for mGluR agonists (bottom) compared with ionotropic GluR agonists (top). Data are derived from 38 neurons studied with (1S,3R)-ACPD (79 exposure periods) and 19 neurons studied with L-CCG-I (27 exposure periods). Thus a total of 57 neurons were studied with either (1S,3R)-ACPD or L-CCG-I and a total of 106 samples were obtained. Some neurons were studied with both agonists. (Ionotopic agonist data from Walsh et al. 1993, Fig. 5.)
cells, during which there is a response enhancement followed by depressed activity (Lingenhohl et al. 1993a). In the rat somatosensory cortex, (1S,3R)-ACPD ionophoresis can either depress or enhance vibrissa-evoked discharge rates (Cahusac 1994). In the rat ventrobasal thalamus, mGluR agonists reduce sensory-evoked inhibition, thus enhancing sensory-evoked responses (Salt and Eaton 1995). The transition of temporal response patterns of neurons in the present study (cf., Fig. 3 raster plots) suggests that the strength of inhibitory synapses may be decreased.

The normal activation of mGluR receptors may involve release of glutamate by parallel fibers. Glutamate is localized to synaptic vesicles in parallel fiber terminals and is depleted in the presence of high potassium concentrations (Osen et al. 1995). This anatomic pattern is consistent with the distribution of mGluR1α (Petralia et al. 1996) and with the excitatory synaptic properties of parallel fibers synapses observed in vitro (Manis 1989; Molitor and Manis 1997). In the present study, glutamate ionophoresis did not activate mGluRs in an obvious way. This may have been due to the powerful endogenous reuptake systems. L-glutamate does mimic mGluR agonists in rat retrotroapezoid neurons (Nattie and Li 1995), but fails to activate (1S,3R)-ACPD-sensitive receptors in rat dorsolateral septal nucleus neurons, suggesting that a novel endogenous ligand may be involved (Zheng and Gallagher 1995).

There is great diversity in the kind of cellular changes that result from mGluR activation. The application of mGluR agonists can variously result in long-lasting depolarizations (Kotak and Sanes 1995; Krieger et al. 1994; Zheng and Gallagher 1991), membrane hyperpolarizations (Raimie et al. 1994), decreased synaptic transmission (Baskys and Malenka 1991; Gereau and Conn 1995; Lovinger and McCool 1995), potentiated synaptic transmission (Gotani et al. 1994; Lachica et al. 1995; Lester and Jahr 1990), and the regulation of calcium entry (Chavis et al. 1994; Lachica et al. 1995; Lester and Jahr 1990).
effects are often consistent with actions at both pre- and postsynaptic receptor locations (Romano et al. 1995). In the present study, a general depression of sound-evoked discharge rates could result from an \((1S,3R)-ACP D\)-activated \(Ca^{2+}\)-sensitive potassium conductance, as shown for neurons in the amygdala (Rainnie et al. 1994), or an inward current, as shown for hippocampal CA3 neurons (Pozzo Miller et al. 1995).

We speculate that the mGluR-dependent modulation of intensity coding constitutes a mechanism for extending the auditory system’s dynamic range in different acoustic environments. This could occur through regulation of intracellular calcium by mGluR-coupled phosphoinositide hydrolysis (Lachica et al. 1995; Zhou and Parks 1991; Zipfel et al. 1994). An analogous mechanism has been proposed to account for retinal photoreceptor gain and contrast sensitivity as background illumination changes (Masu et al. 1995; Miller et al. 1994; Nakajima et al. 1993; Nawy and Jahr 1990, 1991; Shiells and Falk 1990). Finally, although the present results suggest that the mGluR receptors can mediate long-lasting changes in auditory processing in adults, their role in synaptic development and stabilization may be entirely different (Reid et al. 1995; Vázquez et al. 1995) and is the subject of ongoing study.

This work was supported by National Institute of Deafness and Other Communications Disorders Grants DC-01007 to E. J. Walsh and DC-00540 to D. H. Sanes.

Address for reprint requests: E. J. Walsh, Dept. of Physiology, Boys Town National Research Hospital, 555 N. 30th St., Omaha, NE 68131.

Received 19 December 1996; accepted in final form 23 March 1998.

REFERENCES


