Correlation of AMPA Receptor Subunit Composition with Synaptic Input in the Mammalian Cochlear Nuclei

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The composition of AMPA receptors in patches excised from somata and dendrites of six cell types in the mammalian cochlear nuclei was probed and compared electrophysiologically and pharmacologically with the rapid application of glutamate. Cells excited predominantly by auditory nerve fibers had AMPA receptors with exceptionally rapid gating (submillisecond deactivation and desensitization time constants). The nonlinear current–voltage relationship in the presence of spermine showed that few of these receptors had GluR2 subunits, and the insensitivity of desensitization to cyclothiazide indicated that they contained mostly flop splice variants. At synapses made by parallel fibers, AMPA receptors were slowly gating (time constants of deactivation and desensitization >1 msec)

and contained higher levels of GluR2 and flip isoforms. However, receptors at auditory nerve synapses on cells that also receive parallel fiber input, the fusiform cells, had intermediate properties with respect to kinetics and contained GluR2 and flip isoforms. Given the diverse biophysical properties, patterns of innervation, patterns of electrical activity, and targets of each cell type *in vivo*, these data indicate that the kinetics and permeation properties of AMPA receptors are linked to factors associated with synaptic connectivity.

Key words: AMPA receptor; auditory pathways; GluR2; polyamine; rectification; kinetics; flop; cochlear nuclei; cyclothiazide; deactivation; desensitization; targeting

Glutamate receptors of the AMPA subtype vary among and within brain regions (Colquhoun et al., 1992; Raman et al., 1994; Spruston et al., 1995; Gardner et al., 1999). They are combinations of four subunits, GluR1–4 (A–D) that exist in either the flip or flop splice variant (Hollmann et al., 1989; Sommer et al., 1990). The flop isoforms endow receptors comprised of GluR2, GluR3, and GluR4 subunits with faster gating kinetics than their flip counterparts (Mosbacher et al., 1994; Koike et al., 2000). The permeability to Ca^{2+} depends on the absence of the GluR2 subunit (Hollmann et al., 1991; Sommer et al., 1991; Burnashev et al., 1992; Jonas et al., 1994; Geiger et al., 1995).

Postsynaptic receptors are regulated at the level of gene expression as well as by differential targeting to synaptic sites (Tóth and McBain, 2000). Individual CA3 interneurons target the GluR2 subunit differentially to specific synapses (Tóth and McBain, 1998). In addition, electrical activity and elevation of intracellular Ca^{2+} may contribute to differential targeting because excitatory synapses of cerebellar stellate neurons gain GluR2 after periods of repetitive activation (Liu and Cull-Candy, 2000).

In the cochlear nuclei receptors associated with two excitatory fiber systems can be compared in neurons with varied patterns of activity *in vivo*. The auditory nerve drives bushy (Cant and Morest, 1979), octopus (Golding et al., 1995), and T stellate cells (Cant, 1981; Ferragamo et al., 1998) in the ventral cochlear

nucleus and tuberculoventral cells (Wickesberg and Oertel, 1988; Zhang and Oertel, 1993b; Rhode, 1999) in the deep layer of the dorsal cochlear nucleus. Only parallel fibers, the axons of granule cells that are scattered within and around the cochlear nuclei, excite cartwheel cells through AMPA receptors (Kane, 1974; Wouterlood and Mugnaini, 1984; Wouterlood et al., 1984; Zhang and Oertel, 1993a). The two fibers systems converge onto fusiform cells, with auditory nerve fibers contacting basal dendrites and parallel fibers contacting apical dendrites (Kane, 1974; Wouterlood and Mugnaini, 1984). Additional minor excitatory inputs from intrinsic sources have been described in mice to T stellate (Ferragamo et al., 1998), octopus (Golding et al., 1995), tuberculoventral (Zhang and Oertel, 1993b), and possibly fusiform cell basal dendrites (Oertel et al., 1990). Recordings of the sound-driven activity of these neurons *in vivo* have shown that each cell type has a distinct and characteristic response profile (Rhode and Greenberg, 1992).

The rapid application of agonist to excised patches of membrane allows the gating kinetics of receptors to be resolved and their composition to be probed for specific subunits. We find that receptors have identical properties on those cells innervated primarily by the auditory nerve. Receptors on the basal dendrites of fusiform cells that are contacted by the auditory nerve have some properties intermediate between those in cells with predominantly auditory nerve input and those on apical dendrites and cartwheel cells that receive parallel fiber inputs. Moreover, the properties of excised receptors match the properties of synaptic receptors that mediate spontaneous or sucrose-evoked mEPSCs.

MATERIALS AND METHODS

All experiments were done in accordance with the protocols and guidelines of the Animal Care and Use Committee at the University of Wisconsin-Madison Medical School.

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Slice preparation. Inbred ICR mice (Harlan Sprague Dawley, Madison, WI) 18–21 postnatal days old were used for electrophysiological recordings. The dissection and slicing of the cochlear nuclei were done at 34°C in oxygenated, normal physiological saline composed of the following (in mM): 130 NaCl, 3 KCl, 1.3 MgSO₄, 2.4 CaCl₂, 20 NaHCO₃, 3 HEPES, 10 glucose, and 1.2 KH₂PO₄, pH 7.4. Thin slices (175–200 μm) were cut in the coronal plane with a vibrating tissue slicer (Frederick Haer & Co., Brunswick, ME). Slices rested on a nylon mesh in a beaker containing oxygenated saline at 34°C for 10–20 min before they were transferred to the recording chamber. All experiments were performed at 22–24°C, and the slice was superfused with saline at a rate of 6 ml/min through a chamber with a volume of 0.3 ml.

Whole-cell recording. Cells were visualized with a water-immersion lens (63×) using modified dark-field illumination with an upright Zeiss Axioskop microscope (Zeiss, Germany). The condenser was placed slightly out of focus and off-center, and the field diaphragm was closed as far as possible. This technique provided excellent visualization in the absence of infrared illumination and Nomarski optics. Patch pipettes (5–8 MΩ open tip resistance) made from borosilicate glass tubing using a Flaming-Brown Micropipette Puller (Sutter Instrument Company, San Francisco, CA) were filled with a potassium gluconate or cesium sulfate solution composed of the following (in mM): 108 potassium gluconate, 4.5 MgCl₂, 14 phosphocreatinine (Tris salt), 9 EGTA, 9 HEPES, 4 Na₂ATP, 0.3 GTP (Tris salt), 0.1 spermine, pH 7.4, or 70 cesium sulfate, 7 KCl, 1 MgSO₄, 7 H₂O, 1 CaCl₂·2 H₂O, 10 EGTA, 10 HEPES, 2 Na₂ATP, 0.1 spermine, pH 7.3.

Somatic and dendritic whole-cell recordings were made with the Axopatch 200A amplifier (Axon Instruments, Foster City, CA) with the headstage in the whole-cell mode. Dendritic whole-cell recordings were made from primary apical or basal dendrites 5–25 μm from the cell body. The identification of recorded cells was made on the basis of their location in the slice, cell body shape, and responses to depolarizing current steps in current-clamp mode as previously described (Wu and Oertel, 1984; Oertel et al., 1990; Zhang and Oertel, 1993a,b, 1994; Golding et al., 1995). Fusiform apical and basal dendrites were identified by their extension into the molecular and deep layers of the dorsal cochlear nuclei, respectively. In all cells mEPSCs were recorded at a holding potential of −65 mV before patches were excised for comparison of the kinetics to excised-patch responses. The series resistance in whole-cell mode (<10 MΩ) was compensated at 90–95%. The data were acquired with pClamp6 software (Axon Instruments) at a sampling rate of 33 kHz and were low-pass-filtered at 10 kHz. Amplitudes, 10–90% rise, and decay times of mEPSCs were measured using in-house software written by M. I. Banks for comparison with currents evoked from receptors in somatic patches.

Outside-out excised patch recordings. After recording mEPSCs in the whole-cell mode, the patch pipette was slowly drawn away from the cell until a gigohm patch was excised. The patch was then positioned at the interface between two solution streams. The pipette used for the rapid application of solutions to excised patches was made from double-barreled theta glass (World Precision Instruments, Sarasota, FL). The patch was initially placed in front of the barrel containing control solution consisting of normal saline to which 200 μM APV and 10 μM 7-chloro-kynurenic acid were added to prevent the activation of NMDA receptors. The solution bathing the patch was changed rapidly with the use of a piezoelectric device (Burleigh Instruments, Fishers, NY) to the agonist solution that contained normal saline, APV, 7-chloro-kynurenic acid, and 10 mM L-glutamate. The duration of glutamate application was 0.75–1 msec for the deactivation protocol and 100 msec for the desensitization protocol. For some experiments 200 μM cyclothiazide in ethanol was added to both barrels of the pipette. In these experiments, the patch was allowed to remain in the control solution stream for at least 20 sec before each step into the agonist-containing solution. Agonist steps were 1–3 sec in duration in the cyclothiazide experiments. At the end of each experiment, the patch was blown off, and the open-tip junction current was recorded to verify proper positioning and solution exchange times. The 10–90% rise and decay times for solution exchange were 150–200 μsec. The cyclothiazide, APV, and glutamate were obtained from Sigma (St. Louis, MO). 7-chloro-kynurenic acid was obtained from Research Biochemicals International (Natick, MA).

Glutamate-evoked patch currents were analyzed off-line using Clampfit (Axon Instruments) and Origin version 5.1 software (Microcal, Northampton, MA). Single and double exponential fits were made to the decay portion of all currents.

Nonstationary variance analysis. At least 30 consecutive currents

evoked in response to a 100 msec exposure to 10 mM L-glutamate were used to estimate the mean single-channel current, receptor number, and single-channel conductance with nonstationary variance analysis in AxoGraph version 4.4 software (Axon Instruments). The currents from the peaks onward were averaged, the variances were calculated, and the variance was plotted against the mean current. The resulting plots were then fit with the following equation according to Sigworth (1980):

$$\sigma^2 = iI - (I^2/N) + \sigma_n^2 \quad (1)$$

where σ^2 is the variance, i is the single-channel current, I is the mean current, N is the number of channels, and σ_n^2 is the variance of the patch noise in the absence of agonist. Trial-to-trial variances were used to compensate for any rundown in the current over time if present. Ensemble variance was used for patches with no rundown. In addition to fitting the raw data, the data were also binned in 2–20 pA bins depending on the maximum current for a given patch. Fitting the binned data was done to check for biasing of the fits by the majority of data points at the low current values. In all cases the estimates for i and N were similar for fits to the raw and binned data. The maximum open probability was estimated by the equation: $P_{O, \max} = I/iN$.

Sucrose-evoked mEPSCs. To investigate the kinetics and presence of GluR2 in synaptic receptors in fusiform cells, mEPSCs were selectively elicited in either basal or apical dendrites with the application of a hypertonic solution (Bekkers and Stevens, 1995). Somatic whole-cell recordings were made with cesium sulfate-filled pipettes from fusiform cell somata, as described above with 1 μM strychnine (Sigma) in the bath to block glycinergic events. A patch pipette containing normal saline to which 0.5 M sucrose was added was positioned next to either the apical or basal dendrite within 15 μm of the cell body. The solution was applied to the dendrite for 15–90 msec with the use of a Picospritzer (General Valve Corporation, Fairfield, NJ) powered by nitrogen gas at 20 psi. The duration of the sucrose pulse was varied to control the evoked mEPSC frequency. Philanthotoxin-343 (Research Biochemicals International) was used to probe for the presence of the GluR2 subunit in the receptors in each dendrite. Control events were evoked with sucrose before bathing the slice in philanthotoxin. Then 50 μM each of philanthotoxin, kainate, and Cd²⁺ were bath-applied for 2 min. Once the current baseline returned to control levels, events were evoked with sucrose again.

All statistical tests were performed in Origin version 5.0 software. Both t test and nonparametric statistics were performed, and the results were identical. Values are reported for t test results only ($\alpha = 0.01$, for all tests).

RESULTS

AMPA receptors postsynaptic to the auditory nerve have faster decay kinetics compared with those postsynaptic to parallel fibers

All experiments in this study were conducted at 22–24°C; at higher temperatures the activation and deactivation kinetics were too rapid to measure accurately. Examples of currents evoked in response to both short and long steps of 10 mM m-glutamate are shown in Figure 1*A*. The 10–90% rise times of the currents in targets of the auditory nerve, bushy, octopus, T stellate, and tuberculoventral cells were 0.10–0.20 msec and were limited by the solution exchange time. Rise times for currents from the apical and basal dendrites of fusiform cells and cartwheel cells, targets of parallel fibers, were 0.20–0.40 msec. Currents evoked in response to a brief exposure to glutamate (0.75–1 msec) in patches from bushy, octopus, T stellate, tuberculoventral cells, and the basal dendrites of fusiform cells deactivated with single exponential time constants between 0.35 and 0.54 msec (Table 1). Deactivation time constants were not significantly different between bushy, octopus, T stellate, and tuberculoventral cells ($p > 0.05$; t test). The deactivation time constant was fast, but significantly slower in patches from fusiform cell dendrites that are also postsynaptic to the auditory nerve ($p < 0.01$; t test). The deactivation kinetics from individual patches from each cell type matched the decay of the fastest mEPSCs recorded in whole-cell mode before patch excision (Fig. 1*B*). This suggests that if some

Figure 1. *A*, AMPA receptors in auditory nerve targets have faster rates of deactivation and desensitization than receptors in parallel fiber targets. Outside-out patches were exposed to either ≤ 1 or 100 msec pulses of 10 mM L-glutamate. Short exposures led to the opening of the channels followed by deactivation after removal of the glutamate (*light traces*). The channels sometimes started to desensitize during the 1 msec exposure. Single exponential fits to the deactivation of these channels were 0.43, 0.36, 0.37, 0.36, and 0.46 msec for bushy, octopus, T stellate, tuberculoventral cells, and the fusiform basal dendrite, respectively. The time constant of deactivation for the fusiform apical dendrite and the cartwheel cell were 1.17 and 1.99 msec. Time constants for deactivation were significantly faster in auditory nerve targets than in the parallel fiber targets ($p < 0.01$; t test). During long exposures to glutamate (100 msec), channels opened briefly and then desensitized in the continued presence of glutamate (*dark traces*). Single exponential fits to the desensitization of these channels were 0.97, 0.91, 0.90, and 0.82 msec for bushy, octopus, T stellate, and tuberculoventral cells, respectively. Desensitization of receptors from the basal dendrite of a fusiform cell was fit by two exponentials, 1.70 (58%) and 6.36 msec (42%). Receptors in the fusiform apical dendrite and the cartwheel cell had time constants of desensitization of 5.62 and 4.80 msec. *B*, The time constant of decay of the fastest mEPSCs matched the time constant of deactivation. *Left*, Response in a patch from an octopus cell to a 1 msec pulse of glutamate. *Right*, Ensemble average of 127 of the fastest mEPSCs from the same cell before excision of patch. Single exponential fits are superimposed on the current traces.

of the patch receptors were extrasynaptic they had identical kinetics to synaptic receptors. This comparison could not be made in fusiform and cartwheel cells because of dendritic filtering of mEPSCs (Gardner et al., 1999). The desensitization time constants were likewise rapid. Single exponential time constants for desensitization for bushy, octopus, T stellate, and tuberculoventral cells ranged between 0.91 and 0.96 msec (Table 1) and

were not significantly different ($p > 0.05$; t test). In all patches from cells with mainly auditory nerve input, the desensitization time course was well fit by a single exponential and the currents in all patches desensitized by $>99\%$. Desensitization in patches from the basal dendrites of fusiform cells was biexponential and slower than desensitization in the other auditory nerve targets (Table 1) and desensitized by 96% on average.

The time course for deactivation and desensitization in targets of the parallel fibers was significantly slower than in auditory nerve targets ($p < 0.01$; t test). The time constants for deactivation of currents evoked in patches from the apical dendrites of fusiform cells and in cartwheel cells were 1.4 and 1.7 msec, respectively. Desensitization time constants were 3.9 msec for apical dendrites of fusiform cells and 4.4 msec for cartwheel cells. Three (of 20) cartwheel cell patches had currents that decayed with double exponential time constants, of 1.02 ± 0.28 (67%), 4.12 ± 1.29 (33%) msec for deactivation and 3.60 ± 0.38 (45%), 17.75 ± 2.94 (55%) msec for desensitization. The currents from fusiform cell apical dendrites and cartwheel cells desensitized on average to 93 and 95%, respectively. The deactivation and desensitization time constants of the AMPA receptors in fusiform cell apical dendrites and cartwheel cells were not significantly different from one another ($p > 0.05$; t test). Currents elicited in response to 300 μ M AMPA were identical in time course to those in response to glutamate in all cells tested ($n = 6$; data not shown), and 1 mM kainate evoked nondesensitizing currents ($n = 3$; data not shown), verifying that the receptors studied were of the AMPA subtype.

The size of the evoked currents varied widely from patch to patch for each cell type (6–1000 pA), but the time course of those currents was consistent. The coefficient of variation (CV), (the SD divided by the mean) for both kinetic parameters in each cell type was ≤ 0.3 . This suggests that the receptors being examined were from a homogeneous population across many patches for each cell type and that receptors postsynaptic to any minor glutamatergic inputs were identical to those postsynaptic to the auditory nerve. The receptors that were studied in somatic patches from cartwheel cells were homogeneous because the CV for both deactivation, and desensitization was not significantly different from the other cell types ($p > 0.05$; t test).

Flop subunit isoforms are present in rapidly gated AMPA receptors

Coapplication of glutamate and cyclothiazide allowed us to determine whether the rapid desensitization kinetics observed in AMPA receptors in auditory nerve targets were the result of the presence of the flop subunit splice variant. Figure 2 shows that cyclothiazide slowed the desensitization (10–30 times normal) in cartwheel and octopus cells. This result also indicates that AMPA and not kainate receptors were being studied because cyclothiazide blocks desensitization in only the AMPA glutamate receptor subtype (Partin et al., 1994). Long applications of 10 mM glutamate with 200 μ M cyclothiazide to patches from cells with primarily auditory nerve input showed desensitization that was $>90\%$ after 3 sec (Fig. 3A–C). The amount of desensitization in cyclothiazide was not significantly different from in the absence of cyclothiazide ($p > 0.05$; t test), indicating that these receptors contain primarily the flop subunit isoforms. In contrast, the currents in patches from cartwheel cells and the apical and basal dendrites of fusiform cells desensitized to only 54–80% after 3 sec and were significantly different from control ($p < 0.01$; t test) (Fig. 3A–C). Comparison of these results with receptors in ex-

Table 1. Properties of AMPA receptors in outside-out patches from cells in the cochlear nuclei

Cell type	$\tau_{\text{deactivation}}$ (msec)	$\tau_{\text{desensitization}}$ (msec)	Rectification ratio (I ₆₀ /I ₋₆₀)	τ_{recovery} (msec)	Single channel current (pA)	Open probability
Bushy*	0.35 ± 0.07 (n = 14)	0.91 ± 0.08 (n = 17)	0.26 ± 0.04 (n = 6)	19 ± 3 (n = 6)	1.80 ± 0.62 (n = 5)	0.53 ± 0.18 (n = 5)
Octopus*	0.35 ± 0.07 (n = 13)	0.92 ± 0.05 (n = 17)	0.23 ± 0.05 (n = 5)	20 ± 6 (n = 5)	1.58 ± 0.79 (n = 5)	0.62 ± 0.17 (n = 5)
T stellate*	0.41 ± 0.08 (n = 22)	0.92 ± 0.12 (n = 21)	0.23 ± 0.04 (n = 6)	20 ± 6 (n = 5)	1.90 ± 0.78 (n = 5)	0.55 ± 0.15 (n = 5)
Tuberculoventral*	0.39 ± 0.08 (n = 24)	0.96 ± 0.20 (n = 15)	0.29 ± 0.16 (n = 6)	17 ± 4 (n = 5)	1.94 ± 0.74 (n = 5)	0.53 ± 0.16 (n = 5)
Fusiform basal	0.54 ± 0.11 (n = 13)	1.42 ± 0.34 (46%) 6.40 ± 0.34 (54%) (n = 6)	0.97 ± 0.12 (n = 8)	22 ± 6 (n = 5)	0.69 ± 0.32 (n = 6)	0.49 ± 0.11 (n = 6)
Fusiform apical**	1.40 ± 0.45 (n = 16)	3.89 ± 1.05 (n = 10)	0.94 ± 0.17 (n = 5)	36 ± 9 (n = 5)	0.50 ± 0.24 (n = 7)	0.45 ± 0.22 (n = 7)
Cartwheel**	1.65 ± 0.49 (n = 19)	4.40 ± 1.13 (n = 20)	0.91 ± 0.19 (n = 9)	49 ± 6 (n = 7)	0.67 ± 0.25 (n = 6)	0.46 ± 0.10 (n = 6)

*There was no significant difference between these cell types for all parameters ($p > 0.05$; t test).

**There was no significant difference between these cell types for all parameters except recovery from desensitization ($p > 0.05$; t test).

Data are expressed as the mean ± SD.

Figure 2. Cyclothiazide slows desensitization in both auditory nerve targets and parallel fiber targets. *Left*, AMPA receptors in an octopus and cartwheel cell desensitize during a 100 msec exposure to 10 mM glutamate. *Middle*, Glutamate-evoked currents in the presence of 200 μ M cyclothiazide show a dramatic slowing of desensitization, but not a complete block in both cell types, and there was little potentiation of the current peak. Cyclothiazide was less effective in blocking desensitization in the octopus cell. *Right*, Currents evoked with a 3 sec exposure to 10 mM glutamate and 200 μ M cyclothiazide desensitized to >90% in the octopus cell and 82% in the cartwheel cell.

pression systems in which receptors with only flip subunits do not desensitize suggests that receptors in cartwheel cells and in fusiform apical and basal dendrites contain both flip and flop splice variants (Partin et al., 1994; Koike et al., 2000).

Recovery from desensitization

Currents in patches from auditory nerve targets recovered from desensitization faster than currents in patches from cells postsynaptic to parallel fibers. A conditioning pulse of 10 mM glutamate for 50 msec desensitized receptors. Recovery from desensitization was monitored with a test pulse of 5 msec in duration. The

intervals between the conditioning and test pulse varied between 5 and 380 msec. The currents from all cell types recovered with a single exponential time course after a delay of 5 msec for bushy, octopus, T stellate, tuberculoventral, and fusiform cells and 25 msec for cartwheel cells (Fig. 4). The delay is thought to represent the cycling of receptors out of the desensitized state (Raman and Trussell, 1995). The averaged time constants for the recovery from desensitization in targets of the auditory nerve were ~20 msec (Table 1). Currents from fusiform apical dendrites and cartwheel cells recovered from desensitization with time constants of ~36 and 50 msec, respectively (Table 1). These rates differed significantly from one another and were slower than those in auditory nerve targets ($p < 0.01$; t test).

AMPA receptors in cells with only auditory nerve input contain little GluR2

To test for the presence or absence of the GluR2 subunit, 10 mM glutamate was applied for 100 msec to patches whose membrane potentials were stepped from +80 to −100 mV in 20 mV steps in the presence of 100 μ M intracellular spermine (Bowie and Mayer, 1995; Donevan and Rogawski, 1995; Kamboj et al., 1995). The normalized peak current from several patches for each cell type is plotted as a function of the holding potential in Figure 5. Current–voltage relationships were inwardly rectifying at positive holding potentials in patches from cells whose input is mainly from the auditory nerve, but not in patches from fusiform basal and apical dendrites and cartwheel cells. The rectification ratio (I_{+60}/I_{-60}), which provides a measure of the deviation of the current–voltage relationship from linear (a value of 1), was <0.30 in auditory nerve targets and >0.90 in fusiform cell dendrites and cartwheel cells (Table 1). These observations suggest that AMPA receptors in cells with mainly auditory nerve input generally lack GluR2, whereas those in cells that receive some or all of their input from parallel fibers contain more GluR2. This finding is consistent with the result that mEPSCs in cells with mainly auditory nerve fiber input are sensitive to the polyamine-containing toxin, philanthotoxin, whereas mEPSCs in fusiform and cartwheel cells are not (Gardner et al., 1999).

Figure 3. AMPA receptors in auditory nerve and parallel fiber targets contain subunits in the flop isoform. *A*, Glutamate-evoked currents in the presence of 200 μ M cyclothiazide desensitized in all cell types but with a slower time course than under control conditions. *B*, Currents evoked by glutamate in the presence of 200 μ M cyclothiazide desensitized by >90% in receptors from cells with only auditory nerve fiber input. Currents in the fusiform basal dendrites, apical dendrites, and in the cartwheel cell desensitized to ~75%. *C*, Pooled data from at least five patches for each cell type show that AMPA receptors in auditory nerve targets desensitized to a similar extent in cyclothiazide (dark bars) as in the absence (light bars). In contrast, AMPA receptors in fusiform basal and apical dendrites and cartwheel cells desensitized significantly less in the presence of cyclothiazide compared with control ($p < 0.01$; t test). In addition, in the presence of cyclothiazide, AMPA receptors in fusiform and cartwheel cells desensitized significantly less than those in cells with only auditory nerve input ($p < 0.01$; t test). Error bars indicate SD.

AMPA receptors that lack GluR2 have larger single-channel currents than receptors that contain GluR2

It has been shown that AMPA receptors that lack GluR2 have single-channel currents and conductances that are 2–3 times larger than those that contain GluR2 (Koh et al., 1995; Swanson et al., 1997). To determine whether this is true of AMPA receptors in the cochlear nuclei, the single-channel current was estimated with nonstationary variance analysis. At least 30 repetitions of currents evoked with long, desensitizing exposures to glutamate at a holding potential of -65 mV were used for this analysis. Figure 6*A* shows an example of currents in an octopus cell in response to 50 consecutive exposures to glutamate for 100

Figure 4. AMPA receptors in auditory nerve targets recover from desensitization faster than in parallel fiber targets. Recovery from desensitization ($I_{\text{test}}/I_{\text{condition}}$) of the AMPA receptors was plotted as a function of interstimulus interval. A single exponential described the time course of recovery for all cell types after a delay of 5 msec in bushy, octopus, T stellate, tuberculoventral, and fusiform cells and 25 msec in cartwheel cells. Each point represents the average value from at least five different patches from each cell type with the SD (error bars).

msec. The single-channel currents for AMPA receptors in patches from cells with mainly auditory nerve input were similar and not statistically different from one another ($p > 0.05$; t test). Single-channel currents at -65 mV were between 1.6 and 1.9 pA (Fig. 6*B*, Table 1). In contrast, the single-channel current for fusiform cell basal and apical dendrites and cartwheel cell AMPA receptors was ~ 0.7 pA (Table 1). These single-channel currents at a holding potential of -65 mV yield single-channel conductances of 28 pS for bushy, octopus, T stellate, and tuberculoventral cells and 10 pS for fusiform and cartwheel cells, which are consistent with the GluR2 content of these channels. The number of channels in patches from all cells ranged from 7 to 415. The maximum open probability of channels ($P_{O, \text{max}}$) was estimated as I_{max}/iN . The open probability of channels from all cells was in all cases ~ 0.5 and not significantly different ($p > 0.05$; t test), regardless of single-channel current at the glutamate concentration used.

Synaptic receptors have the same features as patch receptors from fusiform cell dendrites

We found that receptors in excised patches from apical and basal dendrites differ with respect to kinetics but that patches from both sets of dendrites contain the GluR2 subunit. To verify that these patch receptors have the same kinetic properties and GluR2 content as known synaptic receptors, we used hypertonic solution to evoke transmitter release from the two sources of input onto the apical and basal dendrites, selectively. While recording in the whole-cell mode at the cell body, a pulse of 0.5 M sucrose solution was applied to the proximal basal or apical dendrites to elicit

Figure 5. In the presence of intracellular polyamines, current-voltage relationships are inwardly rectifying in cells with mainly auditory nerve input and linear in fusiform and cartwheel cells. Patches were exposed to glutamate for 100 msec at a range of holding potentials from +80 to -100 mV in 20 mV steps. Normalized peak currents obtained from patches with 100 μ M spermine in the pipette were plotted as a function of the membrane potential. The current-voltage plots exhibited inward rectification at positive membrane potentials in cells with mainly auditory nerve input, whereas those in fusiform and cartwheel cells were linear throughout the entire voltage range. Each point represents the normalized mean with the SD shown as the error bars.

mEPSCs (Fig. 7A). The rise times for the currents were not significantly different and were 0.35 ± 0.12 and 0.32 ± 0.08 msec for apical and basal dendrites, respectively ($p > 0.05$; t test). mEPSCs evoked in the basal dendrites had significantly faster decays than those in the apical dendrites (Fig. 7B). Single exponential decays were 1.06 ± 0.44 msec in the basal dendrites and 1.98 ± 0.67 msec in apical dendrites ($n = 7$ for both dendrites). These kinetic differences parallel those in patches but are slower, presumably reflecting dendritic filtering of the sucrose-evoked mEPSCs.

To test for the presence of the GluR2 subunit the polyamine-containing toxin, philanthotoxin (PhTX), was applied to open channels. After recording sucrose-evoked mEPSCs in normal bath saline, the slice was bathed in 50 μ M each of PhTX, kainate, and Cd^{2+} for 2 min. After the holding current returned to baseline in normal saline, mEPSCs were evoked. Sucrose-evoked events persisted after application of PhTX at both sets of dendrites, indicating that the receptors were insensitive to PhTX and thus that they contain GluR2 (Fig. 7A). The evoked events were identical with respect to amplitude, rise time, decay time, and frequency before and after PhTX (data not shown). In contrast, PhTX blocked both spontaneous and sucrose-evoked mEPSCs in two T stellate and octopus cells (Fig. 8). This result verifies that

Figure 6. AMPA receptors in cells with mainly auditory nerve input have larger single-channel currents than cells with parallel fiber input. *A*, Left, 50 consecutive currents evoked by 100 msec exposures to glutamate in a patch from an octopus cell at -65 mV. The mean current is superimposed (light gray). Right, The variance of the current for data on the left. *B*, Plots of the variance versus the mean current for at least 30 consecutive currents for single patches from all neurons. The parabolic fits are superimposed on the data.

PhTX can block both spontaneous and sucrose-evoked mEPSCs in cells whose AMPA receptors contain little, if any, GluR2 (Gardner et al., 1999). These data indicate that receptors in excised patches from fusiform cell dendrites have kinetic differences and permeability that parallel those of synaptic receptors. Receptors in the basal dendrites contain more GluR2 than other auditory nerve targets, but have faster decay kinetics than receptors in the apical dendrites that are postsynaptic to the parallel fibers that also contain GluR2.

DISCUSSION

The temporal resolution of the rapid application of glutamate to excised patches was used together with pharmacological probes to compare the molecular composition of AMPA receptors in six

Figure 7. The properties of receptors in fusiform dendrite patches are the same as synaptic receptors. *A, Left*, Four consecutive sweeps of spontaneous mEPSCs recorded in whole-cell mode at -65 mV . *Middle*, mEPSCs evoked in response to a 60 msec spritz of sucrose saline on an apical dendrite. *Right*, mEPSCs evoked in response to a 60 msec spritz of sucrose saline on a basal dendrite. The data are shown before (*top*) and after (*bottom*) application of PhTX. *B*, Sucrose-evoked mEPSCs have faster kinetics in basal dendrites than in apical dendrites. *Left*, Histograms of the single exponential decay time constants fit to individual events from the cells illustrated above. *Right*, Ensemble average currents from 75 events for each dendrite with single exponential fits superimposed.

cell types in the cochlear nuclei. These experiments led to six findings. First, AMPA receptors postsynaptic to the auditory nerve have faster gating kinetics than receptors postsynaptic to the parallel fibers. Second, among the cells whose major source of excitation is the auditory nerve, AMPA receptors were indistinguishable with respect to kinetics, block by polyamines, and sensitivity to cyclothiazide. Third, there was no discernable difference between synaptic and extrasynaptic receptors. Not only were receptors from an individual cell type homogeneous across different patches, but they also had deactivation times similar to those of miniature synaptic currents recorded in these same cells and they had similar polyamine sensitivity (Gardner et al., 1999). Fourth, receptors postsynaptic to auditory nerve fibers in basal dendrites of fusiform cells exhibited faster deactivation and recovery kinetics than receptors postsynaptic to the parallel fibers in

Figure 8. PhTX blocks spontaneously occurring and sucrose-evoked mEPSCs in a T stellate cell. *Left*, Four consecutive current sweeps of spontaneous currents before (*top*) and after (*bottom*) PhTX at -65 mV . *Right*, Sucrose applications for 60 msec to the cell body before and after PhTX at -65 mV .

apical dendrites. Fifth, receptors associated with a source of input are not necessarily identical. Receptors postsynaptic to the auditory nerve on the basal dendrites of fusiform cells contain detectable levels of GluR2 and flip isoforms, whereas those in other auditory nerve targets do not. Receptors postsynaptic to parallel fibers in cartwheel cells recovered from desensitization more slowly than those in fusiform cells. Sixth, receptors on basal dendrites of fusiform cells were kinetically intermediate between those associated with the auditory nerve in cells not innervated by parallel fibers and those apposed to parallel fibers in fusiform and cartwheel cells. Receptors on the basal dendrites of fusiform cells desensitized more slowly and were more sensitive to cyclothiazide than other targets of the auditory nerve, but all exhibited rapid deactivation and recovery from desensitization.

Molecular composition of AMPA receptors

Several lines of evidence are consistent with the conclusion that AMPA receptors associated with auditory nerve fiber synapses are formed mainly from GluR3_{flip} and GluR4_{flip} subunits. The present findings show that glutamate receptors in octopus, T stellate, and tuberculoventral cells have properties that are indistinguishable from those in bushy cells. Electronmicroscopic immunohistochemical localization of AMPA receptor subunits in bushy and octopus cells shows that mostly GluR3 and GluR4 subunits are found opposite auditory nerve fiber terminals (Petrá et al., 1996, 2000; Wang et al., 1998; Korada and Schwartz, 2000; Schwartz et al., 2000). The desensitization kinetics of these rapidly gating receptors resemble those for GluR4_{flip} homomeric

channels (Mosbacher et al., 1994). Finally, Schmid et al. (2001) have shown that GluR3_{flop} and GluR4_{flop} mRNA is high in the ventral and deep layer of the dorsal cochlear nuclei where these cells reside. The similarity in the rate at which receptors recovered from desensitization indicates that the RNA editing at the R/G site near the flip–flop module is the same in these cells (Lomeli et al., 1994). In the brainstem auditory nuclei of birds the AMPA receptors in the auditory pathway are likewise faster than nonauditory neurons (Raman et al., 1994) and contain mRNA (Ravindranathan et al., 2000) and protein predominately in the flop isoforms (Lawrence and Trussell, 2000).

In addition to containing GluR3 and GluR4, bushy and octopus cells appeared also to contain low levels of GluR2 (Korada and Schwartz, 2000; Petralia et al., 2000; Schwartz et al., 2000). Presumably the proportion of GluR2-containing receptors was low because they were not detected functionally; all mEPSCs in octopus and bushy cells were blocked by PhTX and AMPA receptors in excised patches consistently had inwardly rectifying current–voltage relationships in the presence of intracellular spermine (Gardner et al., 1999; present study).

The subunit composition of AMPA receptors differs in the two tufts of dendrites of fusiform cells and includes both flip and flop isoforms. The basal dendrites contain GluR2, GluR3, and GluR4 subunits, whereas the apical dendrites contain only GluR2 and GluR3 and exclude GluR4 (Rubio and Wenthold, 1997). The currents in the basal dendrites have the rapid kinetics predicted by the presence of GluR4 subunits. The presence of GluR2 in both basal and apical dendrites is in agreement with the insensitivity of the current–voltage relationship of glutamate-evoked currents to spermine and the insensitivity of all mEPSCs to philanthotoxin (Gardner et al., 1999; present study). Receptors in both sets of dendrites were somewhat sensitive to cyclothiazide, suggesting that both flip and flop isoforms are present. Schmid et al. (2001) have shown that mRNA for both isoforms is present in the superficial and deep layers of the dorsal cochlear nucleus.

AMPA receptors postsynaptic to parallel fibers on cartwheel cells differ from those on fusiform cells. Cartwheel cell spines contain GluR1, whereas most fusiform cell spines do not (Petralia et al., 1996). The GluR2 and GluR3 subunits have been shown to be present (Petralia et al., 1996, 2000; Schwartz et al., 2000). Our results confirm the presence of GluR2 and show that some subunits are in the flip isoform. The difference in the rate of recovery from desensitization between receptors in cartwheel and in the apical dendrites of fusiform cells could reflect a difference in R/G editing or in which subunits are in the flip isoform.

Potential influences on receptor subunit expression

The association of receptor subtype with the source of input has been documented in several regions of the brain. In the auditory system it has been demonstrated both structurally and functionally (Raman et al., 1994; Petralia et al., 1996, 2000; Rubio and Wenthold, 1997; Wang et al., 1998; Gardner et al., 1999; Lawrence and Trussell, 2000; Ravindranathan et al., 2000). It has also been demonstrated in the hippocampus (Tóth and McBain, 1998). However, the source of input alone does not specify the composition of postsynaptic receptors. The present results show that receptors that oppose the auditory nerve in fusiform cells differ from those in tuberculoventral, octopus, bushy, and T stellate cells. They also confirm the immunocytochemical findings that receptors that oppose parallel fibers in cartwheel and fusiform cells are not identical (Petralia et al., 1996).

Activity has been shown to influence subunit composition (Liu

and Cull-Candy, 2000). Differences in the shapes of neurons, the pattern of the convergence of input from the auditory nerve and from other excitatory and inhibitory sources, and their differing biophysical properties contribute to large differences in the amplitude of synaptic currents that drive these cells and differing responses to sound. Bushy, T stellate, and tuberculoventral cells receive input from few auditory nerve fibers, but the low input resistance of bushy cells in the physiological voltage range requires that they be driven by larger synaptic currents (Wu and Oertel, 1984; Oertel, 1985; Manis and Marx, 1991; Zhang and Oertel, 1993b; Zhang and Trussell, 1994; Ferragamo et al., 1998). Octopus cells require large synaptic currents, but these arise from more auditory nerve fibers (Golding et al., 1995, 1999; Oertel et al., 2000). Cartwheel and fusiform cells are excited not only by sound but also by other sensory modalities (Davis and Young, 1997). The responses to sound are different in the different types of cells (bushy: Bourk, 1976; Rhode et al., 1983; stellate: Smith and Rhode, 1989; octopus: Godfrey et al., 1975; Rhode and Smith, 1986; Oertel et al., 2000; tuberculoventral: Shofner and Young, 1985; Rhode, 1999; fusiform: Smith and Rhode, 1985; Spirou and Young, 1991; Nelken and Young, 1994; cartwheel: Parham and Kim, 1995). It is not known whether there are overall differences in firing rates among these cell types *in vivo* under natural conditions.

It has been suggested that increases in electrical activity with maturation are associated with increases in GluR2 levels and parallel decreases in calcium permeability (Hollmann et al., 1991; Sommer et al., 1991; Jonas et al., 1994; Geiger et al., 1995; Akaïke and Rhee, 1997; Pickard et al., 2000; Liu and Cull-Candy, 2000). Cochlear nuclear neurons seem not to follow this pattern. They would be expected to have high average firing rates but have few GluR2-containing receptors. Furthermore, GluR2 may be down-regulated during development (Lawrence and Trussell, 2000).

The role of the Ca²⁺ flux through AMPA receptors in cochlear nuclear neurons is not understood. In some cells the Ca²⁺ influx regulates the excitatory synapses (Barria et al., 1997; Benke et al., 1998; Liu and Cull-Candy, 2000). Many neurons in the brainstem auditory nuclei stand out for their strong immunostaining for calcium binding proteins that could serve to buffer or localize calcium transients. Bushy, T stellate, and octopus cells contain high levels of parvalbumin and calretinin that are modulated by activity but they contain little calbindin (Lohmann and Friauf, 1996; Caicedo et al., 1996, 1997; Korada and Schwartz, 2000). Cartwheel cells, in contrast, are labeled heavily for calbindin but not for parvalbumin or calretinin (Caicedo et al., 1996; Korada and Schwartz, 2000). Fusiform and tuberculoventral cells are not strongly labeled for any of these calcium binding proteins. The staining pattern is not correlated with the expression of GluR2 and seems to be determined differently in the different types of cells. We suggest that Ca²⁺ flux through AMPA receptors may serve varied functions and can be regulated differently in the different cell types.

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