(7), these three α-chain mutations are responsible for the β-hexosaminidase A deficiency in all Ashkenazi adult G_{M2} gangliosidosis patients and infantile Tay-Sachs patients tested thus far. Diagnosis based on these mutations will be helpful for carrier detection and genetic counseling when the enzymatic assay is inconclusive. Furthermore, it is now possible, with allele-specific probes, to distinguish carriers of the adult mutation from carriers of the infantile mutations, which is not achievable with the conventional enzymatic assay. Finally, it will be of interest to determine if the mutation described here is present in the various clinical forms (juvenile, chronic, and adult) associated with β-hexosaminidase A deficiency in Ashkenazi Jews and in other ethnic groups (*28*) and to assess the clinical consequences of this mutation in a homozygous form.

Note added in proof: We have learned that Paw *et al.* (*29*) have also identified The Gly^{269} to Ser charge in other patients with adult-onset G_{M2} gangliosidosis.

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Quisqualate Activates a Rapidly Inactivating High Conductance Ionic Channel in Hippocampal Neurons

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Glutamate activates a number of different receptor-channel complexes, each of which may contribute to generation of excitatory postsynaptic potentials in the mammalian central nervous system. The rapid application of the selective glutamate agonist, quisqualate, activates a large rapidly inactivating current (3 to 8 milliseconds), which is mediated by a neuronal ionic channel with high unitary conductance (35 picosiemens). The current through this channel shows pharmacologic characteristics similar to those observed for the fast excitatory postsynaptic current (EPSC); it reverses near 0 millivolts and shows no significant voltage dependence. The amplitude of the current through this channel is many times larger than that through the other non-NMDA (*N*-methyl-D-aspartate) channels. These results suggest that this high-conductance quisqualate-activated channel may mediate the fast EPSC in the mammalian central nervous system.

GLUTAMATE IS BELIEVED TO BE AN important excitatory neurotransmitter in the mammalian central nervous system (*1*). The glutamate-evoked current is the result of the behavior of more than one type of glutamate channel coexisting on the same neuron (*2, 3*). These different receptor-channel complexes are postulated to play a variety of physiological roles (*4–7*). A rapidly desensitizing component of the glutamate-evoked current is seen when the agonist is applied rapidly (*8–11*). Here, we identify and characterize the properties

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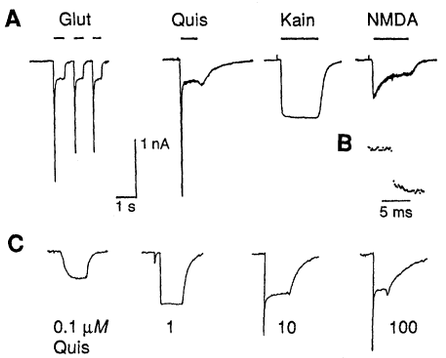

Fig. 1. Voltage-clamped whole-cell currents evoked by rapid step increases in glutamate agonists. (**A**) Glutamate, quisqualate, kainate, and NMDA (at 100 μ*M*) were applied for a sustained period indicated by the bars above the traces. The amplitude calibration bar for NMDA is 0.5 nA. (**B**) Recording showing the speed of solution change with the rapid perfusion system we used for these experiments. A solution containing Cl^- is switched to one without Cl^-. The recording measures the change in diffusion potential at the tip of an electrode. (**C**) Current recordings from one cell sequentially activated by four different concentrations of quisqualate. External solution contained 150 m*M* NaCl, 3 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$ (except for experiments involving NMDA where no Mg^{2+} was added), 2 μ*M* tetrodotoxin, and 10 m*M* Hepes (*p*H 7.3). Pipette solution consisted of 140 m*M* CsCl, 2 m*M* EGTA, 2 m*M* $MgCl_2$, and 10 m*M* Hepes (*p*H 7.3). Experiments were done at room temperature and at a holding potential of −80 mV unless otherwise indicated.

of a quisqualate-activated channel that mediates this fast transient current. This channel may mediate fast excitatory synaptic transmission in the central nervous system.

We used cultured hippocampal neurons from 2- to 4-week-old rats (*12*). Membrane currents were measured by either the whole-cell or the outside-out patch-clamp technique (*13*). Intracellular and extracellular solutions are described in the legends to the figures. Agonists were applied according to a modification of a rapid perfusion system (*14*). This modification allows step changes in the concentration of the agonist within a millisecond to a number of different levels with equal speeds (*15*). The diffusion potential change associated with switching a Cl^--containing solution to a Cl^--free solution is shown in Fig. 1B. With this rapid perfusion system, we measured the whole-cell currents evoked by glutamate and its selective agonists, quisqualate, kainic acid, and NMDA (Fig. 1A). Glutamate repetitively activated a large rapidly decaying current with a maintained component (Fig. 1A). Of the selective glutamate agonists, only quisqualate consistently activated the fast transient current. Kainic acid and NMDA were never found to induce a fast transient current. The glutamate- and quisqualate-evoked fast transient currents had a reversal potential near zero. The current-voltage relation and the rate of decay of the transient current remained constant over membrane potentials between −80 and +80 mV. Unlike the NMDA current, the quisqualate- and glutamate-evoked transient currents were not blocked by extracellular concentrations of Mg^{2+} or by amino-phosphono-valeric acid (APV). In the experiments described below, we used quisqualate to avoid the activation of the other glutamate-sensitive receptor-channel complexes.

Two distinct components of the current may be distinguished at different quisqualate concentrations (Fig. 1C). The maintained current was activated below 50 n*M*, increased with increasing concentrations of quisqualate, and reached maximum near 1 μ*M*. The Hill coefficient of the dose-response relation was 1.71 (*16*). At concentrations higher than 1 μ*M*, the maintained current decreased. The fast transient current, on the other hand, did not appear until the concentration of the agonist was above 1 μ*M*. The transient current was further enhanced at higher concentrations and reached near maximal values around 300 μ*M*. We examined whether these two kinetically distinct currents were mediated by two different sets of channels.

The maintained quisqualate current is carried by a low-conductance channel (8 pS) (*17–19*). However, the transient current was carried by a previously unreported higher conductance channel. We compared the responses of a whole cell and an isolated membrane patch to three different concentrations of quisqualate (Fig. 2). The activation of the transient whole-cell current corresponded with the activation of a high-conductance channel that is recorded at concentrations larger than 1 μ*M*. The high-conductance channel openings occurred only at times corresponding to the activation of the transient current and not throughout the agonist application. Although the low conductance channels were evenly distributed over the cell body (*17*), the higher conductance channels were not. The majority of excised patches did not contain the high-conductance channel; when present, the number of high-conductance channels per patch was large.

The current through the high-conductance channel reversed near 0 mV (Fig. 3A). The conductance of the channel was about 35 pS and did not show significant voltage dependency. The insets to the graph show unitary currents from a single membrane patch activated at indicated membrane potentials. Figures 2B and 3B show examples of unitary events with transitions consistent with the 35-pS channel openings in patches containing more than one channel. Five sequential activations of a patch containing a 35-pS channel are shown (Fig. 3C). In other patches, brief openings with conductance higher than 35 pS as well as lower intermediate conductance openings could be occasionally observed, but the 35-pS opening was the dominant event. The quisqualate-activated 35-pS channel was distinguished from the NMDA-activated channel as it was not blocked by extracellular Mg^{2+} or by APV (*20, 21*). This quisqualate-activated channel differs also from the fast desensitizing glutamate-activated channel found in arthropod muscle, since the latter had a conductance of 150 pS (*22*).

Pharmacological studies of the fast excitatory postsynaptic current (EPSC) have suggested that the fast EPSC is associated with a non-NMDA glutamate receptor-channel complex (*4–7*). The fast EPSC is blocked by the glutamate antagonist, kynurenic acid (*23, 24*), but not by APV (*5, 7*). We found that these antagonists had similar actions on the fast quisqualate-activated current. The reversible block of the transient quisqualate-activated current by kynurenic acid is shown (Fig. 4C). APV at 500 μ*M*, on the other hand, had no significant effect on the transient quisqualate current.

Recovery from "desensitization" of the

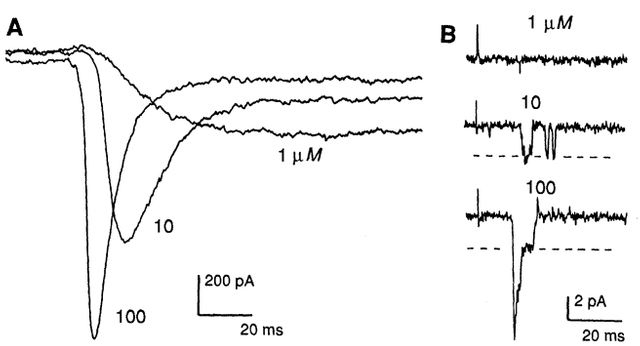

Fig. 2. The transient quisqualate-activated current is mediated by a high-conductance channel. The concentration dependence of activation in the whole cell and in an excised patch is shown. (**A**) A small neuron (diameter about 20 μm) is activated by step applications of three concentrations of quisqualate. These whole-cell currents are displayed on an expanded scale compared to the traces in Fig. 1. (**B**) Recordings from a single excised outside-out patch activated in the same manner as in (A). The time scales in (A) and (B) are the same. The dotted line shows the expected current amplitude of 35 pS transition for currents that reverse at 0 mV.

transient quisqualate current was rapid. Currents from excised patches could be fully reactivated within 350 ms of each other (Fig. 4B). Faster recovery (<100 ms) was observed when glutamate was the agonist. It is likely that reactivation is in part determined by the limitation of the fast perfusion system and the desensitizing effect of submicromolar concentrations of quisqualate and glutamate (*25*).

The relative amplitude of the transient current compared to the maintained current is underestimated when measured in the whole-cell mode. When agonist is applied to the whole cell, all the channels do not open at once because the agonist does not have free access to all the receptors and because the receptors can be separated on the neuron (*8*). This temporal dispersion of activation widens the duration and underestimates the amplitude of the fast transient current. Measurements from the excised membrane patch are probably more representative of the relative contribution of the two current components at the postsynaptic site. In excised patches the transient quisqualate-activated current contributes current many times larger than the maintained current within the few milliseconds required for fast synaptic transmission.

Our studies show that the most unique characteristic of this channel is its high probability of initial opening and its low probability for repeated opening to a sustained agonist exposure. This characteristic translates into a current that inactivates or desensitizes rapidly. When a patch containing large numbers of channels was exposed to a rapid step application of quisqualate, the current decayed with a time constant of 3 ms (Fig. 4A). This rate of desensitization is about the same as that measured for the decay of the fast EPSC (*7*). The physiological role for this fast desensitization is not as yet clear. One possibility is that this intrinsic property of the postsynaptic receptor–channel complex may determine the duration of the fast EPSC under normal conditions. The other possibility is that this rapid desensitization serves a protective role by limiting the duration of the large current carried by these channels under conditions where the neurotransmitter clearance is hindered. The latter hypothesis assumes that normally the duration of neurotransmitter within the synaptic cleft is brief compared to the time course of the EPSC (*26*). Direct information on the time course of neurotransmitter clearance from the synaptic site is not available.

The rapidly activating and inactivating high-conductance channel described here has many of the characteristics expected from the channel underlying the fast EPSC in the mammalian central nervous system. Both the fast EPSC and the current carried by the high-conductance channel reverse near 0 mV and show little or no voltage dependence. Both can be repetitively and rapidly activated. Both are blocked by kynurenic acid and are unaffected by APV. The channel inactivates rapidly and has open times that are consistent with the duration of the EPSC. The clustering of this channel in membrane patches suggests that it is capable of generating a large focal current at the time scale appropriate for fast synaptic transmission. We thus suggest that this 35-pS quisqualate-activated channel is a prime candidate for mediating fast excitatory synaptic transmission in the mammalian central nervous system.

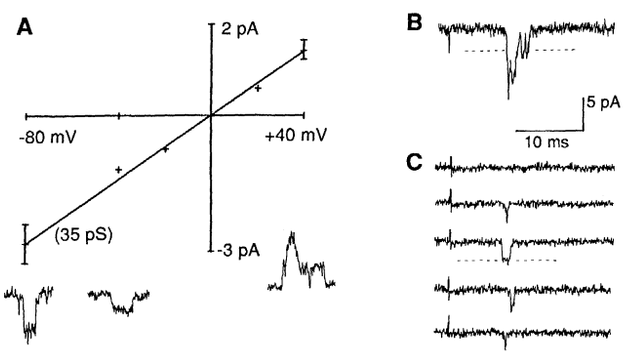

Fig. 3. Single-channel recordings of the channels mediating the transient quisqualate-activated current. (**A**) Determination of unitary channel conductance and reversal potential. Single-channel currents from four different patches were measured at different membrane potentials. The dominant conductance is 35 pS. The currents reverse near 0 mV. (Inset) Recordings from a single membrane patch voltage-clamped at −80, −40, and +40 mV. (**B**) The majority of excised patches contained more than one channel. Large-conductance transitions and not low-conductance transitions can be seen as the dominant events. This trace shows one such example. (**C**) Sequential recordings form a single excised patch containing a 35-pS (dotted line) channel that is repetitively activated by 100 μ*M* quisqualate (100-ms-long pulse with an interpulse delay of 1 s). This and other patches not shown here consistently show that these 35-pS quisqualate-activated channels have high probability of initial opening with very low probability of repeated opening to a sustained quisqualate application. Signals were sampled at 10 kHz and filtered at 2 kHz with an 8-pole Bessel filter.

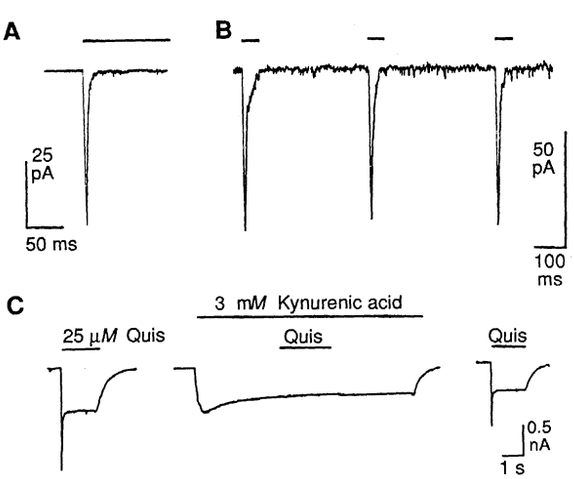

Fig. 4. (**A**) The transient quisqualate-activated current desensitized rapidly. This current recording shows that an excised patch containing a large number of channels can decay with a rate constant of 3 ms when activated by a step application of 100 μ*M* quisqualate. (**B**) The transient current can be repetitively and fully activated. Pulses of 100 μ*M* quisqualate are applied for 50 ms with interpulse delay of 350 ms. (**C**) The transient quisqualate current can be reversibly blocked by kynurenic acid.

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The EGF Receptor Kinase Substrate p35 in the Floor Plate of the Embryonic Rat CNS

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P35 is a calcium- and phospholipid-binding protein that was originally isolated as a substrate for the epidermal growth factor (EGF) receptor tyrosine kinase and later was found to be related to lipocortin I. Immunohistochemistry was used to localize p35 to a raphe of primitive glial ependymal cells in the median one-third of the floor plate in the central nervous system (CNS) of rat embryos. The p35 appears by embryonic day 12 before the arrival of pioneering ventral commissural axons. The unexpected, discrete distribution of this protein during development opens the question of its role in neural morphogenesis.

THE CHARACTERIZATION OF SEVERAL viral oncogene products and the receptors for EGF and other growth factors as enzymes that phosphorylate tyrosine (tyrosine kinases) has led to interest in identifying substrates involved in mediating the effects of these kinases. The molecular and chemical characteristics of putative tyrosine kinase substrates have been reported (*1*); however, most studies have used cells labeled with ^{32}P in culture.

Our laboratories have focused on a 35-kD protein (p35), originally isolated from human carcinoma cells and shown to be a Ca^{2+}-dependent substrate for the EGF receptor kinase (*2*). We have used immunohistochemical methods to determine the cellular distribution of p35 in tissue sections from embryonic rats and have detected a restricted pattern of distribution in the CNS that suggests a role for p35 in neural morphogenesis.

Embryos from pregnant rats under nembutal anesthesia were fixed with Carnoy's fluid (chloroform:ethanol:acetic acid; 3:6:1), embedded in paraffin, and sectioned. Immunohistochemistry was performed with serum from rabbits immunized with human p35. This antiserum was immunoreactive with both p35 and recombinant lipocortin I (*3*); no reactivity was detected against lipocortin II. The immunostaining was blocked by prior incubation of the diluted serum with the original antigen or with p35 isolated from human placenta or with recombinant lipocortin I. Normal rabbit serum controls were negative at a 1:250 dilution of serum.

An antiserum (408) at 1:1000 dilution intensely stained a narrow sector in the ventral midline of the neural tube of a rat fetus at day 16 of gestation (E16) (Fig. 1a). The stained sector is present throughout the spinal cord and hindbrain. It has a consistent width of 40 to 80 μm and forms a longitudinal seam that occupies the median one-third of the floor plate. The ontogeny of p35 in the raphe shows a rostral to caudal progression, with stain appearing in the rhombencephalon at E11 and reaching the lumbar cord by the end of E12. The raphe staining is maximal from E15 to E18 and subsequently decreases until it disappears by postnatal day 5. P35 is not detected in any other regions of the CNS in either embryos or adults.

P35 is primarily concentrated in the bodies and processes of tall cells, each of which spans the thickness of the neural tube from the central canal to the pial surface (Fig. 1b). Both the apical and basal processes of the raphe cells form junctional "membranes" at the free surfaces. Cells in this configuration, which retain the simple pseudostratified epithelial characteristics of the primordial neural plate and early neural tube, have been called "archetypic glial ependymal cells" by comparative anatomists as far back as the early 1900s (*4*). Although the archetypic configuration is typical for all glia in lower vertebrates, it persists in adult birds and mammals only in the ventral midline of the hindbrain and spinal cord.

The absence of mitoses among the p35-positive cells is striking in comparison with the numerous mitotic figures apparent in their sister ependymal cells around the contiguous perimeter of the central canal (Fig. 1a). Studies with [^{3}H]thymidine labeling have shown that median cells of the floor plate in the rat cervical cord are born (cease DNA replication) by day E13 (*5*). Thus, the raphe cells are among the firstborn cells in the nervous system, exiting the mitotic cycle the same day as the firstborn dorsal root ganglion cells and less than a day after the first motor neurons. We do not know whether p35 expression precedes or accompanies the cessation of mitosis.

The regional differences and complexity of the raphe are best appreciated in either sagittal section (Fig. 1c) or stereo pairs of three-dimensional reconstructions from serial sections (Fig. 1d). The raphe runs caudally to the tip of the spinal cord; in the hindbrain, it undulates through both the cervical and pontine flexures to terminate rostrally at the caudal border of the mesencephalon.

The dorsal-ventral distance from central canal to pia in the CNS shows characteristic regional differences; the height of the raphe ranges from less than 150 μm in the cord to 500 μm in the brainstem (Fig. 1c). Although cellular elongation is hardly unusual in neural tissues, the height of the raphe cells at E15 is remarkable when compared to the dimensions of the same cells in adults. The spinal cord and brainstem enlarge considerably as they mature; but the distance from central canal to pia remains constant in the ventral midline, resulting in the deep ventral median fissure. The p35-positive cells attain their adult stature by day E15 and may be the first cells of the organism to do so. At a time when most cells of the embryo are prolifer-

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