Rapidly Deactivating AMPA Receptors Determine Excitatory Synaptic Transmission to Interneurons in the Nucleus Tractus Solitarius From Rat

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Titz, Stefan and Bernhard U. Keller. Rapidly deactivating AMPA receptors determine excitatory synaptic transmission to interneurons in the nucleus tractus solitarius from rat. J. Neurophysiol. 78: 82–91, 1997. Excitatory synaptic transmission was investigated in interneurons of the paravocellular nucleus tractus solitarius (pNTS) by performing patch-clamp experiments in thin slice preparations from rat brain stem. Stimulation of single afferent fibers evoked excitatory postsynaptic currents (EPSCs) mediated by gluta- matase receptors of the DL-α-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA) and N-methyl-D-aspartate types. AMPA-receptor-mediated EPSCs displayed decay time constants of 3.5 ± 1.2 (SD) ms (13 cells), which were slow compared with EPSC decay time constants in neurons of the cerebellum or hippocampus. Slow EPSC decay was not explained by dendritic filtering because the passive membrane properties of pNTS interneurons provided favorable voltage-clamp conditions. Also, the slowness of EPSC decay did not result from slow deactivation of AMPA receptors (0.7 ± 0.2 ms, 5 cells), which was investigated during rapid application of agonist to outside-out patches. Comparison of AMPA receptor kinetics with EPSC decay time constants suggested that the slow time course of EPSCs resulted from the prolonged presence of glutamate in the synaptic cleft.

INTRODUCTION

In central neurons, excitatory synaptic transmission is primarily mediated by glutamate receptors of the DL-α-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA) or N-methyl-D-aspartate (NMDA) types. The time course of excitatory postsynaptic currents (EPSCs) is determined by several parameters, including the release of neurotransmitter from the presynaptic terminal, the lifetime of neurotransmitter in the synaptic cleft, and the distribution and kinetics of postsynaptic neurotransmitter receptors. For many synapses, the residence time of neurotransmitter in the synaptic cleft is thought to be short (~1 ms) and essentially limited by uptake or rapid diffusion of neurotransmitter (see for example Clements et al. 1992; Collquhoun et al. 1992; Hestrin 1992; Taschenberger et al. 1995). The theoretical framework for this process was originally modeled by Eccles and Jaeger (1958) for different synaptic models and parameters. These studies conclude that, for a free diffusion model, neurotransmitter diffuses away from the original release site within a fraction of a millisecond. This view is largely supported by indirect evidence from electrophysiological and pharmacological experiments on cultured hippocampal neurons suggesting a maximum of 1 mM glutamate concentration in the synaptic cleft that rapidly decays within 1.2 ms (Clements et al. 1992). However, many electrophysiological studies established that the time course of excitatory signals can differ substantially between different neuron types. For example, mossy fiber stimulation evokes AMPA-receptor-mediated EPSCs (AMPA EPSCs) in granule cells of the rat cerebellum that display rapid kinetics with decay time constants (τdec) of 1 ms (Silver et al. 1992). Similarly, stimulations of parallel fibers evoke fast AMPA EPSCs in cerebellar interneurons (τdec 1.3 ms) (Barbour et al. 1994), but induce much slower EPSCs in cerebellar Purkinje cells during identical experimental conditions (6.1 ms) (Barbour et al. 1994; Llano et al. 1991). In hippocampal pyramidal neurons, AMPA EPSCs display average τdec ranging from 4 to 7 ms (Schafer collateral–commissural fiber connection to CA1 neurons: 7.1 ms; mossy fiber connection to CA3 neurons: 4.8 ms) (Hestrin et al. 1990; Jonas et al. 1993). These kinetics are slow compared with those observed in other neurons such as, for example, interneurons in the rat visual cortex (τdec 2.5 ms) (Hestrin 1992), cultured retinal ganglion cells (2 ms) (Taschenberger et al. 1995), and calyceal synapses in chick (1.2 ms) (Otis et al. 1996; Trussell et al. 1993). Taken together, these and corresponding observations for other synapses illustrate the substantial kinetic diversity of AMPA EPSCs in the CNS.

Many processes are involved in shaping the time course of AMPA EPSCs in neurons, including the temporal profile of neurotransmitter release, diffusion of neurotransmitter in the synaptic cleft, and the kinetic properties of postsynaptic neurotransmitter receptors. For some neurons the close similarity between EPSC τdec and the time constants of AMPA channel deactivation (e.g., hippocampal CA1 neurons: 2.3 ms; CA3 neurons: 2.5 ms; cortical neurons: 2.15 ms; cerebellar interneurons: 1.25 ms) (Barbour et al. 1994; Collquhoun et al. 1992; Hestrin 1992) has supported a model in which the residence time of neurotransmitter in the synaptic cleft is short and, accordingly, channel deactivation limits the time course of EPSCs. For other neurons such as cerebellar Purkinje cells, EPSC τdec were found to be significantly longer than channel deactivation kinetics (Barbour et al. 1994; Takahashi et al. 1995), suggesting a prolonged presence of glutamate in the synaptic cleft. For the calyceal synapse from chick, a prolonged presence of glutamate in the synaptic cleft has been associated with a “spillover” of glutamate between different release sites, leading to 3e elevated glutamate concentration for several milliseconds (Otis et al. 1996; Trussell et al. 1993). In this system, desen-
sitzation has been shown to participate in shaping the time course of synaptic currents (Otis et al. 1996; Trussell et al. 1993). With respect to the underlying AMPA receptors, these observations clearly demonstrate that there is no unique process that determines EPSC decay, but that, depending on the synapse investigated, either deactivation or desensitization could shape the time course of EPSCs.

In this report excitatory synaptic transmission was investigated by performing patch-clamp experiments on thin transverse slice preparations of the medulla oblongata containing the nucleus tractus solitarius (NTS). The NTS is commonly defined as the primary recipient of visceral afferents originating in the cardiovascular, gastrointestinal, and respiratory systems (Altschuler et al. 1989; Kalia and Sullivan 1982). Both electrophysiological and anatomic studies have established that the NTS consists of specialized subnuclei, including the medial, ventral, and parvocellular NTS, that are commonly classified on the basis of their position relative to the solitary tract (Andresen and Kunze 1994; Kalia and Mesulam 1980; Loewy 1990; Loewy and Burton 1978). Afferents projecting to NTS neurons have been associated, among others, with reflex adjustments during respiration, chewing, swallowing, or salivation (Hamilton and Norgren 1984; Kalia and Richter 1988). In addition, the observation that NTS neurons receive inputs from and also project to higher neural centers has linked the NTS to more complex processing, including endocrine and autonomic responses of the nervous system (Loewy 1990; Van der Kooy et al. 1984).

In this report we performed a detailed electrophysiological analysis of excitatory synapses and glutamate receptor channels in interneurons of the parvocellular NTS (pNTS) (Loewy and Burton 1978). Our results demonstrate that the time course of excitatory synaptic currents is slow compared with the deactivation kinetics of underlying glutamate receptor channels. They suggest that the slow time course is mediated by a prolonged presence of glutamate in the synaptic cleft. Some of the results have been previously published in preliminary form (Titz and Keller 1995; Titz et al. 1996).

METHODS

Preparation of slices

Transverse slices of the brain stem were prepared from 10- to 14-day-old Wistar rats according to previously described methods (Champagnat et al. 1986; Edwards et al. 1989). Animals were anesthetized with ether and subsequently decapitated. Starting from the rostral end of the area postrema up to seven 150-μm-thick slices containing the parvocellular and medial nuclei of the NTS could be received. After preparation slices were maintained at room temperature in continuously bubbled (95% O2 -5% CO2) bicarbonate-buffered saline (composition, in mM: 118 NaCl, 3 KCl, 1 MgCl2, 25 NaHCO3, 1 NaH2PO4, 1.5 CaCl2, and 10 glucose, pH 7.4). Before recording experiments slices were incubated for 1 h to improve recovery. For whole cell recording (Hamill et al. 1981), slices were placed in the recording chamber under a Zeiss upright microscope and continuously superfused with the solution described above (≥2 ml/min). All experiments were carried out at room temperature (21–26°C).

Solutions and drugs

All perfusing solutions contained 10 μM bicuculline (Sigma, Deisenhofen, Germany) to block γ-aminobutyric acid-A (GABA-A)-receptor-mediated inhibitory postsynaptic currents. Additional applied drugs were 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Tocris), 100 μM L-300299 [α-[3-[[2-(3,4-dimethoxyphenyl)-ethyl]methy]aminoproplyl]-3,4,5-trimethoxy-α-(1-methyl-ethyl)-benzenacetonitrile (0–600, Knoll), 1 μM strychnine, 1 mM D-aspartate, 100 μM cyclothiazide, 1 μM glycine, 10 μM AMPA (Sigma), 50 μM kainate (Sigma), and 1 mM L-glutamate. The intracellular pipette solution contained (in mM): 140 CsCl, 10 N-2-hydroxymethyl-piperazine-N’-2-ethanesulfonic acid, 10 ethylene glycol-bis(β-aminoethel ether)-N,N,N’,N’-tetraacetic acid, 1 CaCl2, 2 MgCl2, 4 Na2 ATP, and 0.4 sodium guanosine 5’-triphosphate, pH adjusted to 7.3 with CsOH. Also, 4.5 mg/ml neurobiotin was added to the intracellular solution to allow later anatomic identification of neurons analyzed. d–600 was a generous gift of Knoll, Ludwigshafen, Germany.

Recording and stimulation

Patch pipettes as well as stimulation pipettes were pulled from borosilicate glass tubings (Kimble) and heat polished before use. When filled with intracellular solution, they had a resistance of 2.0–3.5 MΩ. Single-electrode voltage-clamp recordings were performed with a patch-clamp amplifier (EPC-9, HEKA Elektronik, Lambrecht, Germany) employing optimal series resistance compensation as previously described (Llano et al. 1991). The series resistance of pNTS interneurons before compensation was typically 8–15 MΩ. Cells with series resistances >15 MΩ were discarded from further analysis. Values of series resistance and membrane capacitance were obtained from settings of the capacitance cancelation circuitry of the patch-clamp amplifier and monitored during the experiment. Series resistance compensation was set to 50–60%. The membrane potential of the recorded cell was held at -60 mV. No compensation was made for the liquid junction potential. When not stated otherwise, whole cell currents were recorded with sampling frequencies of 5 kHz and filtered (3-pole Bessel filter, 10 kHz; 4-pole Bessel filter, 2.9 kHz) before analysis.

For stimulation of synaptic currents a patch pipette (2–3 MΩ) filled with intracellular solution was used. Pulses of 200 μs and a frequency of 0.3 Hz were delivered with an isolated stimulator (HI-Med, H15). Stimulus intensity was set to minimum (8–30 V) to favor single-fiber stimulation. In most of the experiments the stimulation pipette was positioned under visual control in the ipsilateral pNTS or medial NTS.

Fast application of glutamate on outside-out patches excised from NTS cells was performed with the use of a high-voltage piezoelectric crystal (Physik Instrumente, Waldbronn, Germany) to rapidly move an application pipette (Franke et al. 1987). The flow of solutions from the pipette and from the bath (3 ml/min) was arranged to be parallel to favor laminar flow of the agonist-containing solution.

Analysis

Kinetics of EPSCs and induced currents in outside-out patches were measured with a semiautomatic program by the use of movable cursors (PulseFit, HEKA). The rise time (τrise) was defined as the time from 20 to 80% of the EPSC amplitude. For determining the τdecay a single exponential was fitted from peak to baseline. The peak amplitude was automatically calculated as the difference up to 80% of the amplitude. Statistics are given throughout the text as means ± SD.
Excitatory synaptic transmission was investigated in the pNTS (labeled p in Fig. 1A) by performing patch-clamp experiments in thin slice preparations (Edwards et al. 1989). Patch-clamp recordings were performed on a total of 166 neurons in the pNTS. These cells were characterized by round somata between 8 and 15 μm in diameter (Champagnat et al. 1986) (Fig. 1B), fast capacitive currents with a 3.3 τax of 0.15 ± 0.07 (SD) ms (14 cells), a membrane input resistance as high as 10 GΩ (Fig. 2A), and a resting membrane potential between −70 and −60 mV. The overall electrical behavior during a voltage ramp protocol is illustrated in Fig. 2B. Depolarizations positive to −60 mV induced one or more action potentials depending on the cell investigated and the number of voltage ramp protocols applied. More positive voltages led to the opening of voltage-dependent calcium channels (237 ± 97 pA at 0 mV, 7 cells) that were partially blocked by bath application of 100 μM of the L-type calcium channel antagonist d–600 (68 ± 27% block, 7 cells). Cells that did not display action potentials were excluded from the analysis.

In the whole cell recording configuration afferents connecting to pNTS interneurons were activated by focal electrical stimulation with conventional patch pipettes. By systematically varying stimulus location and intensity within the stimulation area (labeled s in Fig. 1A), activation of afferent fibers was detected by their postsynaptic current response. For a given cell the number of independent locations that evoked postsynaptic responses was remarkably small (3–4). After one or several fibers had been activated, the stimulation intensity was turned to a minimum. Under such conditions the mean amplitude of the postsynaptic response depended sharply on the stimulation intensity and the precise position of the stimulation pipette, suggesting that single fibers were activated (“unitary” EPSCs, Fig. 5).

**Glutamate-receptor-mediated EPSCs in brain stem interneurons**

EPSCs recorded from pNTS interneurons are displayed in Fig. 3. At −60 mV, EPSCs were dominated by the fast AMPA receptor component that was completely blocked after bath application of 10 μM CNQX (Fig. 3, A and B). Depolarizations up to +40 mV revealed a second, NMDA-receptor-mediated component that could be distinguished by slower kinetics, insensitivity to CNQX, and Mg2+ block. Amplitudes of NMDA-receptor-mediated EPSCs were remarkably small and ranged from 9 to 44 pA (average 18.1 ± 17.8 pA, measured at a holding potential of +40 mV, 4 cells) even when 1 μM glycine was present in the bath solution. Their τax was slow and the maximum amplitude was reached within 4.4 ± 2.5 ms (20–80%, 4 cells). Their decay was best approximated by the sum of two exponentials in which the second (slow) component displayed a τax of 320 ms (4 cells, +40 mV). Peak amplitudes of EPSCs showed a linear current-voltage relationship (Fig. 3D) with a reversal potential of 6.7 ± 1.1 mV.

AMPA EPSCs were studied at a membrane potential of −60 mV (Fig. 4A). This allowed us to separate them reliably from NMDA-receptor-mediated EPSCs in the absence of any glutamate receptor antagonist. To maximize the probability of single-fiber stimulations, we performed a minimum stimulation that was defined by the smallest stimulus intensity that consistently evoked a synaptic response. A significant delay was observed between the stimulation of the presynaptic fiber and the onset of the postsynaptic response (delay time = 3.87 ± 0.92 ms, 11 cells). An EPSC latency distribution is shown in Fig. 4B with a mean latency of 3.3 ± 0.15 ms. Short latencies <5 ms together with small SDs <0.4 ms were taken as further evidence that a single fiber was activated. Stimulation of several fibers often resulted in a multimodal distribution and/or a steplike rising phase of the EPSC. The detection of small EPSCs depended strongly on their discrimination from the background noise of our current recording, which was 1.5 ± 0.3 pA (root mean square, 8 cells). Correspondingly, EPSCs were discriminated by threshold detection if their amplitude exceeded 3 pA. Under constant stimulation conditions, unitary
EPSC amplitudes varied significantly for repetitive stimuli. Amplitude fluctuations in normal saline are illustrated in Fig. 4A. Figure 4C displays the peak amplitude distribution at −60 mV recorded from a single cell. The average amplitude was 44.3 ± 24.1 pA (excluding failures) in this particular experiment, and the largest EPSC displayed a peak amplitude of 142 pA. For a total of 13 cells the mean amplitude was 42 ± 21 pA (−60 mV), corresponding to a unitary EPSC conductance level of 698 ± 344 pS (13 cells).

**Kinetic properties of AMPA EPSCs**

Figure 5A displays the synaptic current response of a presynaptic fiber stimulated repetitively at an intensity of 18 V. This resulted in all-or-none type synaptic responses that could be reliably separated from synaptic failures. Figure 5B displays an analysis of EPSC amplitudes as a function of stimulation intensity. For stimulations <13 V no synaptic response was observed for a total number of 200 stimuli. Stronger stimulations evoked EPSC responses in 18% of a total of 600 stimuli analyzed. As expected for unitary events, EPSC amplitudes showed a sharp unimodal onset and were independent of further increases in stimulation intensity. EPSC τrise of 0.3 ± 0.1 ms (20–80% maximum amplitude, Fig. 6B) were observed in 14 cells, suggesting that excitatory synapses were located electrically close to the soma. The decay of AMPA EPSCs was well approximated by a single exponential function as exemplified in Fig. 6A. bottom trace. The average τdec of unitary EPSCs was 3.5 ± 1.2 ms (13 cells). Figure 6C compares the τdec of EPSCs for different current amplitudes. EPSC amplitudes ranged from 20 to 150 pA during constant stimulation conditions. Their τdec varied between 2 and 5 ms but did not depend on the current amplitude (Fig. 6C, slope = 0.5 ms/100 pA). This result was confirmed in four other neurons, indicating that the massive release of neurotransmitter associated with large EPSC amplitudes did not significantly overrule the mechanisms that shaped smaller EPSCs. Taken together, the distributions of latencies, τrise, and τdecay suggested that stimulated EPSCs in pNTS interneurons resulted from synapses located electrically close to the soma and were mediated by single presynaptic afferents.
An alternative way to investigate the mechanisms that control EPSCs is to pharmacologically manipulate the transmission process. For example, cyclothiazide has been shown, among other things, to affect the desensitization properties of AMPA receptors in different neuron types (Yamada and Tang 1993). Cyclothiazide also affected AMPA EPSCs in pNTS interneurons by slowing the decay approximately twofold (6.5 ± 2.2 ms, 7 cells). Cyclothiazide did not affect the rate of spontaneous EPSCs, suggesting little or no pre-synaptic effect in pNTS interneurons (control: 0.54 per s; 100 μM cyclothiazide: 0.36 per s; 2 cells). To investigate the role of glutamate uptake transporters, we also performed EPSC recordings in the presence of 1 mM D-aspartate, known to effectively block glutamate uptake (Balcar and Johnston 1972; Barbour et al. 1994). As demonstrated in Fig. 7, the presence of D-aspartate in the bath solution increased the background noise of our current recording (control: 2.2 ± 0.8 pA; 1 mM D-aspartate: 4.4 ± 2.0 pA, root mean square; 4 cells), most likely explained by the accumulation of glutamate in the extracellular space and subsequent tonic activation of glutamate receptors. Concentrations as high as 1 mM D-aspartate did not affect EPSC activation (control: \( \tau_{\text{rise}} = 0.4 \pm 0.1 \) ms; 1 mM D-aspartate: \( \tau_{\text{rise}} = 0.4 \pm 0.1 \) ms), amplitude (control: 37 ± 27 pA; 1 mM D-aspartate: 34 ± 19 pA), or EPSC \( \tau_{\text{dec}} \) (control: \( \tau_{\text{dec}} = \))
FIG. 6. Kinetics of AMPA-receptor-mediated EPSCs in pNTS interneurons. A: individual AMPA-receptor-mediated EPSCs in response to standard stimulation protocol (i). Bottom trace: exponential fit is superimposed on decay phase of EPSC. B: EPSCs selected for fast rise times (\(\tau_{\text{rise}} = 0.32 \pm 0.05\) ms) show slow decay time constants (\(\tau_{\text{dec}} = 3.9 \pm 0.5\) ms, \(n = 14\) cells). C: \(\tau_{\text{dec}}\) of AMPA-receptor-mediated EPSCs as a function of EPSC amplitude for a total of 85 signals. EPSC \(\tau_{\text{dec}}\)S were determined as described in Fig. 8. \(\tau_{\text{dec}}\) did not show a significant dependence on amplitude.

3.4 ± 0.5 ms; 1 mM d-aspartate: \(\tau_{\text{dec}} = 3.6 ± 0.5\) ms; \(n = 82; 4\) cells), suggesting that glutamate uptake did not significantly shape size and time course of EPSCs.

Spontaneous synaptic currents are commonly thought to reflect the basal activity of single presynaptic afferents. In pNTS interneurons, two types of spontaneous postsynaptic currents were repeatedly observed. The first population was completely blocked by application of 10 \(\mu\)M bicuculline, suggesting that they were mediated by GABA\(_A\) receptors. The second population was blocked by 10 \(\mu\)M CNQX, identifying them as AMPA receptor mediated. Because these measurements were performed at a membrane potential of −60 mV, the occurrence of NMDA-receptor-mediated signals was suppressed by Mg\(^{2+}\) block. Additional spontaneous currents were not observed, indicating that the basal activity of other synapses was absent or extremely rare. To prevent any ambiguities in the analysis, measurements were performed in the presence of 10 \(\mu\)M bicuculline and 1 \(\mu\)M strychnine to block postsynaptic GABA\(_A\) and glycine receptors. Under such conditions, we found spontaneous AMPA-receptor-mediated responses with amplitudes ranging from 5 to 95 pA (20.6 ± 10.9 pA, 6 cells, Fig. 8A). Amplitudes and \(\tau_{\text{dec}}\)S (3.0 ± 1.1 ms, 6 cells) were almost indistinguishable from those of stimulus-evoked unitary EPSCs, suggesting that they were controlled by similar synaptic mechanisms.

Rapid kinetics of glutamate receptor channels during fast application of agonist

The functional properties of postsynaptic AMPA receptor channels were further investigated in the presence of varying agonist concentrations (10 \(\mu\)M AMPA, 50 \(\mu\)M kainate). During bath application of agonist, AMPA-receptor-mediated currents displayed linear current-voltage relationships with a reversal potential close to the Nernst potential for monovalent cations (−0 mV). Currents were completely blocked by 10 \(\mu\)M CNQX (7 cells). To correlate the kinetic properties of synaptic signals with those of underlying AMPA receptors, we performed patch-clamp experiments on outside-out patches pulled from pNTS interneurons. In some cases we optimized our current responses by recording from nucleated patches (Paoletti and Ascher 1994), giving rise to agonist-induced current amplitudes of >300 pA. Figure 9A displays the result of a fast application of glutamate to a nucleated outside-out patch with the use of a computer-controlled piezolectric device. As demonstrated in earlier reports (Barbour et al. 1994), that system allowed to completely exchange agonist solutions around outside-out membrane patches within 0.2 ms. The desensitization properties of AMPA receptors were investigated by performing a rapid application of saline containing 1 mM glutamate (Fig. 9A).
FIG. 8. Kinetics of spontaneous AMPA-receptor-mediated EPSCs. Holding potential was −60 mV. A: examples of spontaneous EPSC recordings. B and C: decay and rise kinetics were determined analogous to kinetics of stimulated EPSCs ($\tau_{\text{dec}} = 3.0 \pm 1.1\, \text{ms}$, $\tau_{\text{rise}} = 0.6 \pm 0.2\, \text{ms}$, $n = 124$, 7 cells).

AMPA receptors activated within $0.4 \pm 0.2\, \text{ms}$ (5 cells, 20–80% maximum amplitude) but rapidly desensitized according to a single exponential time constant of $5.4 \pm 1.3\, \text{ms}$ (8 cells). After 16 ms, the majority of receptors was desensitized, as indicated by a steady-state current of <5% of the original current response. Channel deactivation was investigated by a pulse application protocol lasting 1 ms (Fig. 9B). Channel activation was equally rapid, but channel closure on removal of agonist occurred with a time constant of $0.7 \pm 0.2\, \text{ms}$ (5 cells). Interestingly, a second pulse application of glutamate showed a reduced current amplitude that most likely reflected a desensitization of receptors after the short application pulse (Colquhoun et al. 1992; Hestrin 1992; Jonas and Sakmann 1992). A repeated application after 2 s showed that AMPA receptors had completely recovered from desensitization within this time interval.

DISCUSSION

Excitatory synaptic currents in brain stem interneurons

Excitatory synaptic transmission was studied in interneurons of the pNTS (Loewy and Burton 1978) in thin slices of the rat brain stem (Champagnat et al. 1986). Synaptic currents could be recorded under well-defined voltage-clamp recording conditions, which was evident from several observations. First, pNTS interneurons displayed capacitive currents that were 1 order of magnitude faster than capacitive currents observed in hippocampal CA1 neurons (Brown et al. 1981; Spruston and Johnston 1992) and cerebellar Purkinje cells (Llano et al. 1991). The absence of a second exponential component suggested that pNTS interneurons were electrically compact. Another argument for favorable voltage-clamp conditions is derived from the fast onset of synaptic currents with $\tau_{\text{rise}}$ as fast as 0.2 ms. Similar $\tau_{\text{rise}}$ have been observed in other neurons with favorable voltage-clamp conditions, such as cerebellar granule cells (0.3 ms) (Silver et al. 1992) and cortical stellate cells (0.4 ms) (Stern et al. 1992).

Kinetic properties of glutamate (AMPA) receptor channels

By performing agonist applications we characterized the functional properties of postsynaptic glutamate receptors. AMPA receptors displayed current responses both to AMPA and kainate with linear current-voltage relationships, a reversal potential close to the Nernst potential of monovalent cations, and a CNQX block in the micromolar concentration range. These properties were similar to those in other neuronal systems previously studied (Hestrin 1992; Hestrin et al. 1990; Jonas and Sakmann 1992; Keller et al. 1991; Llano et al. 1991; Schneggenburger et al. 1992). To investigate the kinetic parameters we performed a fast application of agonist to outside-out membrane patches (Barbour et al. 1994). During application of 1 mM glutamate we found fast activation times, suggesting that essentially our perfusion system limited the onset of the current response. For longer, steplike applications of glutamate we found desensitization time constants of 5.4 ms. These were comparable with desensitization time constants in cerebellar Purkinje cells and interneurons (3.9 and 5.1 ms, respectively) (Barbour et al. 1994), but significantly faster than the desensitization time in hippocampal neurons (granule cells: 9.4 ms; CA1: 9.3 ms; CA3: 11.3 ms) (Colquhoun et al. 1992; Jonas and Sakmann 1992). Short pulse applications of glutamate demonstrated a deactivation time constant of 0.7 ms in pNTS interneurons. This was similar to values observed in cerebellar Purkinje cells and interneurons (0.8 and 1.25 ms, respectively) (Barbour et al. 1994), but notably faster than those recorded in hippocampal cells (granule cells: 3.0 ms; CA1: 2.3 ms; CA3: 2.5 ms) (Colquhoun et al. 1992) and cortical neurons (2.15 ms) (Hestrin 1992; Llano et al. 1991). These differences could be well explained by a molecular diversity of glutamate receptor channels in different neuron types. For example, a similar kinetic diversity has been stated for recombinant glutamate receptors GluR1–GluR4 after expression in cell lines (Mosbacher et al. 1994).
Kinetic properties of glutamate (AMPA)-receptor-mediated EPSCs

The decay of unitary AMPA EPSCs in brain stem interneurons was well described by a single exponential function with a time constant of 3.5 ms. This was comparable with $\tau_{\text{dec}}$ for hippocampal synapses connecting mossy fibers to CA3 neurons (4.8 ms) (Jonas et al. 1993), hippocampal granule cells during stimulation of the perforant path (3–9 ms) (Keller et al. 1991), the Schaffer collateral–commissural fiber connection to CA1 neurons (7.1 ms) (Hestrin et al. 1990), cortical cells (2.4 ms) (Stern et al. 1992), and spinal cord motoneurons (3.7 ms) (Konnerth et al. 1990), but was notably faster than EPSC $\tau_{\text{dec}}$ in cerebellar Purkinje cells (7 ms) (Barbour et al. 1994; Llano et al. 1991). EPSC decay in pNTS interneurons was remarkably slow compared with $\tau_{\text{dec}}$ of $\sim$1 ms described for cerebellar granule cells and interneurons (1.2 and 1.3 ms, respectively) (Barbour et al. 1994; Silver et al. 1992).

The central question posed by our results is related to the synaptic structures and mechanisms that would sufficiently prolong the residence time of neurotransmitter in the synaptic cleft to account for slow EPSC decays. A prolonged presence of glutamate could be explained by several independent mechanisms. One possibility is that the diffusion of glutamate from the synaptic cleft is slow. For example, Barbour et al. (1994) suggested that for cerebellar Purkinje cells the complex geometry of synaptic spines could severely restrict the diffusion of neurotransmitter accounting for slower EPSC decays. Indeed, numerical simulations showed that plausible synaptic structures and parameters could prolong the residence time of neurotransmitter in the synaptic cleft up to several milliseconds (Barbour et al. 1994; Holmes 1995). In an extension of this model, suitable glutamate buffers or surrounding glia cells could further prolong the residence time of neurotransmitter in the synaptic cleft. Because there is only little information about ultrastructural morphology of synapses in the NTS (Kalia and Richter 1995), it is an unresolved question whether such structures or molecular components do in fact exist in sufficient density to account for the observed kinetic parameters. From our experiments there was little or no evidence for a significant role of glutamate uptake transporters in shaping the time course of EPSCs. This was evident from experiments performed in the presence of 1 mM D-aspartate, known to effectively block the uptake of glutamate in the CNS (Balcar and Johnston 1972; Barbour et al. 1994).

An independent model for a prolonged residence time of glutamate has been previously proposed for calyceal synapses in chick (Otis et al. 1996; Trussell et al. 1993). In neurons of the nucleus magnocellularis the prolonged presence of glutamate in the synaptic cleft was explained by transmitter diffusion from multiple release sites into a large synaptic cleft and a spillover of glutamate between different release sites. In this case, EPSCs were significantly shaped by rapid channel desensitization and a late, retarded EPSC component reflected a slow “tail” of glutamate in the synaptic cleft (Otis et al. 1996). In a similar model the slow decay time course of EPSCs in pNTS interneurons could be explained by multiple release of neurotransmitter at neighboring release sites. One prediction of the model is that miniature EPSCs recorded in the presence of tetrodotoxin should display fast $\tau_{\text{dec}}$ if they resulted from the release of a single synaptic vesicle (Diamond and Jahr 1995; Trussell et al. 1993). To test the validity of this model, we are currently investigating the kinetic parameters of miniature glutamate release.
EPSCs in more detail with an improved (pA) resolution of small events.

Another factor that could, under appropriate synaptic conditions, prolong the time course of AMPA EPSCs is an asynchronous release of neurotransmitter in later phases of the synaptic signal (Diamond and Jahr 1995; Isaacson and Walmsley 1995; Katz and Miledi 1965). If this were the case for pNTS interneurons, miniature EPSC should display faster \( \tau_{\text{rise}} \) than stimulated EPSCs presumably resulting from several asynchronously released vesicles. Also, the \( \tau_{\text{dec}} \) of miniature EPSCs should be accelerated compared with larger synaptic signals (Diamond and Jahr 1995; Isaacson and Walmsley 1995). Both possibilities are currently investigated by performing minimum stimulations for anatomically defined synapses close to the somatic recording site. For the basic electrophysiological results presented in this report, both the concept of glutamate spillover between multiple release sites and an asynchronous release of neuro-EPSCs at the mossy fibre synapse on CA3 pyramidal cells of rat hippocampus.

We thank B. Lage for excellent technical assistance and C. de Buhr for help with photographic material. We also thank Drs. A. Marty, D. W. Richter, and S. Schwarcz for comments on the manuscript.

This research was supported by Deutsche Forschungsgemeinschaft Grant Ke 403/5–2, Ke 403/6–1, the Graduiertenkolleg “Organisation and Dynamics of Neuronal Nets,” and the Sonderforschungsbereich 406. Address for reprint requests: B. U. Keller, Zentrum Physiologie und Pathophysiologie, Universität Göttingen, Humboldtallee 23, 37073 Göttingen, Germany.

Received 11 October 1996; accepted in final form 20 February 1997.

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