Mechanisms shaping glutamate-mediated excitatory postsynaptic currents in the CNS

Peter Jonas and Nelson Spruston

Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany

Excitatory postsynaptic currents in neurones of the central nervous system have a dual-component time course that results from the co-activation of AMPA/kainate-type and NMDA-type glutamate receptors. New approaches in electrophysiology and molecular biology have provided a better understanding of the factors that determine the kinectics of excitatory postsynaptic currents. Recent studies suggest that the time course of neurotransmitter concentration in the synaptic cleft, the gating properties of the native channels, and the glutamate receptor subunit composition all appear to be important factors.

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Introduction

Chemically mediated synaptic transmission results from the fusion of neurotransmitter-containing synaptic vesicles with the presynaptic plasma membrane, the release of the vesicular contents into the synaptic cleft, and the subsequent activation of postsynaptic receptor channels. Although there is little doubt that this concept — originally established for the neuromuscular junction — applies to central synapses, many details of synaptic transmission in the mammalian central nervous system (CNS) remain unclear. The current thinking is that the majority of excitatory synapses are glutamatergic, but it is not yet known precisely how much glutamate is in a synaptic vesicle or what is the time course of glutamate concentration in the synaptic cleft, although attempts have been made to simulate or measure these [1,2,3••]. We also need to learn more about the action of glutamate on the postsynaptic receptors, since neither the kinetic properties of these receptors nor their subunit composition are sufficiently understood. Important information is just beginning to be obtained about the ultrastructure of central synapses (e.g. vesicle size, number of release sites and distance between them, width of the synaptic cleft, and size of postsynaptic densities) [4•]. A more detailed knowledge of all these cellular and molecular factors will help us understand more complex aspects of brain function, such as the integration of synaptic signals and synaptic plasticity.

In this review, we will summarize recent attempts to infer, from excitatory postsynaptic current (EPSC) kinetics, the mechanisms underlying excitatory synaptic transmission in the CNS. Whereas the time course of the excitatory postsynaptic potential (EPSP) is largely determined by the passive properties of the postsynaptic cell, the EPSC amplitude, at any given point in time, should be proportional to the number of open postsynaptic receptor channels, which allows one to make inferences about the molecular mechanisms of synaptic transmission [5–7]. In order to address such questions, new techniques have been applied to central neurones, including patch-clamp methods to record EPSCs in brain slices, fast application of glutamate to outside-out patches to mimic EPSCs, and the use of molecular biology to elucidate the subunit composition of the postsynaptic receptor channels.

Kinetics of EPSCs at central synapses

In most types of mammalian CNS neurones, EPSCs consists of two components that differ in kinetics by almost two orders of magnitude. The fast component is selectively blocked by CNQX (a specific AMPA/kainate receptor channel antagonist), but is insensitive to D-APV (a specific NMDA receptor channel antagonist). Conversely, the slow component is blocked by D-APV but not by CNQX; it is also blocked by Mg^{2+} in a characteristic voltage-dependent manner [8]. These results strongly suggest that the two components of the EPSC are mediated by two different types of glutamate-gated ion channels: AMPA/kainate-type (fast) and NMDA-type (slow) glutamate receptor (GluR) channels [9••,10–13].

Voltage- and space-clamp problems

Patch-clamp techniques allow one to record from central neurones in the intact synaptic environment of a brain slice, at high temporal resolution and with low

Abbreviations

AMPA—α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; **APV**—2-amino-5-phosphonovaleric acid; **CNQX**—6-cyano-7-nitroquinoxaline-2,3-dione; **CNS**—central nervous system; **EPSC**—excitatory postsynaptic current; **GluR**—glutamate receptor; **MF**—mossy fiber; **NMDA**—*N*-methyl-D-aspartate; τ—time constant.

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background noise [9••,10,11,13,14,15••,16,17]. The majority of the excitatory synapses in the CNS are located on dendrites remote from the cell soma, which causes serious distortions of measured synaptic currents, particularly of the fast AMPA/kainate component of the EPSC. Simulations based on realistic models of neuronal morphology reveal that filtering by the dendritic cable and imperfect somatic voltage clamping (resulting from the series resistance of the recording pipette) severely attenuate and slow the time course of the recorded current [17–21]. The only way to circumvent these problems is to focus on excitatory synapses that are located close to, or at, the cell soma, and to use patch pipettes with the lowest possible resistance.

Focus on electrotonically close synapses

A number of excitatory synapses that are located electrotonically close to the cell soma have been studied in brain slices using patch-clamp techniques; the growing list includes mossy fiber (MF) synapses on cerebellar granule cells [9••,14], synapses on neocortical interneurones [10], calyceal synapses on neurones in the the cochlear nucleus [15••] and the medial nucleus of the trapezoid body [11], synapses on medial septal neurones [16], and MF synapses on hippocampal CA3 pyramidal cells [17]. The fast AMPA/kainate receptor-mediated components of the EPSCs at these synapses have rise times that are only a few hundred microseconds; the decay is approximately mono-exponential, with time constant (τ) values that vary between 0.5 ms (cochlear nucleus neurones [15••]) and 3 ms (CA3 pyramidal neurones [17]) at different synapses. It has been suggested that generally the EPSC decay may be faster in relay neurones (e.g. cochlear nucleus neurones [15••]) than in neurones specialized to integrate multiple synaptic signals. Moreover, it has been noted that the decay of the AMPA/kainate receptor-mediated EPSC component is faster in inhibitory than in excitatory postsynaptic neurones [22•–24•]. Whether or not these principles apply throughout the CNS remains to be determined. The kinetics of the NMDA receptor-mediated EPSC component are much slower than that of the AMPA/kainate-generated EPSC. The rise time is around 10 ms (see, however, [25•]). The decay appears to be double-exponential with τ values of about 50 ms and 250 ms, respectively. When the NMDA component reaches its peak, the AMPA/kainate component has decayed to a negligible value [9••,10,25•,26••,27•].

Determinants of EPSC kinetics

The rise time of an ideally voltage-clamped synaptic current is determined by both the rate of rise of neurotransmitter concentration and the activation kinetics of the channels. However, the factors that determine the rate of the decay of the synaptic current are more complicated; three basic scenarios have been put forward (Fig. 1). In hypothesis I, it is postulated that the decay of neurotransmitter concentration is very rapid, and that the synaptic current is terminated by channel deactivation following removal of neurotransmitter from the synaptic cleft (EPSC τ = deactivation τ),

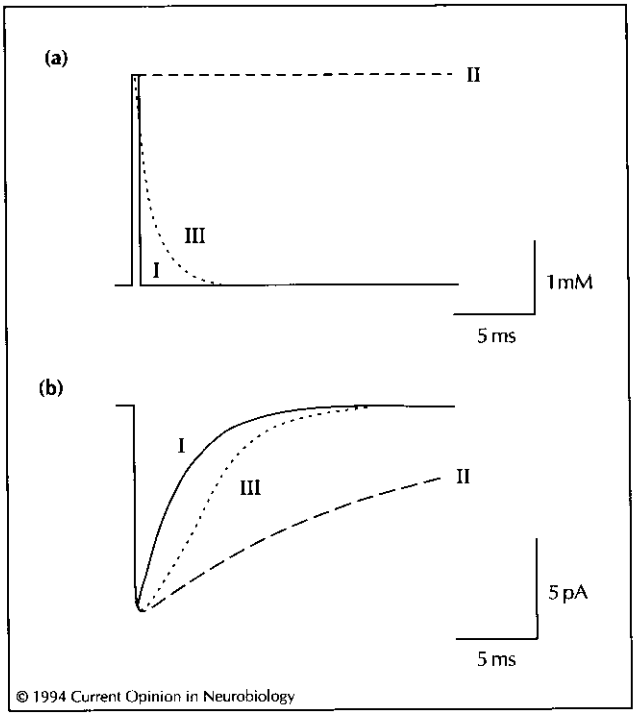

Fig. 1. Influence of the time course of neurotransmitter concentration in the synaptic cleft on the shape of the glutamate-activated current. The three hypotheses discussed in the text (I, II, and III) are illustrated with the corresponding numbers next to the type of curve they refer to. Simulations are based on the kinetic model of AMPA/kainate-type GluR channel gating described by Jonas *et al.* [17]: **(a)** glutamate concentration plotted against time, and **(b)** glutamate-activated current (25 channels, 8 pS, −70 mV, rate constant set 1) versus time.

as has already been proposed for the neuromuscular junction [5,6]. In hypothesis II, it is envisioned that the decay of neurotransmitter concentration is slow, and the synaptic current is terminated by channel desensitization in the maintained presence of neurotransmitter (EPSC τ = desensitization τ). As AMPA/kainate-type GluR channels desensitize more rapidly than any other ligand-gated ion channel, this hypothesis is not unreasonable [28]. In hypothesis III, it is proposed that the neurotransmitter concentration decays with a time course that is intermediate between these extremes, implying that the decay of the synaptic current is influenced by the time course of neurotransmitter concentration, deactivation, and desensitization in a complicated manner (deactivation τ < apparent EPSC τ < desensitization τ).

Mimicking synaptic events using fast application

At an intact synapse, it is difficult at an intact synapse to investigate which molecular steps are rate-limiting for the EPSC time course. Fast application of the putative neurotransmitter glutamate to excised outside-out membrane patches (within about 100 µs) allows one to mimic synaptic release [15••,22•–24•,26••,27•,28,29•,30•,31,32••,33]. The fast application system and the membrane patch may together be regarded as a surrogate synapse where

'presynaptic' parameters can be readily controlled. The influence of pulse length and concentration on the time course of the glutamate-activated current can thus be investigated systematically, something which is much more difficult at native synapses.

Which factors determine the rapid kinetics of the AMPA/kainate component of the EPSC ?

The rapid rise time of the AMPA/kainate receptor-mediated membrane currents activated by fast application of millimolar glutamate concentrations [15••,22•–24•,29•,30•,31] is similar to the rise time of the well-clamped AMPA/kainate EPSCs [9••,10,11,14,15••,16,17]. Measured rise times in fast application and synaptic experiments, however, represent upper limits rather than true values, because they appear to be strongly affected by the speed of solution exchange and the quality of the voltage clamp, respectively [17–21,29•]. In any case, the available data allow for the conclusion that the rise of neurotransmitter concentration in the synaptic cleft must be very rapid. Whereas it was originally assumed that instantaneous and complete neurotransmitter release occurs by fusion of synaptic vesicles [1], recent data on non-neuronal secretory cells challenge this view and suggest that partial release via a narrow fusion pore is possible [34,35]. If glutamate is discharged via a fusion pore at central excitatory synapses, the fast rise of the AMPA/kainate EPSC implies that either the lifetime of this pore is brief or the conductance is high [36].

Deactivation and desensitization time constants of AMPA/kainate receptors have been studied in a variety of CNS neurones with the aim of gaining a better understanding of the determinants of EPSC decay. Rapidly gated AMPA/kainate-type GluRs (deactivation $\tau \approx 1$ ms; desensitization $\tau \approx 3$ ms) have been found in cochlear nucleus neurones [15••,31], in cerebellar granule cells (B Edmonds, RA Silver, D Colquhoun, SG Cull-Candy, abstract 624.5, 23rd Annual Meeting of the Society of Neuroscience, Washington DC, November 1993), and in inhibitory interneurones of neocortex [23•,30•] and hippocampus [24•]. More slowly gated AMPA/kainate-type GluRs (deactivation $\tau \approx 2.5$ ms; desensitization $\tau \approx 10$ ms) have been reported in hippocampal granule cells [29•], hippocampal pyramidal neurones [29•], and neocortical pyramidal neurones [22•,23•,30•]. A comparison of the kinetics of EPSCs to that of currents activated by glutamate pulses in the same cell type reveals that the majority of cells expressing rapidly gated AMPA/kainate receptors show rapidly decaying AMPA/kainate EPSCs ([9••,10,15••,23•,30•,31]; B Edmonds, RA Silver, D Colquhoun, SG Cull-Candy, abstract 624.5, 23rd Annual Meeting of the Society of Neuroscience, Washington DC, November 1993). In all the cell types investigated, desensitization of GluRs in membrane patches is two- to four-fold slower than the decay of the well-clamped AMPA/kainate EPSC [9••,10,15••,17,22•,23•,29•,30•,31]. Under the assumption that the functional properties of postsynaptic GluR channels and extrasynaptic channels in patches are identical, this excludes hypothesis II and rules out desensitization as the major determinant of EPSC decay. In many cell types, the deactivation τ is very close to the synaptic decay τ, but usually is slightly slower [10,17,29•,30•]. It is difficult to distinguish unequivocally between hypothesis I and III solely on the basis of these data, because the apparent EPSC decay time course may have been slowed by voltage-clamp imperfections. In addition, the decay of evoked multiquantal EPSCs, on which the reported τs are largely based, may be slower than that of uniquantal EPSCs as a result of asynchronous release of individual neurotransmitter quanta [9••].

The synaptic glutamate pulse may be prolonged under some conditions

Pharmacological tools that inhibit the desensitization of AMPA/kainate-type GluR channels provide an approach for distinguishing between hypothesis I and III. Cyclothiazide, which is probably the most potent and specific agent available, slows the decay of miniature EPSCs in hippocampal neurones [37]. At the calyceal synapse on cochlear nucleus neurones, which is characterized by a large number of active zones for neurotransmitter release, cyclothiazide slows the decay of evoked multiquantal EPSCs more strongly than that of miniature EPSCs [15••]. This suggests that the simultaneous release of several quanta from nearby sites may slow the clearance of neurotransmitter from the cleft at this type of synapse. Interpretation of these results is, however, complicated by the observation that cyclothiazide also affects deactivation kinetics [38], as has been well established for other desensitization modifiers, such as aniracetam [22•].

Another approach to distinguish between hypotheses I and III is to map the time course of glutamate concentration in the synaptic cleft using low-affinity competitive antagonists of the NMDA receptor [3••,39•]. It has been estimated that under normal conditions the synaptic glutamate pulse experienced by NMDA receptors is characterized by a peak concentration of 1.1 mM and a decay τ of 1.2 ms [3••]. When released onto AMPA/kainate-type GluR channels, a glutamate pulse of this length may be considered prolonged, because it results in an AMPA/kainate EPSC decay τ that is slightly slower than the deactivation τ in response to a brief pulse of glutamate (see Fig. 1, hypothesis III). Moreover, under conditions of increased release probability glutamate remains at an effective concentration for longer periods of time [39•]. This may occur as a result of multivesicular release at a single site [39•], or, alternatively, as a result of simultaneous release at neighbouring sites.

In further support of the idea of a prolonged presence of glutamate in the synaptic cleft (hypothesis III) is the fact that a high-turnover enzyme for neurotransmitter degradation, analogous to acetylcholinesterase at the neuromuscular junction, is lacking at glutamatergic synapses. Experiments with specific inhibitors suggest that the decay of the AMPA/kainate receptor-mediated EPSC component is not dependent on glutamate uptake [40•,41]. Thus, the only mechanism available for rapid neurotransmitter elimination appears to be dif-

fusion. Assuming instantaneous release and free diffusion, the time course of neurotransmitter concentration in the synaptic cleft is expected to decay with a multiexponential time course, showing an initial rapid falling phase followed by more slowly decaying components, depending on synaptic geometry [1,42].

The mechanisms shaping EPSCs in the CNS are thus probably more complicated than at the neuromuscular junction, where the decay of the endplate current is suggested to be largely governed by a single rate constant [5,6]. Deactivation kinetics of AMPA/kainate-type GluR channels appears to be the major determinant of EPSC decay, however, both the time course of neurotransmitter concentration in the synaptic cleft and GluR channel desensitization may also contribute to the EPSC falling phase under some conditions. The functional role of desensitization may depend on pre- and postsynaptic peculiarities of the synapse. Firstly, the contribution of desensitization to EPSC decay is probably more significant at synapses comprised of 'giant' boutons with several release sites and a high probability of release (e.g. the calyceal synapse [15••]) than at synapses with only a single release site and a low probability of release. Secondly, the contribution of desensitization is possibly also more important in cells that express rapidly desensitizing AMPA/kainate-type GluR channels, such as cochlear nucleus neurones [15••,31], than in cells where GluR channel desensitization is slow, such as hippocampal granule or pyramidal neurones [29•].

Subunit composition may determine AMPA/kainate GluR channel kinetics

Molecular cloning studies have identified a family of AMPA receptor subunits (GluR-A, GluR-B, GluR-C and GluR-D, also known as GluR-1, GluR-2, GluR-3 and GluR-4) and a family of kainate receptor subunits (GluR-5, GluR-6 and GluR-7, probably co-assembled with KA-1 or KA-2) [43,44]. These subunits are widely expressed in the mammalian CNS and thus constitute the candidate proteins from which the native AMPA/kainate-type channels in the postsynaptic density are assembled. The native channels are probably heteromeric channels, as suggested by electrophysiological [44,45] as well as immunocytochemical [46•] data. The functional characteristics of native AMPA/kainate-type GluR channels in somata [30•,38,45] and dendrites (N Spruston, P Jonas, B Sakmann, abstract 261.1, 23rd Annual Meeting of the Society of Neuroscience, Washington DC, November 1993) of CNS neurones suggest that they are predominantly assembled from subunits of the GluR-A to GluR-D family [47•].

Experiments on recombinant GluR channels have demonstrated that subunit diversity is functionally relevant in determining the kinetics of AMPA/kainate-type GluR channels. Desensitization is fast for GluR-A or GluR-D homomeric channels, slow for GluR-B or GluR-C homomers, and intermediate for heteromeric channels, e.g. GluR-A/B [44,48]. In addition, channels assembled from flip splice version subunits desensitize more slowly than channels built up from flop version subunits [44]. Although the deactivation kinetics of recombinant channels have not been studied, they may be affected by subunit composition as well. In native GluR channels, deactivation and desensitization kinetics vary among different cell types. The putative subunit composition of the native channels has been partly determined by *in situ* hybridization [43], immunocytochemistry [49,50], and single-cell mRNA analysis using the polymerase chain reaction [30•,51,52], and correlations with the kinetic properties of the channels have been noted [29•,30•,45]. It is thus tempting to speculate that the large differences in EPSC decay time constant between different cells (see above) result primarily from differences in GluR subunit gene expression.

Functional determinants of the slow kinetics of the NMDA component of the EPSC

Deactivation and desensitization of NMDA receptors occur much more slowly than for AMPA/kainate receptors; thus, a neurotransmitter pulse that is long for AMPA/kainate receptor channels may still be brief for NMDA receptor channels. On the other hand, the affinity of NMDA receptors for glutamate is much higher than that of AMPA/kainate receptors [45,53,54••]; thus, a neurotransmitter concentration that is low for the AMPA/kainate receptors may be high for the NMDA receptors. Originally, the slow NMDA EPSC decay was explained either by the prolonged presence of neurotransmitter in the synaptic cleft (hypothesis II) or by the slow diffusion of glutamate to NMDA receptors located at remote sites. Several pieces of experimental evidence now argue against these possibilities and favour deactivation of NMDA receptor channels as the major determinant of NMDA EPSC decay. Firstly, the application of D-APV to excitatory synapses on cultured neurones during the NMDA EPSC does not change its time course. This suggests that rebinding of glutamate does not occur during the NMDA EPSC decay phase [55]. Secondly, brief pulses of glutamate applied to membrane patches activate NMDA components with slow rise and decay times ([26••,27•,32••,55,56••]; N Spruston, P Jonas, B Sakmann, abstract 261.1, 23rd Annual Meeting of the Society of Neuroscience, Washington DC, November 1993). Deactivation kinetics of NMDA responses are dependent on the type of agonist (e.g. glutamate, aspartate or cysteate) [32••], as well as on the channel subunit composition [57]. Thirdly, the decay of the NMDA EPSC mediated by the false neurotransmitter D-glutamate (loaded into autaptic glutamatergic synapses) is much faster than that mediated by the natural neurotransmitter L-glutamate, which is consistent with the much lower affinity of NMDA receptors for D-glutamate [58••]. Fourthly, partial agonists for the glycine site of the NMDA receptor speed up the NMDA EPSC decay and the deactivation of NMDA receptor-mediated currents activated by brief glutamate pulses [33]. Fifthly, kinetics of the NMDA EPSC and those of currents activated by brief pulses of glutamate in patches change in parallel during development or plasticity [26••,27•]. Finally, the time course of the neurotransmitter concentration in the cleft that is experienced by NMDA receptors was estimated for

excitatory synapses on cultured hippocampal neurones using competitive antagonists [3••]; the estimated decay of neurotransmitter concentration is much faster than that of the NMDA component (peak concentration 1.1 mM, decay τ 1.2 ms). All lines of evidence thus converge and lead to the conclusion that the deactivation of NMDA receptor channels alone is sufficiently slow to explain the decay time course. It seems probable that postsynaptic AMPA/kainate-type and NMDA-type GluR receptors sense glutamate pulses with very similar time courses, suggesting the co-localization of both types of channels.

Co-localization and co-activation

Further evidence that AMPA/kainate and NMDA channels are co-localized microscopically, possibly in the same postsynaptic density, is as follows: firstly, AMPA/kainate and NMDA components are present in miniature EPSCs, which are believed to be caused by the release of a single quantum of neurotransmitter [9••,12,13]; secondly, AMPA/kainate and NMDA receptors appear to be co-localized in hot spots corresponding to synaptic sites [59]; thirdly, amplitudes of AMPA/kainate and NMDA components of fluctuating unitary EPSCs vary in concert [10]; and finally, brief pulses of glutamate applied to dendritic membrane patches co-activate AMPA/kainate and NMDA components (N Spruston, P Jonas, B Sakmann, abstract 261.1, 23rd Annual Meeting of the Society of Neuroscience, Washington DC, November 1993). If the two types of channels are strictly co-localized, additional information can be inferred from the amplitude ratio of the two EPSC components. All tested neurotransmitter candidates, other than glutamate, either do not co-activate AMPA/kainate and NMDA receptor types or do not mimic the decay of the NMDA component of the EPSC [32••]; thus, one can hardly escape the conclusion that glutamate is the natural neurotransmitter. The amplitude ratio of the two components of the EPSC is virtually constant when the release probability is changed experimentally [39•,60•]. This implies that the absolute value of neurotransmitter concentration in the cleft may be high enough to nearly saturate both types of receptors [39•].

Conclusions and perspectives

The understanding of excitatory synaptic transmission in the CNS has greatly increased through the analysis of the factors that determine the kinetics of AMPA/kainate and the NMDA components of the EPSC, using combinations of patch-clamping, fast application, and various molecular biology methods. However, many questions remain. On the presynaptic side, more needs to be learned about the exact amount of neurotransmitter contained in a synaptic vesicle and the nature of the release process. On the postsynaptic side, more information is needed about the subunit composition and the functional properties of the native synaptic channels. Finally, attempts should be made to integrate these findings into quantitative models of synaptic transmission that incorporate detailed descriptions of GluR channel gating, as well as information about synaptic geometry.

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rotransmitter occurs at single release sites, but that AMPA/kainate and NMDA receptor channels are normally saturated by the release of the contents of a single vesicle.

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P Jonas and N Spruston, Max-Planck-Institut für Medizinische Forschung, Abteilung Zellphysiologie, Jahnstrasse 29, D-69120 Heidelberg, Germany.