

# Electrophysiological description of mechanisms determining synaptic transmission and its modulation

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Signal integration in neurons is a complex process that depends on e.g. the kinetics of synaptic currents, distribution of synaptic connections as well as passive and excitatory membrane properties. The time course of synaptic currents is largely determined by the kinetics of the postsynaptic receptors and the time course of synaptic neurotransmitter concentration. The analysis of current responses to rapid agonist applications provides the means to study the ligand-gated receptor gating but experimentally based estimation of neurotransmitter transient at central synapses was an important challenge during the last decade. Both theoretical as well as experimentally based approaches indicated that synaptic agonist transient is very brief, implying that the activation of postsynaptic receptors occurs in conditions of extreme non-equilibrium. Such a dynamic pattern of activation of postsynaptic receptors has a crucial impact not only on the kinetics of synaptic currents but also on their susceptibility to pharmacological modulation.

**Key words:** receptor gating, non-equilibrium, modifiers of gating, kinetics, pharmacology, synaptic agonist transient

## INTRODUCTION

Signal integration on a neuron critically depends on several factors including the kinetics of synaptic currents, distribution of synaptic connections on the soma and dendritic tree, passive and excitatory properties of the neuronal membrane and the homeostasis of neurotransmitters in the neuronal surroundings (due to e.g. activity of the uptake systems). The time course of synaptic currents is believed to largely rely on the agonist release dynamism and the kinetics of postsynaptic receptors. During development, kinetic properties of ionotropic receptors as well as their distribution dramatically change with a predominant trend to accelerate the synaptic currents (Tia et al. 1996, Hollrigel and Soltesz 1997, Dingledine et al. 1999, Rumbaugh and Vicini 1999, Okada et al. 2000, Cherubini and Conti 2001, Cull-Candy et al. 2001, Vicini et al. 2001, Cathala et al. 2003, Fritschy and Brunig 2003). Such an acceleration of synaptic currents is commonly ascribed to a refinement of

neural network leading to an enhancement of its capacity to detect the coincident events. Activation of postsynaptic receptors clearly depends on the amount of released agonist and on duration of its presence in the synaptic cleft. Recent investigations indicated, that the time course of synaptic agonist transient is a crucial factor in shaping the kinetics of synaptic currents and their susceptibility to modulation by various endogenous and exogenous factors. The present review will focus on electrophysiological approaches in describing the pharmacological properties of synaptic transmission, mainly GABAergic one. This relatively novel issue has attracted considerable attention during the last decade and brought an increased awareness that the dynamism of agonist release, its time course and variability is an important factor that needs to be taken into account when describing the mechanisms shaping the time course of synaptic currents and its modulation.

## ELECTROPHYSIOLOGICAL RECORDINGS

Membrane voltage is routinely recorded using the classical sharp electrodes technique (Fig. 1). The idea is analogous to that employed in a classical voltmeter

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used to measure the voltage in e.g. concentration cell (Fig. 1) but there is a technical challenge to impale the cell with a sharp electrode and thereby to “connect” the recording device to the cell interior to reliably measure the intracellular potential without excessively damaging the membrane or affecting the cytoplasmic milieu. When correctly configured, the major advantage of this technique is a high fidelity of membrane voltage recordings with potentially small interference with the cell properties. However, the value of the membrane voltage can be affected by several factors, often functionally coupled with each other, and for this reason it is usually hard to unambiguously dissect the information on a specific effector (e.g. different membrane conductances) or to describe a specific modulatory process. For this reason current recordings using the patch clamp technique in the voltage-clamp mode (current recordings at a defined electrode potential) became the primary tool in the pharmacokinetic studies. Depending on the purposes of specific experiments, patch-clamp technique can be used in different configurations (Fig. 2). In the cell-attached mode, the current recording is made from a tiny membrane patch with a diameter of a micron or so and the single channel activity can be revealed. In these conditions the cell interior is nominally intact while the membrane voltage is manipulated by altering the extracellular potential by the recording electrode. The most commonly used is the whole-cell configuration that enables to measure the current flowing through the entire cell membrane while controlling its voltage. In particular, using this recording mode, the synaptic currents can be recorded. A disadvantage of this recording mode is that the pipette solution dialyses the cell and therefore the intracellular solution is substituted in a matter of minutes by artificial saline that gives rise to a washout of several potentially important intracellular messengers. The advent of slice recordings and improved optics enabled electrophysiologists to perform not only somatic but also dendritic electrophysiological recordings. These new experimental possibilities revealed a number of observations, some of them going against somehow dogmatic view of dendrites as purely passive elements. Discovery of robust back propagation of action potentials into dendritic tree and, in some cells (e.g. mitral cells), a capacity to elicit dendritic action potentials substantially enriched our knowledge on the mechanisms of signal integration in neurons. In pharmacological studies so called excised patch (outside-out and inside out, Fig. 2) are of great

advantage as a rapid solution exchange (see also below) around these tiny membrane pieces is much easier than around a large cell (in the whole-cell mode). It needs to be taken into account that excised patch configuration brings about a physical detachment of studied channels from its natural milieu that may substantially alter the properties of these proteins.

### ESTIMATING THE TIME SCALE OF THE SYNAPTIC AGONIST TRANSIENT

As already mentioned, synaptic currents strongly depend on for how long and how many neurotransmitter particles appear in the vicinity of the postsynaptic receptors. The time course of agonist clearance from

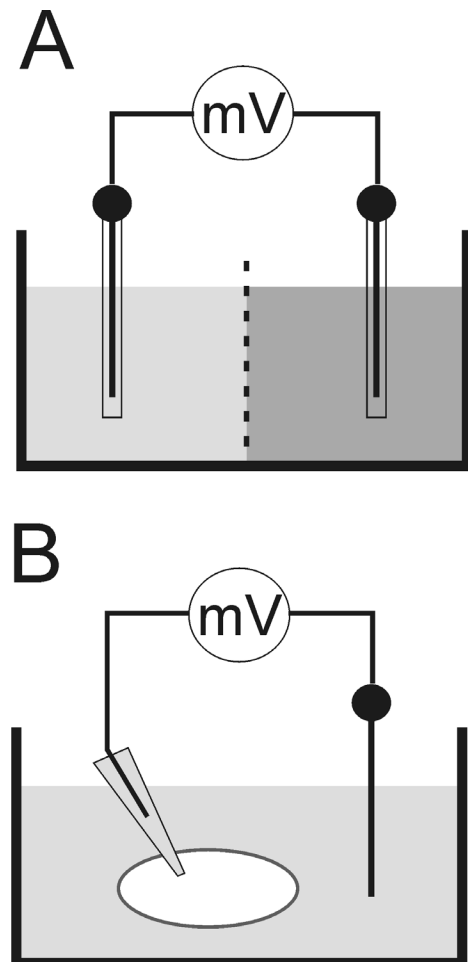


Fig. 1. Schematic view of the intracellular recording of membrane voltage with sharp electrodes. The idea is analogous to e.g. measurement of voltage in a concentration cell (A) but in the case of membrane voltage one electrode should connect the cell interior with the recording device (B) via a sharp electrode.

the synapse can be roughly estimated by solving the so called diffusion equation or by simulations using the Monte-Carlo algorithms for boundary conditions reflecting the geometry of the synapse. Calculations based on these approaches indicate that most of agonist molecules released from a synaptic vesicle are cleared out from the cleft within at most hundreds of microseconds (Holmes 1995, Clements 1996, Kleinle et al. 1996, Silver et al. 1996, Wahl et al. 1996, Glavinovic' 1999, Franks et al. 2002, Overstreet et al. 2002, Ventriglia and Di Maio 2003). The major weakness of the analytical approaches aiming at estimating the agonist transient is that the value of the crucial parameter – diffusion coefficient of neurotransmitter within the synaptic cleft is basically unknown. Assumption that it is equal to that measured in the bulk solution is clearly wrong as the structure and content of the synaptic cleft gives rise to several limitations of the diffusion process. There is a general agreement that this parameter is considerably smaller in the cleft than in the bulk solution but choosing its value in a specific computer simulations remains a matter of guessing. Nevertheless, the estimations based on model simulations clearly imply that during synaptic transmission the postsynaptic receptors are exposed to the neurotransmitter for considerably less than 1 ms, meaning that the activation of these receptors occurs in conditions characterized by a high degree of non-equilibrium. It is likely that there could be a minor component of the agonist

transient lasting for milliseconds (e.g. Overstreet et al. 2002) but there is a general agreement that, as already mentioned, the predominant phase of this process is considerably shorter than 1 ms. In Fig. 3, results of simulations are shown that illustrate how strong is dependence of the synaptic currents on the velocity of agonist clearance. The current response (simulated using the model in Fig. 3a, Mozrzymas et al. 2003b) to “nearly saturating” GABA concentration (1 mM, Fig. 3b) applied for 0.1 ms gives rise to a response with amplitude close to one third of that for the current evoked by the same GABA concentration applied for 1 ms (Fig. 3c,d). In addition, in Fig. 3e, a comparison is made between the response to square-like impulse of GABA with duration of 0.1 ms and exponentially decaying GABA transient with  $\tau = 0.1$  ms (more similar to a presumed synaptic agonist application). Notably, in spite of the fact that the averaged time exposure of receptors in both cases (Fig. 3e) is the same, the amplitudes and the time course of currents are markedly different. This example shows that extremely dynamic clearance of agonist from synapse renders synaptic transmission very sensitive to variation in the synaptic agonist waveform. Thus, it may be expected that a relatively minor variation in agonist time course due to e.g. modulation of uptake system or a presynaptic effect that modifies quantal content or release mechanism, can result in substantial alteration of synaptic transmission. A fundamental question, that is closely related to the investigations of the agonist transient kinetics and that is still a matter of debate, is whether or not the central synapses are saturated (see below).

Description of mechanisms underlying the time course of synaptic currents basically requires knowledge of two factors: gating of postsynaptic receptors and the time course of the synaptic agonist transient. As explained above, standard whole-cell recordings reveal the time course of synaptic currents but are insufficient to explore the underlying mechanisms because for any specific synaptic current, the agonist transient is unknown. Moreover, a thorough determination of the pharmacokinetics of the postsynaptic receptors, basing solely on the analysis of synaptic currents is not possible because it requires determination of dose-response relationships that cannot be obtained because synaptic agonist transient cannot be easily manipulated. For this reasons, the kinetic analysis of ligand-gated receptor kinetics requires recordings of current responses to exogenously applied ligand. However, to make such

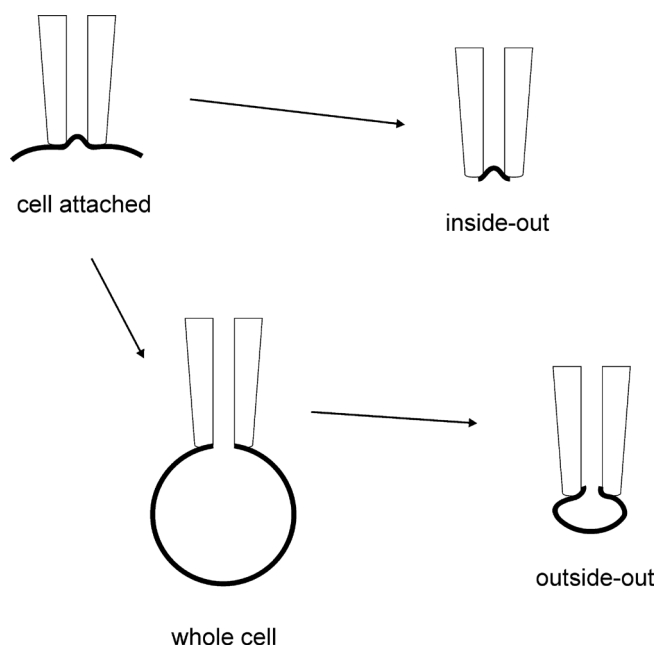


Fig. 2. Configurations of the patch-clamp technique

studies relevant to synaptic transmission the agonist application must be able to mimic the synaptic conditions. The difficulties in construction of the application system able to apply the ligands in a submillisecond time scale was the major limitation in determining the kinetics of ligand-gated receptors. A successful attempt to construct such an ultrafast perfusion systems was achieved by Franke and Dudel (1987) and this method was later mastered by the group of Peter Jonas (Jonas 1995). These systems are able to exchange solutions within less than 100  $\mu$ s allowing thus precise studies of receptor gating with time resolution corresponding to the time scale of synaptic events. However, it needs to be born in mind that using these systems it is still difficult to closely mimic synaptic agonist transient for at least two reasons: (i) the rapid application systems deliver a “square shaped” impulse of agonist while the synaptic agonist transient is likely to show exponential-like decay (see example in Fig. 3e) and (ii) even if the “on” and “off” application events can be faster than 100 ms, administration of neurotransmitter for less than 1  $\mu$ s often encounters technical problems (Mozrzymas et al. 2003a,b). Thus, in spite of a possibility to describe the receptor gating with high temporal resolution, the synaptic agonist transient remains a critical factor that cannot be either directly monitored or reliably mimicked in electrophysiological experiments.

The experimental estimation of the agonist time course in glutamatergic and GABAergic synapses is difficult because no direct detection method of these neurotransmitters is available. For instance, an attempt to deduce peak of synaptic GABA concentration by measuring the onset rate of synaptic currents was misleading because low agonist concentration applied for a very short time may produce a response with a rapid onset.

The lack of direct methods to assess the time course of synaptic agonist transient stimulated alternative strategies. One of them was to induce a defined “perturbation” of the system (postsynaptic receptors) and to deduce the time course of the synaptic agonist transient from quantitative elaboration of effects induced by such “perturbation” on synaptic currents. A prerequisite for this approach is the description, with highest possible temporal resolution, of receptor gating in control conditions and in the presence of the “perturbing factor” (e.g. competitive antagonist). The final deduction of the synaptic agonist transient is made by using model simulations. Below, examples of successful applications of such strategies are briefly described.

### LOW-AFFINITY, QUICKLY UNBINDING COMPETITIVE ANTAGONISTS REVEAL AGONIST TRANSIENT KINETICS

A very clever method applied to assess the time course of glutamate released in the glutamatergic synapses was to “perturbate” the time course of synaptic currents with competitive, quickly dissociating antagonist (Clements et al. 1992, Clements 1996). The basic idea of this method is that if duration of synaptic agonist transient is comparable to that of antagonist dissociation, then a non-equilibrium displacement of competitive blocker would be unmasked by a current flowing through unblocked postsynaptic receptors. Thus, displacement of competitive antagonist would be associated also with a modification of the synaptic current rising phase. However, application of this approach to the synaptic currents requires collection of substantial information. In particular, the receptor gating in control conditions and the blocking mechanism must be precisely described. This goal is achieved by recording current responses to ultrafast agonist applications and by optimizing the respective rate constants. Such a method has been applied to determine the time course of synaptic agonist transient in glutamatergic synapse (Clements et al. 1992, Clements 1996) and it was concluded that synaptically released glutamate peaks at approximately 1.1 mM and is cleared out with a time constant of ca. 1.2 ms (Clements et al. 1992).

More recently, the method based on the use low-affinity antagonist (SR95531) has been applied to estimate the time course of agonist in the GABAergic synapses (Overstreet et al. 2002) yielding an estimation of the synaptic GABA transient: peaks at 3–5 mM and is clearance time constant within 300–600  $\mu$ s. Importantly, in spite of a high peak GABA concentration, postsynaptic GABA<sub>A</sub> receptors were implicated not to be saturated due to rapid agonist clearance.

The method of quickly dissociating competitive antagonists, although conceptually brilliant, is endowed with considerable limits resulting from the speed of dissociation of available competitive antagonists. The dissociation rate of D-amino adipate (D-AA) used by Clements and coworkers (1992) is 160 s<sup>-1</sup> that corresponds to the dissociation time constant of approximately 6.25 ms. SR95531 used by Overstreet and others (2002) to determine agonist transient in GABAergic synapse has the dissociation time constant of approximately 2 ms. Thus, the time constants of agonist clear-

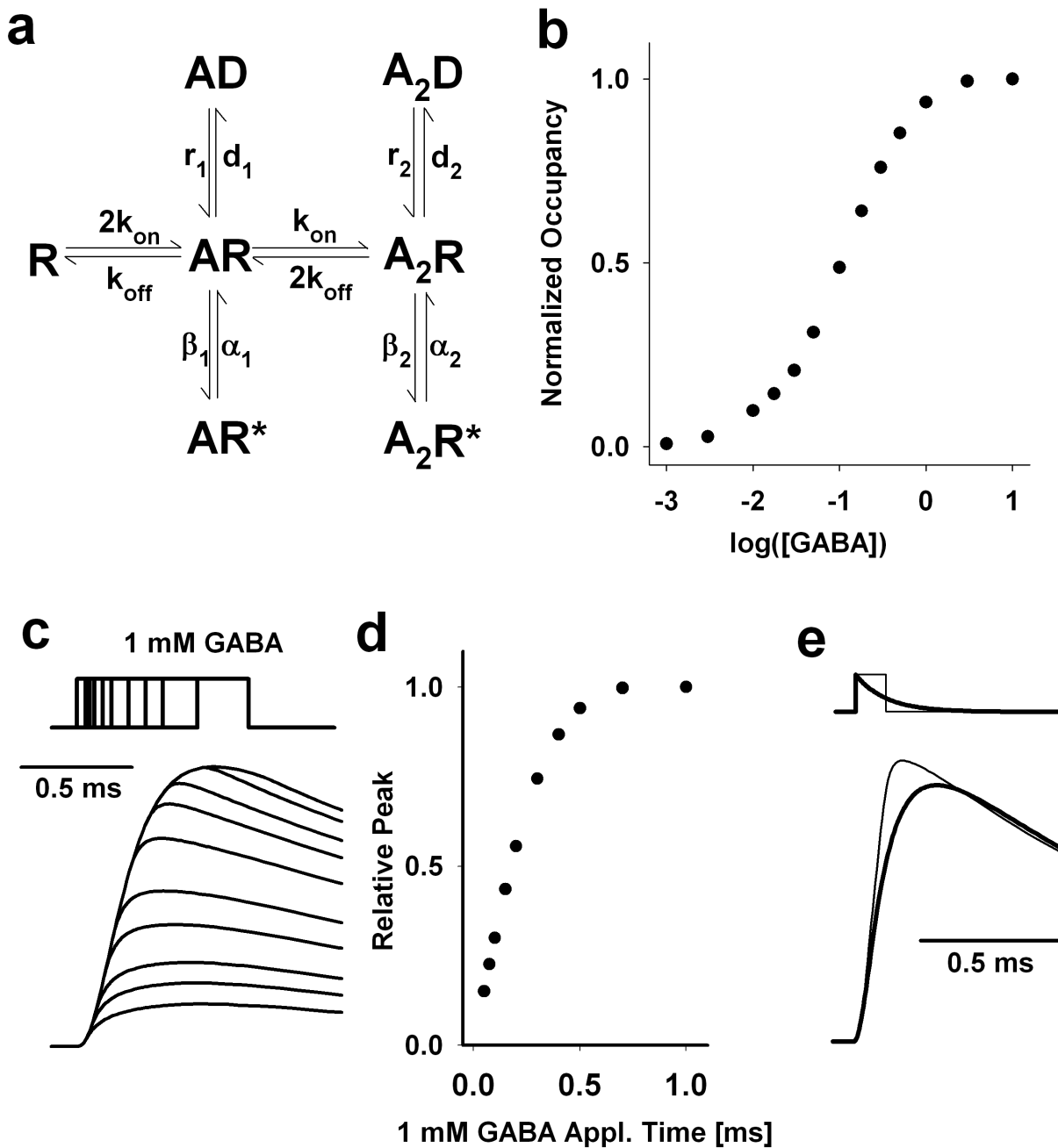


Fig. 3. Characteristics of current responses to applied agonists depend on the time exposure of the agonist and on the temporal profile of the agonist transient. (a) the frame of the Jones and Westbrook's model of GABA<sub>A</sub> receptor gating (Jones and Westbrook 1995). Rate constants for simulations are from Mozrzymas and others (2003b):  $k_{\text{on}} = 6 \text{ ms}^{-1}\text{mM}^{-1}$ ;  $k_{\text{off}} = 1 \text{ ms}^{-1}$ ;  $\beta_1 = 0.15 \text{ ms}^{-1}$ ,  $\alpha_1 = 1.5 \text{ ms}^{-1}$ ,  $d_1 = 0.045 \text{ ms}^{-1}$ ,  $r_1 = 0.014 \text{ ms}^{-1}$ ;  $\beta_2 = 3 \text{ ms}^{-1}$ ,  $\alpha_2 = 0.4 \text{ ms}^{-1}$ ,  $d_2 = 12 \text{ ms}^{-1}$ ,  $r_2 = 0.07 \text{ ms}^{-1}$ . (b) dose-response relationship for peak currents (simulated as the sum of open states occupancies) evoked by GABA applications sufficiently long to reach maximum value at given agonist concentration. At 1 mM current amplitude reaches 93 % of maximum response. (c) simulated current responses to rectangular GABA pulse (1 mM) of various duration from 0.05 to 1 ms. Note that for 0.1 ms pulse duration, current response reaches less than one third of response elicited by 1 ms application. In (d) the peak values of simulated currents presented in (c) are presented *versus* time duration of GABA pulse (1 mM). (e) comparison of simulated currents elicited by a rectangular GABA pulse (1 mM, 0.1 ms, thin line) to the current evoked by exponentially decaying GABA application ( $A \times \exp(-t/\tau)$  where  $A = 1 \text{ mM}$ ,  $\tau = 0.1 \text{ ms}$ , thick line). Note that averaged exposure to the agonist is the same in both cases but the time course of currents show marked differences.



ance in glutamatergic (1.2 ms, Clements et al. 1992) and in GABAergic synapse (0.3–0.6 ms, Overstreet et al. 2002) are at the borderline of resolution of this method. Thus, if there were components of agonist clearance characterized by time constants of hundreds of microseconds or shorter, their detection would be limited by the lack of blockers that dissociate sufficiently quickly to reveal it. On the other hand, very fast clearance components of ca. 100  $\mu$ s have been postulated by several Monte Carlo and model simulations (Holmes 1995, Clements 1996, Kleinle et al. 1996, Wahl et al. 1996, Glavinovic' 1999, Franks et al. 2002, Ventriglia and Di Maio 2003). The experimental verification of these predictions requires thus a modified strategy.

### MODIFIERS OF GATING

As an alternative strategy to the quickly dissociating competitive antagonists, recently, allosteric modifiers of receptor gating have been employed to assess the synaptic agonist time course (Mozrzymas et al. 1999, 2003b, Barberis et al. 2000). In particular, modulators affecting the binding rate (such as chlorpromazine – CPZ, protons or benzodiazepine receptor agonists) turned out to be particularly useful. The starting point for this approach is the idea that if the time exposure of the postsynaptic receptors is very brief then alteration in the binding rate would be expected to have a major impact on the activation of these receptors. Thus, if the agonist transient is very fast (e.g.  $\tau = 100 \mu$ s), then the reduction of the binding rate (by CPZ or decrease in pH) results in a decreased entrance into the bound states because the exposure time of receptors to agonist becomes insufficient to complete the binding step (or to bring it to the same level as in control conditions). This prediction indicates that the shorter the agonist transient, the larger is the sensitivity of synaptic currents to alteration of the binding rate. These considerations help to realize that whether or not the applied agonist is saturating depends not only on its concentration but also on the time duration of its application. This appears to be particularly important in the case of synaptic transmission.

As explained above, chlorpromazine (CPZ), variation in extracellular pH and benzodiazepines strongly affect the binding rate making them particularly suitable to probe the kinetics of synaptic GABA transient (Mozrzymas et al. 1999, 2003b, 2007). Model simulations based on recordings of synaptic currents as well as of current responses to ultrafast GABA applications

in control conditions and in the presence of these modulators consistently revealed that the predominant component of synaptic GABA clearance is very fast. The best reproduction of the experimental data was obtained for the time constant of approximately 100  $\mu$ s. However, the main methodological problem with the use of the modifiers of gating is that it is difficult to find drugs that modify specifically only the binding step. In most cases, modulators affect also other transitions (e.g. opening/closing, desensitization, unbinding) and it can be difficult to distinguish the effect on the binding rate from modification of other transition rates. This means that the use of a modulator to probe the kinetics of the agonist transient, must be preceded by a thorough and complete determination of the modulatory mechanisms. An additional outcome of these studies was an evidence that at least in the considered model (cultured hippocampal neurons) the mIPSCs were considerably distant from saturation. This is an important point as the saturation issue remained a matter of debate. So far, the major tool to test the saturation of synaptic currents was the effect of compounds known to enhance the GABA<sub>A</sub>R affinity (benzodiazepines) but results obtained using these drugs were not consistent as some investigators reported potentiation of synaptic currents (Segal and Barker 1984, Frerking et al. 1995, Perrais and Ropert 1999, Cohen et al. 2000, Hajos et al. 2000) while others did not (Otis and Mody 1992, DeKoninck and Mody 1994, Mody et al. 1994). In addition, as it has been demonstrated in our laboratory (Mozrzymas et al. 2007), benzodiazepines besides affecting binding, modulate also the gating properties (mainly desensitization).

### CONCLUSIONS

Signal integration in neurons is an extremely complex process that is known to critically depend on dendrite morphology, kinetic properties of synaptic and extrasynaptic ligand-gated channels as well as on passive and excitatory properties of neuronal membrane. In the last decade, studies on the spatiotemporal profile of the synaptically released agonist have emphasized the crucial impact of agonist transient kinetics on the kinetic shape and susceptibility to pharmacological modulation of synaptic currents. This factor strongly depends not only on the presynaptic releasing machinery but also on the geometry of synapse and its surroundings (e.g. presence of glial cells adjacent to the synapse) as well as

on the activity of agonist uptake or cleavage mechanisms. Thus, agonist transient might be a subject to modulation by several direct or indirect mechanisms affecting thereby the signal integration. The major difficulty in exploring the synaptic agonist transient is that its experimentally based assessment is indirect, requires separate experiments and substantial quantitative elaboration yielding eventually an averaged values of agonist transient characteristics. In conclusion, agonist transient, although difficult to track in the everyday experimental practice, turns out to be one of the most critical steps in signal integration in neurons.

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