Positive α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) Receptor Modulators Have Different Impact on Synaptic Transmission in the Thalamus and Hippocampus

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ABSTRACT

Earlier studies showed that positive modulators of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors enhance synaptic responses and facilitate synaptic plasticity. Those studies focused mainly on hippocampal functions. However, AMPA receptors have regionally distinct subunit compositions and thus potencies and efficacies of modulators may vary across the brain. The present study compared the effects of CX546 [1-(1,4-benzodioxan-6-ylcarbonyl)piperidine], a benzamide-type modulator, on synaptic transmission in neurons of the reticular thalamic nucleus (RTN), which regulates the firing mode of relay cells in other thalamic nuclei, and on hippocampal CA1 pyramidal cells. CX546 greatly prolonged synaptic responses in CA1 pyramidal cells, but at the same concentration it had only weak modulatory effects in RTN neurons. Effects on miniature excitatory postsynaptic currents (mEPSCs) were similar to those on EPSCs in both regions, suggesting that variations in neuronal morphology and transmitter release kinetics do not account for the differences. Relay cells in the ventrobasal thalamus also exhibited weak modulatory effects that were comparable with those in RTN neurons. Regionally different effects on response duration were also observed with CX516 [BDP-12, 1-(quinoxalin-6-ylcarbonyl)piperidine], a second benzamide drug. In contrast, 100 μM cyclothiazide produced comparable synaptic enhancements in hippocampus and RTN. The regional selectivity of benzamide drugs (ampakines) may be explained, at least in part, by a lower potency at thalamic AMPA receptors, perhaps due to the prevalence of the subunits GluR3 and 4. Although regional preferences of the ampakines were modest in their extent, they may be sufficient to be of relevance when considering future therapeutic applications of such compounds.

Drugs that up-modulate AMPA receptors constitute a new family of potential therapeutics, and they also provide tools to examine how specific aspects of AMPA receptor kinetics contribute to synaptic transmission and neuronal function. These modulators have been shown to increase to various extents the amplitude and the duration of synaptic responses (Arai et al., 2004), to facilitate synaptic plasticity (Arai and Lynch, 1992; Arai et al., 1996a, 2004), and to improve many extents the amplitude and the duration of synaptic responses and thus potencies and efficacies of modulators may vary across the brain. The present study compared the effects of CX546 [1-(1,4-benzodioxan-6-ylcarbonyl)piperidine], a benzamide-type modulator, on synaptic transmission in neurons of the reticular thalamic nucleus (RTN), which regulates the firing mode of relay cells in other thalamic nuclei, and on hippocampal CA1 pyramidal cells. CX546 greatly prolonged synaptic responses in CA1 pyramidal cells, but at the same concentration it had only weak modulatory effects in RTN neurons. Effects on miniature excitatory postsynaptic currents (mEPSCs) were similar to those on EPSCs in both regions, suggesting that such as memory encoding. However, with AMPA receptors being abundant throughout the brain, the range of functions potentially susceptible to such modulation may be much wider. Moreover, since AMPA receptors can assemble in virtually any combination from the four constituent subunits GluR1 to 4 and their alternative splice variants called flip and flop, cell types differ widely in the composition of these receptors. The effect of a given AMPA receptor modulator may thus potentially vary between brain regions for several reasons. First, the modulator may discriminate among subunits. Second, receptors vary in their kinetic properties, such as in the rates at which receptors desensitize in the presence of glutamate or deactivate after removal of glutamate. Thus, if a modulator changes a particular rate constant underlying these processes, its efficacy may be different across receptors, depending on the relative importance of that rate constant for response dynamics. For example, desensitization seems to make a significant contribution to response termination in

ABBREVIATIONS: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; RTN, reticular thalamic nucleus; CX546, 1-(1,4-benzodioxan-6-ylcarbonyl)piperidine; CX516, 1-(quinoxalin-6-ylcarbonyl)piperidine; ACSF, artificial cerebrospinal fluid; NMDA, N-methyl-D-aspartate; MK-801, dizocilpine maleate; VB, ventrobasal nucleus of the thalamus; EPSC, excitatory postsynaptic current; mEPSC, miniature excitatory postsynaptic current; D-AP5, d-2-amino-5-phosphonovaleric acid; GYKI 52466, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine.
some neuron types (Gardner et al., 2001) but not in others (Arai and Lynch, 1998; Lin et al., 2002), and drugs that modulate desensitization would accordingly be expected to have more prominent effects in the former. Last, integration of multiple synaptic events may be influenced differentially by modulators, depending on extrasynaptic factors such as dendritic morphology (for review, see Spruston et al., 1994) or frequency and synchrony of afferent activity (Diamond and Jahr, 1995).

The present study examined the effects of some prototypical modulators on synaptic responses in the thalamus and compared them with those in pyramidal cells of hippocampal area CA1. The thalamus was selected because synaptic events often have a faster time course and AMPA receptors have different subunit compositions, with GluR3 and 4 subunits being more prevalent than the GluR1 and 2 subunits that are abundant in the hippocampus (Mineff and Weinberg, 2000; for review, see Jones, 2002). Tests were conducted mainly in the reticular thalamic nucleus (RTN), an area that exerts a complex integrative control over thalamic function, with some additional tests in thalamic relay cells, which are involved in communicating sensory information to the cortex.

A further factor to be taken into consideration is that existing AMPA receptor modulators are heterogeneous in structure and in the way they modify receptor kinetics and synaptic processes (Johansen et al., 1995; Arai and Lynch, 1998; Arai et al., 2000, 2002, 2004). Regional preferences may accordingly be specific for a given subtype of drug. The main focus of this study was on CX546, a first generation benzamide drug (ampakine) of relatively low potency but with high efficacy for prolonging synaptic responses (Nagarajan et al., 2001; Arai et al., 2002). Additional tests were conducted with CX516, another benzamide drug that enhances response amplitudes more prominently than duration (Arai et al., 1996b, 2002) and that has been the drug most extensively examined in behavioral and clinical tests (Lynch, 2003). A third modulator was cyclothiazide, which is particularly effective in blocking receptor desensitization (Yamada and Tang, 1993). A preliminary report of this work was published in abstract form (Xia et al., 2002).

Materials and Methods

Slice Preparation. Hippocampal and thalamic slices (350–400 μm) were prepared from Sprague-Dawley rats (postnatal day 12–15). The animals were anesthetized with halothane before decapitation according to an institutionally approved protocol and in observation of the guidelines of the National Institutes of Health. All efforts were made to minimize any discomfort of the animals. The brain was removed from the skull and immersed into ice-cold artificial cerebrospinal fluid (ACSF). The anterior and posterior parts of the brain, including frontal cortex and cerebellum, were cut off, and the brain block was glued on the stage of a Leica VT1000S vibratome. The block was cut horizontally in ice-cold ACSF, and slices were cut into blocks 270–285 mOsm. Synaptic transmission in the CA1 area was induced by activation of Schaffer commissural fibers (see details in Arai et al., 2002). Stimulation intensity was adjusted so as to obtain 30 to 50% of the maximum amplitude, and constant current stimulation was delivered every 15 s. In addition, a brief voltage jump of −10 mV for 60 ms was applied before every stimulation to monitor access resistance. Experiments with changes in access resistance of more than 30% were excluded from analysis. After establishing a stable baseline, the drug was introduced in the recording chamber. The time for solution exchange of the small-volume recording chamber was 3 min. Excitatory postsynaptic currents (EPSCs) were recorded with AxoPatch 200B and digitized at 10 kHz with Digidata1200B/pClamp 9. The holding potential was maintained at −70 mV, and all the experiments were carried out at room temperature (22–24°C).

Data analysis was carried out off-line. The decay phase of the response was fitted with a biexponential function. The quality of fitting was assessed from correlation statistics. Fast and slow decay time constants and the corresponding fractional amplitude contributions were compared before and in the presence of the drug. To obtain a weighted decay time constant, the fast and slow decay time constants were multiplied by their respective fractional amplitude and summed. In some experiments, spontaneous miniature excitatory postsynaptic currents (mEPSCs) were recorded from CA1 pyramidal cells and RTN neurons. Access resistance was monitored throughout the experiments. Spontaneous events were selected with a template event detection tool in pClamp 9 (Clements and Bekkers, 1997). Experiments with average rise times larger than 2.5 ms were excluded from final analysis. Decay time constants were calculated by fitting the response decay phase with a single exponential function.

Drugs. The AMPA receptor modulators CX546 and CX516 were provided by Dr. G. Rogers (Cortex Pharmaceuticals, Irvine, CA). All other agents, including cyclothiazide, were purchased from Sigma-Aldrich (St. Louis, MO). Stock solutions of AMPA receptor modulators were prepared in dimethyl sulfoxide and diluted at least 1000 times for every experiment. Stock solutions for D-AP5, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline, and MK-801 were prepared in water or aqueous buffers.

Results

Effects of CX546 on Excitatory Synaptic Transmission in the RTN and Hippocampus. Stimulation in the internal capsule produced a fast inward current in RTN neurons with a very short time to peak. The decay phase contained a fast as well as a slow component at a holding potential of −70 mV. Application of 50 μM D-AP5 abolished some of the slow component, suggesting that NMDA receptors in RTN neurons participate in synaptic transmission, even when the membrane potential is clamped at −70 mV (Warren and Jones, 1997; Gentet and Ulrich, 2003). As a consequence, all subsequent experiments were carried out in the presence of 10 μM MK-801 and 50 μM D-AP5. The remaining current was abolished by 20 μM 6-cyano-2,3-dihydroxy-7-nitroquinoxaline and 40 μM GYKI 52466 (data not shown), indicating that the currents are mediated through hippocampal CA1 pyramidal cells. Neurons were visualized with an infrared microscope (BX50WI, Olympus, Tokyo, Japan) with differential interference contrast configuration, and whole-cell recording was established with borosilicate glass electrodes (5–10 MOhm) filled with the internal solution containing 130 mM CsF, 10 mM EGTA-K, 2 mM ATP-Na, 2 mM MgCl2, and 10 mM HEPES, pH 7.35 (270–285 mOsm). Synaptic responses in the RTN were evoked by a bipolar nichrome stimulation electrode positioned in the internal capsule. For recordings in the VB, the stimulation electrode was positioned adjacent to the recording cell. Synaptic transmission in the CA1 area was induced by activation of Schaffer commissural fibers (see details in Arai et al., 2002). Stimulation intensity was adjusted so as to obtain 30 to 50% of the maximum amplitude, and constant current stimulation was delivered every 15 s. In addition, a brief voltage jump of −10 mV for 60 ms was applied before every stimulation to monitor access resistance. Experiments with changes in access resistance of more than 30% were excluded from analysis. After establishing a stable baseline, the drug was introduced in the recording chamber. The time for solution exchange of the small-volume recording chamber was 3 min. Excitatory postsynaptic currents (EPSCs) were recorded with AxoPatch 200B and digitized at 10 kHz with Digidata1200B/pClamp 9. The holding potential was maintained at −70 mV, and all the experiments were carried out at room temperature (22–24°C).

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AMPAs. As illustrated in Fig. 1A, AMPA receptor-mediated EPSCs in RTN had a faster time course than those at synapses between Schaffer commissural fibers and pyramidal cells in hippocampal area CA1. On average, the rise time (1.76 ± 0.13 ms) and half-width (6.2 ± 0.6 ms; n = 40) were about 2.5 times smaller than in the hippocampus (4.4 ± 0.5 and 17.3 ± 1.3 ms, respectively; n = 29) (also see Arai et al., 2004).

CX546 was shown previously to produce robust increases in AMPA receptor currents in patches excised from hippocampal pyramidal cells and from human embryonic kidney 293 cells expressing recombinant AMPA receptors (Arai et al., 2002). Kinetic analysis revealed that its main effect was to slow response deactivation up to 10-fold. The most prominent effect on synaptic transmission accordingly was to increase response duration, both in hippocampal slices (Arai et al., 2002, 2004) and primary cultures (Nagarajan et al., 2001). In agreement with these previous studies, CX546 at 400 μM greatly prolonged response duration at CA1 pyramidal cell synapses as indicated by a 168 ± 29% (n = 7) increase in response half-width, with comparably modest effect on amplitude (Fig. 1, A and B). By comparison, effects on RTN cells were remarkably weak at all concentrations tested (Fig. 1, A and B). Response half-width was increased by only 68 ± 16% (n = 8) at 400 μM, and even at 800 μM the increase was smaller (104 ± 18%; n = 9) than that obtained in the hippocampus with 400 μM drug. CX546 at 200 μM increased the amplitude by 40 ± 13% (n = 10) in the hippocampus but by only 5 ± 6% (n = 6) in RTN neurons; the increase over baseline in the hippocampus (p = 0.016; two-tailed) as well as the difference to the RTN (p = 0.029; one-tailed) were significant.

Fig. 1. Differential effects of CX546 on synaptic transmission in the RTN versus hippocampus. EPSCs were recorded from RTN neurons and hippocampal CA1 pyramidal cells. Slices were equilibrated with 50 μM picrotoxin, 50 μM D-AP5, and 10 μM MK-801. The holding potential was −70 mV. A, representative traces before and in the presence of 400 μM CX546 (top). The graphs underneath show percentage of changes in the response half-width and input resistance over the course of drug infusion. After establishing a stable baseline, 400 μM CX546 was applied to the slices for 10 to 15 min, as indicated with the horizontal bar. Symbols represent the mean and S.E.M. of 12 (RTN) and seven (hippocampus) experiments. The mean input resistance was 183.3 (RTN) and 83.1 (hippocampus) Ωm. B, concentration-effect relations for EPSC amplitude and response half-width. The y-axes indicate the percentage of change over predrug baseline. The columns represent the mean and S.E.M. of six to nine (RTN) and seven to 10 (hippocampus) experiments. *, p = 0.011; **, p = 0.008 for comparisons with the corresponding concentration tested in the RTN (t test, two-tailed). †, p = 0.036 (one-tailed) versus effect of 400 μM CX546 on hippocampal EPSCs. ††, p = 0.029 (one-tailed) for comparison with the corresponding concentration in the RTN. C and D, decay phase of EPSCs fitted with a biexponential function. The columns show the magnitude of the fast and slow decay time constants (τ) and the percentage of contribution of the slow decay component to total response amplitude, before and after infusion of 400 μM CX546. The data are means and S.E.M. from seven (RTN) and six (hippocampus) experiments. *, p < 0.05, two-tailed paired t test; **, p < 0.01, t test, unpaired.
Closer inspection of the traces showed that response decay often is biphasic, before and after infusion of drug. According to biexponential curve fittings, the slow component accounted for about 20% of total amplitude in control responses of both brain regions (Fig. 1, C and D). The dominant fast component had a significantly shorter decay in RTN neurons (4.6 ± 0.8 ms) than in the hippocampus (10.2 ± 1.7 ms; p < 0.01), as expected from the comparisons of response half-width discussed above, but the slow decay components were not statistically different, although the time constant tended to be larger in the RTN. Drug effects on these measures of response wave form differed again between the two brain regions. In the hippocampus, CX546 significantly increased both the fast (1.4 ± 0.3 ms) and the slow (2.1 ± 0.5 ms) decay time constants, but the most prominent change was an increase in the contribution of the slow component from 23 to 69% of total response amplitude (Fig. 1D; p < 0.01) (also see Arai et al., 2004). The remaining fast component probably reflects in part the presence of receptors in which the drug sites were not or only partially occupied, owing to the fact that the drug concentration of 400 μM is not saturating. In the RTN, changes in response parameters were much smaller. The fast decay time constant increased 1.3 times (p < 0.05), but the time constant of the slow component seemed to be reduced (0.75 ± 0.1 ms), and the contribution of the slow component to total response amplitude increased to merely 34 ± 5% (from 23% before drug). Differences in the latter values did not reach significance due to the variability inherent in such fittings. To quantify drug effects further, we determined for each experiment a weighted decay time constant and the total charge transfer. The values are summarized in Table 1, along with other response parameters and input resistance. In CA1 pyramidal cells, the weighted time constant increased by a factor of 4.3 ± 0.6 after infusion of 400 μM CX546, whereas RTN neurons exhibited virtually no change. Similarly, charge transfer increased 2.5 times in hippocampal neurons but only 1.2 times in the RTN. Thus, CX546 influences the duration of excitatory responses in RTN neurons to a lesser extent, regardless of the parameter used to express response duration. No significant effects were observed, however, on response rise time or amplitude, and no differences were evident in the input resistance (Table 1).

Effects of CX546 on Miniature Spontaneous EPSCs in the RTN and Hippocampus. As shown above, the time course of evoked responses in hippocampal pyramidal cells is considerably slower than in RTN neurons. The reasons are not yet fully understood. Differences in the subunit composition of AMPA receptors may play a role because RTN cells mainly contain GluR3 and 4 subunits (Golshani et al., 2001), which may have faster kinetics (Mosbacher et al., 1994; Sekiguchi et al., 1997). However, other factors may be of similar or greater importance, such as dendritic filtering and regional differences in transmitter release kinetics. It has also been suggested that asynchronous release of transmitter broadens the waveform of hippocampal EPSCs (Diamond and Jahr, 1995). It is thus conceivable that the seemingly more robust effects of CX546 in the hippocampus result from factors unrelated to AMPA receptors, such as an enhanced visibility of the drug effect due to cumulation of early and delayed response components. If this were the case then regional differences in drug effects should disappear when measuring mEPSCs. Drug effects on hippocampal mEPSCs in particular would be expected to become smaller and closer in magnitude to those seen in EPSCs from RTN neurons.

This was examined in the experiments shown in Fig. 2. Spontaneous miniature events were recorded under blockade of NMDA and GABA<sub>A</sub> receptors. In hippocampal recordings, the time course of the averaged spontaneous events varied considerably between cells, and only experiments in which a majority of events (>85%) had a rise time faster than 2.5 ms were chosen for analysis. The spontaneous events in these selected experiments showed a mean rise time of 1.6 ± 0.3 ms (n = 5), which is 2.8 times shorter than that of evoked EPSCs (Table 1); this rise time was comparable with the average value obtained in RTN neurons in both spontaneous (1.7 ± 0.2 ms; n = 10; Table 2) and evoked responses (1.4 ± 0.4 ms; Table 1). Fitting an exponential function to the decay phase of these spontaneous mEPSCs yielded mean decay time constants of 4.2 ± 0.9 ms for hippocampal responses and 4.1 ± 0.5 ms for RTN responses. Thus, time course and waveform of the spontaneous mEPSCs before drug was similar in both brain regions. Given that the time course of hippocampal mEPSCs is much faster than for evoked EPSCs, the miniature events probably originated from synapses close to the soma, which would minimize the influence of dendritic filtering.

The effects of 400 μM CX546 on spontaneous events are illustrated in Fig. 2 and summarized in Table 2. The time course of mEPSC recorded in the RTN remained similar to that before drug (Fig. 2, A and B), with little change in the

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<tr>
<th>RTN (n = 7)</th>
<th>Hippocampus (n = 6)</th>
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<tr>
<td><strong>Rise Time</strong></td>
<td><strong>Weighted Area</strong></td>
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<tr>
<td>10-90%</td>
<td>μs</td>
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<tr>
<td>Control</td>
<td>202 ± 9</td>
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<tr>
<td>CX546 (400 μM)</td>
<td>244 ± 25</td>
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<tr>
<td>Average ratio ± CX546</td>
<td>1.2 ± 0.1</td>
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*hp, hippocampus; N.S., not significant.

One case that differed by more than 10 standard deviations from the mean of the remaining values (n = 6) was excluded; ***p < 0.001 versus control (predrug value), paired t test (two-tailed).
rise time and with decay time constants being increased to only a modest degree (44 ± 14%; \( n = 10 \); Fig. 2D). In contrast, miniature events in hippocampal neurons were greatly prolonged. The decay time constant for these events was increased on average by a factor of 3.0 ± 0.7 to a value of 13 ± 4 ms (Fig. 2D). The difference in the changes of the decay time constant between hippocampus and RTN is significant at \( p = 0.037 \) (Table 2). In either region, the drug had no...
TABLE 2
Effects of CX546 on spontaneous miniature EPSCs recorded from RTN neurons and from hippocampal CA1 pyramidal cells

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<tr>
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<th>RTN (n = 10)</th>
<th>Hippocampus (n = 5)</th>
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<tr>
<td></td>
<td>pA</td>
<td>10-90% Rise Time</td>
</tr>
<tr>
<td>Control</td>
<td>22.6 ± 2.4</td>
<td>1.4 ± 0.3</td>
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<tr>
<td>CX546 (400 µM)</td>
<td>25.1 ± 3.2</td>
<td>1.6 ± 0.3</td>
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<tr>
<td>Average ratio ± CX546</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.02</td>
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<tr>
<td>Comparison hp vs. RTN</td>
<td>N.S.</td>
<td>N.S.</td>
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hp, hippocampus; N.S., not significant.

*p < 0.05; *** p < 0.005 versus control (predrug value), paired t test (two-tailed).

significant effect on the frequency of the events or on the mean amplitude (Fig. 2, C and D). The rise time was increased in the hippocampus (p < 0.05), but comparison of the averaged ratios in RTN versus hippocampus was not significant.

Together, the effects of CX546 on spontaneous mEPSCs are in quantitative agreement with the results from evoked EPSCs even though the time course of the control spontaneous event is about 2.8 times faster than that of evoked EPSCs. The larger effects of CX546 in the hippocampus are thus likely to reflect a genuine property of its synapses and their AMPA receptors.

Effects of CX546 on Excitatory Synaptic Transmission in Thalamocortical Neurons of the Ventrobasal Complex. Differences in drug effects may be the consequence of variations in subunit composition, including the relative prevalence of the splice variants flip and flop. AMPA receptors in hippocampal pyramidal cells from rats older than postnatal day 5 are mainly composed of GluR2 subunits in combination with GluR1 and 3 (Keinanen et al., 1990; Zhu et al., 2000). In contrast, receptors in the RTN and most other thalamic nuclei seem to consist mostly of GluR3 and 4 (Mineff and Weinberg, 2000; Ibrahim et al., 2000; Golshani et al., 2001; Jones 2002) with notable absence of GluR1 and 2, at least in the rodent thalamus (Liu et al., 2001). However, receptor composition varies to some extent also between thalamic nuclei. For instance, corticothalamic synapses in the RTN contain a 3 to 4 times higher amount of GluR4, but not GluR3, than those on thalamic relay cells in the VB, which has been suggested to correlate with the higher amplitude of unitary EPSCs seen in the former (Golshani et al., 2001). We therefore also examined the effects of CX546 on relay cells in the VB. Excitatory synaptic transmission was recorded from these cells in response to activation of the fibers within the VB. The time course of the EPSCs was considerably slower than that typically seen in RTN neurons; the rise time of 4.2 ± 0.7 ms and the half-width of 18 ± 3 ms (n = 11) were in fact similar to the values obtained in the hippocampus (Fig. 3, A and B). However, the effect of 400 µM CX546 was comparable in magnitude to that in RTN cells, with a 54 ± 18% increase in half-width (n = 7); the difference to the effect obtained at hippocampal synapses is significant at p < 0.01 (Fig. 3, C and D). Also, the contribution of the slow decay component to total response amplitude increased to only about 30% in the presence of the drug, as it had been the case in RTN neurons (data not shown). These findings suggest that CX546 may have low effectiveness at synapses throughout the thalamus, regardless whether EPSCs recorded in those neurons have fast or slow response waveforms. It also tentatively suggests that it is the general lack of GluR1 and 2 subunits, which accounts for the low effectiveness in the thalamus, rather than local variations in densities of GluR3 and 4.

Effects of CX516 and Cyclothiazide on Excitatory Synaptic Transmission in the RTN and Hippocampus. To test whether AMPA receptor modulators in general have weaker effects in RTN neurons, two other drugs were examined that were previously found to be effective in the hippocampus. Cyclothiazide potentiates both amplitude and duration in hippocampal whole-cell recordings (Rammes et al., 1994; Lin et al., 2002; Arai et al., 2004), and CX516 (BDP-12)
showed a preference for enhancing response amplitude more than duration, a preference that is opposite to that of CX546 (Arai et al., 1996b, 2002, 2004). In RTN neurons, the onset of drug action and the time to achieve plateau effects were generally the same as in hippocampal neurons. As shown in Fig. 4, cyclothiazide was highly effective in enhancing both amplitude and half-width of responses in the RTN, and the magnitude of its effects were similar to those in the hippocampus. This rules out the possibility that drug access to synapses on RTN neurons is generally impaired. Results with 1 mM CX516 differed somewhat for amplitude and half-width measures. The amplitude increase in the RTN was sizable (116 ± 16%; n = 7), although it tended to be smaller than in the hippocampus. On the other hand, the drug had virtually no effect on half-width in the RTN but prolonged responses significantly in the hippocampus (by 111 ± 38%). These results suggest that benzamide-type drugs (CX546 and CX516) may have generally weaker effects on response duration at RTN synapses.

### Discussion

This study has shown that the sensitivity of synaptic AMPA receptors to benzamide-type modulators such as CX546 can differ substantially across brain regions. The drug greatly increased the duration of AMPA receptor-mediated currents at hippocampal synapses but had comparatively weak effects at synapses in the reticular and ventrobasal nucleus of the thalamus. An important question is whether these differences are due to variations in drug potency (EC₅₀) or in the extent to which the drug changes receptor kinetics, i.e., in its efficacy. Fitting of EPSC waveforms with biexponential decay functions showed that the increase in response width produced by the drug correlates to some extent with the increase in the fractional contribution of the slow response component. In control responses, the slow decay component accounted for only about 20% of total response amplitude in both brain regions. Upon infusion of 400 μM CX546, this component became dominant in hippocampal synapses but remained minor in reticular and thalamic relay cells. If one takes the slow component as an approximate indicator for the percentage of receptors that have bound the drug at a given concentration, then about two-thirds of the hippocampal receptors but only one-third of the thalamic receptors were occupied by CX546 at 400 μM. This would suggest that the EC₅₀ in the hippocampus is lower than 400 μM, whereas that of RTN receptors would have to be in the millimolar range. The main reason for the regional differences in drug effects thus probably was an at least 3-fold higher potency of CX546 at hippocampal synapses. This is also supported by the observation that amplitude increases in the RTN at 800 μM drug were comparable with those at 200 μM in the hippocampus. We did not attempt to construct more extended saturation curves since higher drug concentrations often evoked increased noise, presumably by acting on nearby unclamped neurons. Nonetheless, the above-potency estimates agree in widest terms with previous reports that found EC₅₀ values between 200 μM and 2 mM, depending on preparation and test conditions (Baumbarger et al., 2001; Arai et al., 2002). It should be noted, however, that the magnitude of the drug effect could not be examined at truly saturating drug concentrations and hence the possibility of additional differences in drug efficacy cannot be ruled out.

A potentially confounding factor has been that basal AMPA receptor-mediated responses differ in their time

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**Fig. 4.** Effects of cyclothiazide and CX516 on synaptic transmission in RTN versus hippocampus. EPSCs were recorded from RTN neurons and pyramidal cells in area CA1, as in Fig. 1. Typical examples of traces recorded before and in the presence of 100 μM cyclothiazide (top) or 1 mM CX516 (bottom) are shown on the left. Note that both compounds have substantially larger effects on the peak amplitude compared with CX546. The bar graphs show summary data for the changes in amplitude and half-width over the predrug response; the data are the means and S.E.M. of seven experiments for each drug and brain region. *p < 0.05.
course between RTN and hippocampus, with rise and decay times being 2 to 3 times slower in the latter. Importantly, however, miniature events are similarly fast in both structures. This suggests that the kinetic properties of their AMPA receptors are not very different and that EPSC waveforms in the hippocampus are instead more strongly influenced by factors such as asynchrony in transmitter release (Diamond and Jahr, 1995), ineffective removal of glutamate (Lawrence et al., 2003), and dendritic filtering. Our data indicate, however, that the higher effectiveness of CX546 in the hippocampus is not the result of such nonsynaptic factors. Spontaneous miniature EPSCs in the hippocampus exhibited a 3-fold widening in the presence of the drug, which is comparable in magnitude with the effect on evoked EPSCs, whereas miniature EPSCs in the RTN again exhibited only modest changes. Since mEPSCs represent single release events and mostly originate from synapses close to the cell body, one can conclude that the drug-induced large changes in the waveforms of hippocampal responses are not artifactually caused by poor space clamp efficiency or by asynchronous transmitter release and instead reflect genuine receptor properties. These conclusions are further supported by the observation that the changes produced by CX546 in ventralbasal thalamic neurons were weak like in RTN neurons, in spite of the fact that their EPSCs had a slow time course like hippocampal neurons.

If the observed differences in drug effects reflect true receptor properties, then they presumably result from variations in the subunit composition of the AMPA receptor or from secondary receptor modifications. It may be significant in this context that neurons throughout the thalamus contain mainly GluR3 and 4 subunits (Spreafico et al., 1994), whereas receptors in hippocampal pyramidal cells contain GluR1–3 with GluR2 being dominant. Leever et al. (2003) indeed observed that CX546 had a preference for GluR2 over GluR3 and 4 in oocyte recordings, which would in broadest terms agree with our observations that drug effectiveness was lower in both RTN and ventrobasal nucleus. However, regional differences in splice variants may also have contributed since benzamides often exhibit a mild preference for flop variants (Johansen et al., 1995; Partin et al., 1996; Arai et al., 2000), and flop variants are prominent in area CA1 (Sommer et al., 1990) even at a relatively early age (Arai and Lynch, 1996). Unfortunately, data about splice variant prevalence in the thalamus and elsewhere are still sparse. The original in situ hybridization study by Monyer et al. (1991) on flip-flop variant distributions in the brain indicated a particularly strong GluR3 flip signal in the RTN region of postnatal day 8 animals, but beyond this it does not allow for quantitative comparisons with other subunits and splice variants. Nonetheless, it may be instructive that CX546 exhibited a 3-fold lower potency in modulating [3H]AMPA binding to GluR3 flip receptors than to GluR3 flop and GluR2 receptors (Table 3; Arai et al., 2000). Thus, the lower susceptibility of the thalamus for CX546 perhaps resulted from a combination of having more GluR3 than GluR2 subunits and being enriched in the flip splice variant of this subunit.

Drug potencies could also be influenced in ways that are not readily apparent from tests with recombinantly expressed homomeric receptors, for example, due to secondary receptor modifications such as phosphorylation or specific receptor interactions with local synaptic proteins. For example, the negative AMPA receptor modulator GYKI 53655 exhibited up to 10-fold lower potencies for modulating recombinantly expressed subunits than for native receptors (Partin and Mayer, 1996), and the latter showed positive modulatory effects in addition to the negative modulatory action typically seen with recombinant receptors (Arai, 2001). No disparities of such magnitude have been reported for positive AMPA receptor modulators, but there is evidence for general differences in kinetic properties between recombinant and native AMPA receptors, for example, in their binding affinities (Suzuki et al., 2003), and thus modulatory drug effects on native synaptic receptors could also differ in ways that cannot be predicted from subunit characteristics alone.

CX516 consistently enhanced response amplitude more than duration, as in previous reports (Arai et al., 1996a, 2002). At the concentration used here, the amplitude increase in RTN neurons was smaller than in the hippocampus, but the difference was modest. However, effects on response half-width differed greatly with essentially no change in RTN neurons versus a doubling in the hippocampus. This can again be potentially explained by a lower potency of this drug at thalamic synapses. CX516 in hippocampal field recordings had a lower EC50 for enhancing amplitude (180 μM; Arai et al., 1996b) than for enhancing half-width (>1 mM). Thus, the concentration of 1 mM may have been sufficiently high to cause a substantial increase in the amplitude of RTN responses, even with reduced potency, but it may have been too low to significantly enhance response duration if the EC50 for response duration was increased accordingly. Cyclothiazide produced comparable increases in amplitude and half-width in both brain regions. It is possible, however, that the concentration of cyclothiazide used here was closer to saturation and thus regional differences in potency may have been less evident (also see Arai and Lynch, 1996).

Because of the growing interest in their therapeutic potential, many AMPA receptor modulators have been screened for subunit preferences with the expectation that such preferences could be used to direct drug action to particular brain regions. The general observation has been that most modulators exhibit only modest discrimination, with subunit affinities usually differing by factors far smaller than 10. Nonetheless, the present study has documented that the effects of such drugs on synaptic transmission can differ severalfold.

### Table 3

<table>
<thead>
<tr>
<th>Subunit</th>
<th>EC50 (mM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>GluR2 flop</td>
<td>0.44 ± 0.03</td>
<td>7</td>
</tr>
<tr>
<td>GluR2 flip</td>
<td>0.35 ± 0.01</td>
<td>2</td>
</tr>
<tr>
<td>GluR3 flop</td>
<td>0.42 ± 0.23</td>
<td>2</td>
</tr>
<tr>
<td>GluR3 flip</td>
<td>1.37 ± 0.13***</td>
<td>4</td>
</tr>
</tbody>
</table>

**Notes:**

- ***p < 0.001 for all pairwise comparisons according to analysis of variance with Tukey’s post test.

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between brain regions, and this may be sufficiently distinct to be of importance when considering therapeutic applications. Our results would tentatively suggest, then, that AMPakine modulators such as CX546 are more suitable to enhance hippocampal functions, which include memory encoding, than to modulate thalamic functions, which include wakefulness and vigilance. We can at this point not rule out, however, that changes in AMPA receptor subunit expression later in development, such as the progressive expression of GluR4 subunit in the thalamus (Spaepen et al., 1994), or potential changes in the balance between flip and flop variants, would alter again the effectiveness of these drugs and perhaps reduce the degree of discrimination between hippocampus and thalamus. Such predictions will not be feasible unless it is clarified whether the regional selectivities reported here indeed result from differences in subunit expression patterns or from secondary receptor modifications. In addition, more precise information is needed about the molecular makeup of AMPA receptors across brain regions and developmental stages, both in animals andhumans.

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References


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