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Effects of the Novel NMDA Receptor Antagonist, CGP 39551, on Field Potentials and the Induction and Expression of LTP in the Dentate Gyrus In Vivo

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ABSTRACT The effects of the novel competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, CGP 39551 [the carboxyethylester of CGP 37849; DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid], on extracellular field potentials and long-term potentiation (LTP) induced in the dentate gyrus by stimulation of the perforant path were studied in anesthetized rats. CGP 39551 attenuated the population spike (PS) and excitatory postsynaptic potential (EPSP) amplitude of dentate field potentials, reduced the NMDA receptor-mediated component of train-evoked burst potentials, and prevented the induction of LTP. The decrease in PS and EPSP amplitude produced by CGP 39551 was observed mainly in non-potentiated synaptic populations; potentiated field potentials were only minimally affected by drug treatment. These results are consistent with the in vivo blockade of NMDA receptors by CGP 39551. They also indicate that NMDA receptors may contribute in a tonic manner to the state of dentate granule cell excitability. Finally, the differential modulation of potentiated and non-potentiated synapses by CGP 39551 suggests that a change in some properties of postsynaptic AMPA receptors is involved in the expression of LTP.

INTRODUCTION

Long-term potentiation (LTP) is a form of synaptic enhancement produced by high-frequency activation of excitatory afferent pathways in the mammalian brain. The initial steps required to induce LTP in the hippocampus (at least in area CA_1 and the dentate gyrus) include the activation of *N*-methyl-D-aspartate (NMDA) receptors (a subclass of glutamate receptors) under conditions of strong postsynaptic depolarization, a resultant influx of calcium through opened NMDA receptor-gated channels, and the activation of calcium-dependent enzymes [see Massicotte and Baudry (1991) for a recent review]. Although the mechanisms involved in LTP induction are now well-understood, the mechanisms responsible for LTP expression remain controversial (Baudry and Davis, 1991).

Assuming that LTP results from strengthening existing synaptic contacts (rather than the formation of new synapses or unveiling of quiescent ones), three mechanisms could account for the increase in synaptic efficacy (Lynch and Baudry, 1991): (1) an increase in presynaptic transmitter release, (2) a decrease in postsynaptic spine resistance (resulting in an effectively greater synaptic conductance), or (3) a change in the properties of postsynaptic receptors. Of these mechanisms, disparate results from quantal analyses [cf. Malinow and Tsien (1990) and Foster and McNaughton (1991)] and unsuccessful attempts to validate the spine-resistance hypothesis (Jung et al., 1991; Larson and Lynch, 1991) have left changes in the properties of postsynaptic receptors as the most plausible substrate for LTP expression. Accordingly, a considerable body of literature indicates that enhanced synaptic efficacy following LTP is associated with a postsynaptic change in some properties of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subclass of glutamate receptors, in the absence of NMDA receptor modification (Kauer et al., 1988; Muller and Lynch, 1988; Muller et al., 1988; Staubli et al., 1990; Tocco et al., 1991, 1992).

Recently, Staubli et al. (1990) tested whether or not aniracetam, a nootropic compound that selectively increases the conductance of AMPA receptors, differentially affected potentiated and non-potentiated field po-

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; APV, D-2-amino-5-phosphonovaleric acid; CGP 37849; DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid; CPP, 3-[(±)-2-carboxypiperazin-4-yl-]-propyl-1-phosphonic acid; NMDA, N-methyl-D-aspartate; MK-801, [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate].

tentials in area CA_1 in vitro. In theory, aniracetam was expected to have relatively smaller effects on synapses expressing LTP if both LTP and aniracetam modified the same synaptic variable [e.g., AMPA receptors; see Staubli et al. (1990) for detailed arguments]. As expected, aniracetam had significantly smaller effects on potentiated synapses relative to non-potentiated synapses. Although these results provide strong support for a change in AMPA receptors following LTP induction, they do not exclude the possibility that LTP is also accompanied by a change in NMDA receptors, as suggested by Bashir et al. (1991).

Using a strategy similar to that of Staubli et al. (1990), the present study was designed to investigate the effects of NMDA receptor blockade in potentiated and non-potentiated synaptic populations in vivo. CGP 39551 (the carboxyethylester of CGP 37849; DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid) is a potent and selective competitive antagonist of the NMDA subclass of glutamate receptor (Fagg et al., 1990). It has no effect on responses to iontophoretically applied quisqualate or kainate in hippocampal neurons, the uptake of L-[^{3}H]-glutamate, or the release of endogenous glutamate from rat hippocampal slices (Fagg et al., 1990). Unique among competitive NMDA receptor antagonists, CGP 39551 and CGP 37849 exhibit significant central effects following peripheral administration. The ability of CGP 39551 to cross the blood-brain barrier allows one to examine the effects of competitive NMDA receptor blockade in vivo. In the present study, we first verified NMDA receptor blockade by CGP 39551 in the perforant path-dentate granule cell system of anesthetized rats. Secondly, we determined the impact of NMDA receptor blockade on potentiated and non-potentiated perforant path synaptic transmission in vivo.

MATERIALS AND METHODS

Subjects and surgery

Twenty-three male Long-Evans rats (250–300 g; Simonsen Labs) were pair-housed and maintained on ad libitum water and rat chow on a 12-hour light/dark cycle.

Prior to electrophysiological testing, rats were anesthetized with an intraperitoneal (i.p.) injection (60 mg kg^{-1}) of sodium pentobarbital (65 mg ml^{-1}) and mounted in a Kopf stereotaxic frame; the head position was adjusted to place bregma and lambda in the same horizontal plane. After retraction of the scalp, burr holes of approximately 2 mm diameter were drilled bilaterally in the skull for the placement of stimulating and recording electrodes. The recording electrodes were implanted in the hilus of the dentate gyrus (3.3 mm posterior, 2.4 mm lateral, and 2.8–3.0 mm ventral to bregma) and the bipolar stimulating electrodes in the medial perforant pathway (8.1 mm posterior, 4.4 mm lateral, and 2.5–4.0 mm ventral to bregma). The electrodes consisted of Epoxylite-coated stainless-steel pins with the recording and stimulating surfaces formed by removing the insulation at the tips (tip lengths = 50 and 500 μm for the recording and stimulating electrodes, respectively). The ventral locations of both the recording and stimulating electrodes were adjusted to maximize the amplitude of the perforant path-evoked hilar responses. After placement, the electrodes were affixed to the skull with dental acrylic. Body temperature was kept at approximately 37°C with a heating pad; the subjects were not artificially respirated. Surgical anesthesia was maintained with booster injections of pentobarbital as was necessary.

Electrophysiology

Electrophysiological testing (100 μsec pulses; voltage adjusted to elicit an approximately 2 mV population spike [PS]) began after stable dentate hilar field potentials had been maintained for at least 30 min. Field potentials from the left and right hippocampi were sampled independently every 30 sec with stimulation of the right perforant path 15 sec after the left. The responses were amplified (gain = 100), band-pass filtered (1 Hz–10 kHz), digitized at 10 kHz, and stored on disk (AST Premium 386c computer/BrainWave Systems, Inc., Boulder, CO).

Evoked dentate hilar responses were recorded during a 30 min interval before and a 150 min interval following i.p. injection. Animals received either 0, 10, or 30 mg kg^{-1} CGP 39551 ($n=6$ in all groups; CGP 39551 was a generous gift from Cesare Mondadori, CIBA-GEIGY, Ltd., Basel, Switzerland). High-frequency theta burst stimulation (TBS; ten 40 msec 400 Hz bursts at 5 Hz; voltage adjusted to elicit a 2 mV PS) was delivered to the left and right perforant pathways 30 and 120 min post-injection, respectively. This stimulation protocol permitted a within animal assessment of the time course of NMDA receptor blockade.

Data analysis

The 180 min recording period was divided into six 30 min periods. An averaged field potential was computed for each hemisphere and period. Three measures, the population spike (PS) amplitude, excitatory postsynaptic potential (EPSP) slope and EPSP amplitude, were computed from the averaged field potentials. To examine the effect of CGP 39551 on single perforant path-evoked responses, the mean electrophysiological data for five 30 min intervals (one pre-injection and four post-injection intervals) from the right (non-potentiated) hemisphere were submitted to a repeated measures ANOVA with time (five levels: −30–0, 0–30, 30–60, 60–90, and 90–120 min intervals) as a within-subjects factor; independent ANOVAs were performed on each treatment group. To determine the effects of CGP 39551 treatment on LTP induction, the mean percent changes in PS amplitude and EPSP slope (calculated as the relative change from the 30 min pre-teta-

TABLE I. Effects of CGP 39551 on perforant path-evoked field potentials[1]

Group	n	Interval (min)				
		−30	0	30	60	90
0 mg kg^{-1}	6 (PS)	2.26 ± 0.32	2.57 ± 0.40	3.32 ± 0.80	3.42 ± 0.81	3.73 ± 1.12
	6 (ES)	5.24 ± 0.30	5.16 ± 0.40	5.26 ± 0.50	5.09 ± 0.52	5.23 ± 0.68
	6 (EA)	10.75 ± 1.20	10.72 ± 1.33	10.95 ± 1.62	10.65 ± 1.60	10.73 ± 1.81
10 mg kg^{-1}	6	2.53 ± 0.12	3.01 ± 0.21	2.70 ± 0.39	3.41 ± 0.64	2.90 ± 0.54
	6	5.54 ± 0.77	5.52 ± 0.72	5.43 ± 0.79	5.64 ± 0.77	5.50 ± 0.76
	6	11.51 ± 0.64	12.04 ± 0.67	11.91 ± 0.80	12.12 ± 0.67	11.69 ± 0.84
30 mg kg^{-1}	6	3.03 ± 0.47	3.72 ± 0.53	4.34 ± 0.84	4.18 ± 0.79	2.23 ± 0.78**
	6	4.55 ± 0.28	4.56 ± 0.28	4.71 ± 0.33	4.82 ± 0.41	4.77 ± 0.52
	6	10.39 ± 0.46	10.51 ± 0.55	10.69 ± 0.66	10.81 ± 0.71	9.72 ± 0.86*

[1]Values represent the mean (±S.E.M.) PS amplitude (PS; mV), EPSP slope (ES; mV/ms), and EPSP amplitude (EA; mV) for five 30 min recording intervals (labels indicate the times at the beginning of the intervals). The first interval (−30 min) represents the pre-injection baseline. **$P < .01$ and *$P < .02$ indicate significant differences to the preceding 30 min interval.

nus interval to the following 30 min post-tetanus interval) for the left (30 min post-injection TBS) and right (120 min post-injection TBS) hemispheres were submitted to an ANOVA with the between-subjects factor of group (three levels: 0, 10, and 30 mg kg^{-1} CGP 39551). Lastly, Student's *t*-tests (independent groups) were used to evaluate the hypothesized differential effects of CGP 39551 on potentiated and non-potentiated responses. For these tests, the percent change in the electrophysiological measures was calculated between two 30 min intervals (60–90 min and 90–120 min) following drug administration. These intervals were chosen based on the effects of the drug on single evoked responses (see Results). All values are presented as the mean ± the standard error of the mean (S.E.M.).

RESULTS

Effects of CGP 39551 on perforant path-evoked field potentials

Averaged field potentials for representative subjects in each treatment group are displayed in Figure 1; the corresponding group means are shown in Table I. CGP 39551 did not have significant effects on either the EPSP slope (10 mg kg^{-1}, $F_{4,20} = 0.59$, $P = .68$; 30 mg kg^{-1}, $F_{4,20} = 0.47$, $P = .76$) or EPSP amplitude (10 mg kg^{-1}, $F_{4,20} = 0.87$, $P = .50$; 30 mg kg^{-1}, $F_{4,20} = 2.32$, $P = .09$) of single evoked field potentials recorded in the right (non-potentiated) hemisphere up to 120 min following administration as indicated by nonsignificant main effects of time in the ANOVAs. Similarly, the field EPSPs recorded from control (0 mg kg^{-1}) subjects were stable over the recording period (slope, $F_{4,20} = 0.16$, $P = .96$; amplitude, $F_{4,20} = 0.15$, $P = .96$). In contrast, the PS amplitude of perforant path-evoked potentials recorded from subjects receiving 30 mg kg^{-1} CGP 39551 was sensitive to drug treatment ($F_{4,20} = 8.31$, $P < .001$). Planned comparisons revealed that the mean PS amplitude recorded in these subjects was markedly attenuated ($P < .008$) 90–120 min post-injection relative to the preceding post-injection intervals. The PS amplitude 90–120 min post-injection was attenuated approximately 50% relative to the preceding 30 min interval and approximately 30% relative to the pre-injection baseline (although the latter decrease was not significant). Animals treated with 10 mg kg^{-1} CGP 39551 did not exhibit an attenuation of PS amplitude ($F_{4,20} = 1.37$, $P < .28$) and responses recorded from control (0 mg kg^{-1}) subjects were similarly stable over the recording period ($F_{4,20}$ 2.71=, $P < .06$). The strong attenuation of PS amplitude in subjects treated with 30 mg kg^{-1} CGP 39551 led us to perform planned comparisons on the EPSP means of the same subjects (Wilcox, 1987). In accordance with the PS data, these comparisons indicated that the EPSP amplitude, but not the EPSP slope, was significantly attenuated ($P < .02$) 90–120 min following drug administration relative to the preceding 30 min interval, but not relative to the pre-injection baseline. The EPSP amplitude 90–120 min post-injection was attenuated by approximately 10% relative to the preceding 30 min interval. The failure to identify significant changes in either the PS or EPSP amplitude relative to the pre-injection baseline was most likely due to the nonsignificant increase in both measures over the course of the recording period observed in all animals.

Effects of CGP 39551 on the induction of long-term potentiation

To assess the time course of CGP 39551-mediated NMDA receptor blockade with respect to LTP induction, high-frequency TBS was delivered either 30 (left hemisphere) or 120 (right hemisphere) min following drug administration. Thirty minutes following CGP 39551 treatment, TBS induced comparable LTP in all of the subjects (Figs. 1, 2). This was indicated by a nonsignificant main effect of Group in both the EPSP slope ($F_{2,15} = 0.85$, $P = .45$) and PS amplitude ($F_{2,15} = 0.71$, $P = .51$) ANOVAs. The mean increase in EPSP slope (30 min following TBS) in the three treatment groups was 0 mg kg^{-1}, 50.86 ± 2.97%; 10 mg kg^{-1}, 48.61 ± 7.20%; 30 mg kg^{-1}, 40.13 ± 7.26%; and the mean PS LTP was 0 mg kg^{-1}, 612.87 ± 94.35%; 10 mg kg^{-1}, 443.46 ± 160.30%; 30 mg kg^{-1}, 429.24 ± 97.64%.

One hundred twenty minutes following administration, however, CGP 39551 significantly suppressed in-

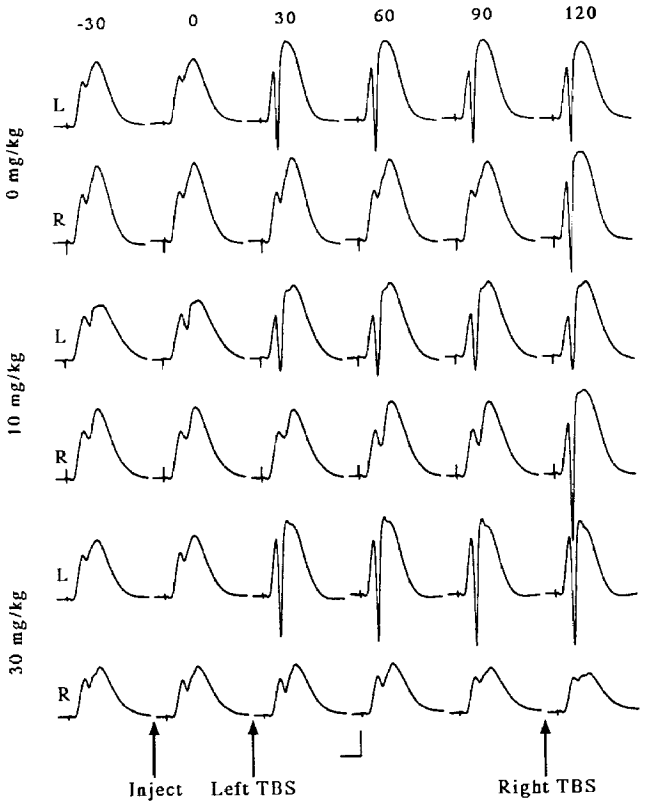

Fig. 1. Averaged perforant path-evoked dentate hilar field potentials recorded from representative subjects in each of the treatment groups (0, 10, and 30 mg kg^{-1}). Bilateral perforant path stimulation and hilar recording permitted data collection from both the left (L) and right (R) hemispheres. Each trace represents an average of 60 field potentials collected over a 30 min interval (the beginning of which, relative to drug injection, is indicated in the top portion of the graph). In all subjects, the left hemisphere received theta burst stimulation (TBS) 30 min post-injection, whereas the right hemisphere received TBS 120 min post-injection. Note that TBS delivered 30 min post-injection produced robust LTP in all of the subjects (left hemisphere averages). In contrast, 30 mg kg^{-1} CGP 39551 prevented LTP induction when the TBS was delivered 120 min post-injection (right hemisphere averages). Note also the attenuation of EPSP and PS amplitude in the non-potentiated field potential (right side), but not the potentiated field potential (left side), between the 60 and 90 min intervals in the subject treated with 30 mg kg^{-1} CGP 39551. Calibrations: 5.15 msec, 4.25 mV.

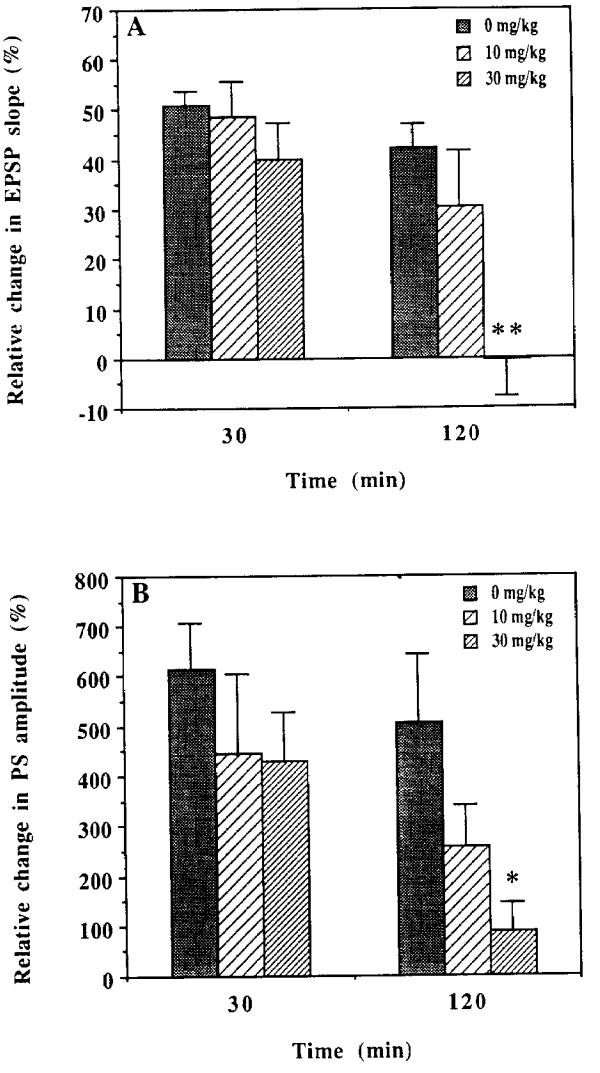

Fig. 2. Mean EPSP slope (A) and PS amplitude (B) LTP in each of the treatment groups (0, 10, and 30 mg kg^{-1}; $n = 6$ in each group). TBS was delivered either 30 or 120 minutes following drug administration. Double asterisks indicate that EPSP LTP in the 30 mg kg^{-1} group was significantly attenuated ($P < .05$) relative to both the control (0 mg kg^{-1}) and 10 mg kg^{-1} groups. The single asterisk indicates that PS LTP in the 30 mg kg^{-1} group was significantly attenuated ($P < .05$) relative to only the control group. No significant drug effects were apparent with delivery of TBS 30 min post-injection.

duction of both EPSP slope ($F_{2,15} = 6.95, P < .008$) and PS amplitude ($F_{2,15} = 4.63, P < .03$) LTP Fig. 1, 2). The mean EPSP slope LTP for the treatment groups was 0 mg kg^{-1}, 42.24 ± 4.68%; 10 mg kg^{-1}, 30.36 ± 11.39%; 30 mg kg^{-1}, −0.17 ± 7.41%. The mean PS LTP was 0 mg kg^{-1}, 506.06 ± 138.22%; 10 mg kg^{-1}, 257.55 ± 80.74%; 30 mg kg^{-1}, 85.33 ± 58.14%. On both the EPSP slope and PS amplitude measures, post hoc comparisons (Newman-Keuls; $P < .05$) revealed that subjects receiving 30 mg kg^{-1} CGP 39551, but not those receiving 10 mg kg^{-1}, significantly differed from control (no drug) animals, confirming the dose-dependent nature of the NMDA receptor blockade observed on single perforant path-evoked field potentials.

Effects of CGP 39551 on train-evoked responses

Waveforms representing the difference between the first and second train-evoked burst potentials (i.e., the second burst potential minus the first burst potential) evoked during TBS are displayed in Figure 3. CGP 39551 (30 mg kg^{-1}) noticeably reduced the amplitude of train-evoked burst potentials recorded 120 min following drug administration. Specifically, the drug eliminated the late depolarization (onset latency ≈ 10 msec) associated with NMDA receptor activation (Larson and Lynch, 1986, 1988; Mott and Lewis, 1991). Train-evoked burst potentials were not attenuated 30 min

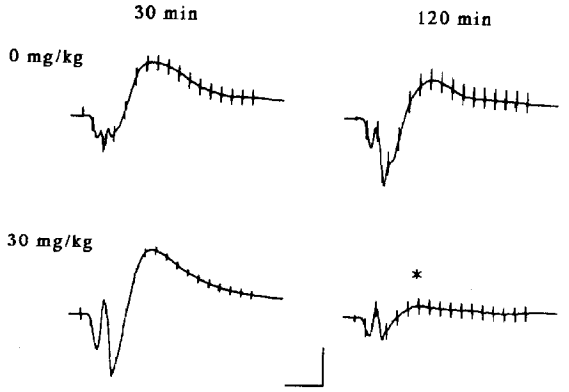

Fig. 3. Waveforms representing TBS-evoked burst potential subtractions from representative subjects in the control (0 mg kg^{-1}) and 30 mg kg^{-1} CGP 39551 groups. Each trace represents the second train-evoked burst potential (evoked during TBS) minus the first burst potential. The large depolarization (onset latency ≈ 10 msec) reflects NMDA receptor activation. The asterisk indicates that 30 mg kg^{-1} CGP 39551 completely eliminated the NMDA receptor activation associated with TBS 120 min following drug administration. Calibrations: 9.15 msec, 4.00 mV.

following CGP 39551 administration (Fig. 3), and were not attenuated in subjects treated with 10 mg kg^{-1} CGP 39551 (data not shown).

Effects of CGP 39551 on potentiated and non-potentiated perforant path synaptic transmission

The preceding analyses revealed that CGP 39551 required approximately 120 min following i.p. administration to achieve significant NMDA receptor blockade. We therefore compared the effects of CGP 39551 on potentiated synapses and non-potentiated synapses 120 min following drug injection. For these comparisons, the percent change in the electrophysiological variables was calculated between two 30 min intervals beginning 60 and 90 min following drug injection (or 30 and 60 min following LTP induction in the left hemisphere), respectively. Student's *t*-tests (independent groups) revealed that the EPSP amplitude and PS amplitude (but not the EPSP slope) of non-potentiated responses were significantly attenuated following 30 mg kg^{-1} CGP 39551 relative to control animals (Table II; EPSP amplitude, $t_{10} = 2.53, P < .05$; PS amplitude, $t_{10} = 4.03, P < .005$). The mean reductions (± S.E.M.) in EPSP and PS amplitude amounted to 10.56 (± 3.34)% and 50.59 (± 9.02)%, respectively. Surprisingly, CGP 39551 did not significantly affect electrophysiological responses following LTP induction. That is, the reductions in EPSP and PS amplitude associated with potentiated responses in subjects treated with CGP 39551 were not significantly different from those in control (no drug) subjects. Thus, the slight reductions in EPSP and PS amplitude observed in subjects treated with 30 mg kg^{-1} CGP 39551 were most likely due to the decay of LTP, rather than a drug effect per se.

To examine this effect more thoroughly, five additional subjects were run as previously except that input/output (I/O) functions were generated 60 and 120 min following CGP 39551 (30 mg kg^{-1}) administration. As before, the left perforant path received TBS 30 min following drug administration. The I/O functions for both the left (potentiated) and right (non-potentiated) hemispheres were constructed from 10 different stimulation intensities (3 sweeps per intensity) adjusted for each animal to generate a range of field potentials. In general, the lowest stimulation intensity was subthreshold for PS generation, whereas the highest intensity produced a field potential with an asymptotic PS. The I/O data were averaged across subjects and plotted (top half of Fig. 4). As was expected, CGP 39551 differentially affected potentiated and non-potentiated dentate field potentials across a range of stimulation intensities (PS: $F_{3,96} = 4.76, P < .001$; EPSP: $F_{3,96} = 2.22, P < .05$). Linear regression equations describing the data were calculated and used to estimate the percent change in both EPSP and PS amplitude across a range of initial (x) amplitudes (i.e., amplitudes 60 min post-injection). Plots of these functions are displayed in the bottom half of Figure 4. These "theoretical" graphs allowed us to compare the effects of CGP 39551 on the EPSP and PS amplitude of potentiated and non-potentiated responses for any initial response magnitude. We first estimated the percent decrease in EPSP and PS amplitude for potentiated and non-potentiated responses given the mean initial PS and EPSP amplitudes obtained from the six original subjects treated with 30 mg kg^{-1} CGP 39551. For both the EPSP and PS measures, the estimated values we obtained were remarkably similar to the actual values reported in Table II. However, the "theoretical" graphs indicate that the differential effect of CGP 39551 on the PS amplitude of potentiated and non-potentiated response reported in Table II was slightly overestimated. That is, a non-potentiated response with an initial PS amplitude comparable to that of a potentiated response (e.g., 12.5 mV) would be expected to decrease by approximately 25%. On the other hand, differential effects of CGP 39551 on the EPSP amplitude of potentiated and non-potentiated responses may have been underestimated in the original subjects because the mean initial EPSP amplitudes in these animals fell in a region of the graph where differences between potentiated and non-potentiated responses would not be expected to be maximized. In both cases, the graphs indicate that the differential effects of CGP 39551 on potentiated and non-potentiated responses of comparable initial magnitude would be expected to be more apparent at high rather than low stimulation intensities, an effect consistent with increased NMDA receptor activation at higher stimulation intensities.

Clearly, the contribution of NMDA receptors to the population synaptic potential is *relatively* smaller in

TABLE II. Effects of CGP 39551 on potentiated and non-potentiated synapses[1]

Group	n	EPSP slope		PS amplitude		EPSP amplitude	
		Potentiated	Non-potentiated	Potentiated	Non-potentiated	Potentiated	Non-potentiated
0 mg kg^{-1}	6	−5.50 ± 3.31	1.77 ± 4.09	−7.63 ± 3.21	−0.84 ± 8.45	−2.82 ± 2.51	−0.10 ± 2.43
10 mg kg^{-1}	6	−1.86 ± 2.65	−2.67 ± 1.62	−10.05 ± 3.44	−13.25 ± 7.14	−1.77 ± 1.00	−3.89 ± 3.15
30 mg kg^{-1}	6	−4.08 ± 2.32	−2.23 ± 2.85	−15.61 ± 6.58	−50.59 ± 9.02**	−5.75 ± 0.80	−10.56 ± 3.34*

[1] Values represent the percent change (mean ± S.E.M.) between two 30 min intervals (60–90 and 90–120 min) following CGP (or vehicle) injection; the potentiated responses received high-frequency stimulation 30 min following CGP 39551 treatment; the non-potentiated responses did not receive high-frequency stimulation. $^{**}P < .005$ and $^{*}P < .05$ indicate significant differences from control (0 mg kg^{-1}) values.

synapses expressing LTP as compared to non-potentiated synapses. An important question that must be considered is whether or not this phenomenon is due to a modification of NMDA receptors (or the NMDA receptor-mediated conductance) per se, or to some other synaptic mechanism that leaves the properties of NMDA receptors intact. We have addressed this question using the approach described above. Assuming an initial EPSP amplitude of 11 mV for both the left and right responses prior to both LTP induction and drug administration, and a 24% increase in EPSP amplitude following the induction of LTP (these values correspond to those typically observed in the present study), the regression equations predict that the EPSP amplitude of the potentiated response would decrease by 1.03 mV and that of the non-potentiated response by 1.15 mV following CGP 39551 treatment. Hence these calculations indicate that the *absolute* contribution of NMDA receptors to the population synaptic potential would be expected to remain the same before and after LTP induction.

DISCUSSION

The present experiments demonstrate that CGP 39551 antagonizes NMDA receptors in a dose- and time-dependent fashion following intraperitoneal administration in vivo. The dose required to elicit total NMDA receptor blockade was 30 mg kg^{-1}, and the latency to onset of blockade was from 90 to 120 min following intraperitoneal administration; 10 mg kg^{-1} did not produce significant blockade of NMDA receptors, and neither dose of CGP 39551 had noticeable effects 30 min following administration.

One consequence of NMDA receptor blockade by CGP 39551 that we observed in the present study was an attenuation of EPSP and PS amplitude (but not EPSP slope) in perforant path-evoked dentate field potentials. The latency to onset of PS and EPSP attenuation was approximately 90 min following drug injection, and consisted of an approximately 50% reduction in the PS amplitude and a 10% reduction in EPSP amplitude. The latency to onset of NMDA receptor blockade reported here agrees with the values published by Fagg et al. (1990) and Pozza et al. (1990), who found that 30 mg kg^{-1}CGP 39551 (delivered via an esophageal cannula) antagonized responses in hippocampal neurons to iontophoretically applied NMDA with a latency of 80–90 min. Similarly, the magnitude of PS attenuation observed in the present experiments is remarkably similar to the 30% PS attenuation reported by Sah et al. (1989) following APV application in area CA_1 in vitro. It is noteworthy that the reduction in PS amplitude produced by NMDA receptor blockade is not uncommon; both competitive (e.g., APV and CPP) and noncompetitive (e.g., ketamine and MK-801) NMDA antagonists have been reported to attenuate the population spike in hippocampal regions in vivo and in vitro (Abraham and Mason, 1988; Errington et al., 1987; Maren et al., 1991; Pozza et al., 1990; Sah et al., 1989; Stringer and Guyenet, 1983). Sah et al. (1989) proposed that this effect was due to a reduction of tonic NMDA receptor activation resulting in reduced cell excitability and, consequently, a reduced population spike. The differential effects of CGP 39551 on the slope of the rising phase of the field EPSP (measured approximately 2–5 msec following the onset of the response) and EPSP amplitude (latency ≈ 10 msec) is most likely related to the relatively long rise time of the NMDA receptor-mediated current (8–20 msec; Hestrin et al., 1990a, b). The protracted rise time of the NMDA receptor-mediated current indicates that NMDA receptor activation would be expected to be minimal during the rising phase of the field EPSP and near maximal during the peak amplitude of the field EPSP. Accordingly, Muller and Lynch (1988) have reported that APV reduces the amplitude, but not the slope, of field EPSPs recorded in low magnesium in area CA_1 in vitro.

In addition to its effects on single evoked perforant path field potentials, CGP 39551 (30 mg kg^{-1}, 120 min post-injection) had significant effects on train-evoked burst potentials. In particular, CGP 39551 significantly attenuated train-evoked burst potentials, completely eliminating the late depolarization which has been shown to involve NMDA receptor activation (Larson and Lynch, 1986; 1988; Mott and Lewis, 1991). Because the induction of LTP was prevented when there was also a concomitant suppression of train-evoked burst potentials, the blockade of the NMDA receptor-dependent component of the burst potential by CGP 39551 is likely to be responsible for the blockade of LTP observed in the present experiments. In agreement with the effects of CGP 39551 on single evoked responses, the prevention of LTP induction and the elimination of the NMDA receptor-mediated component of train-

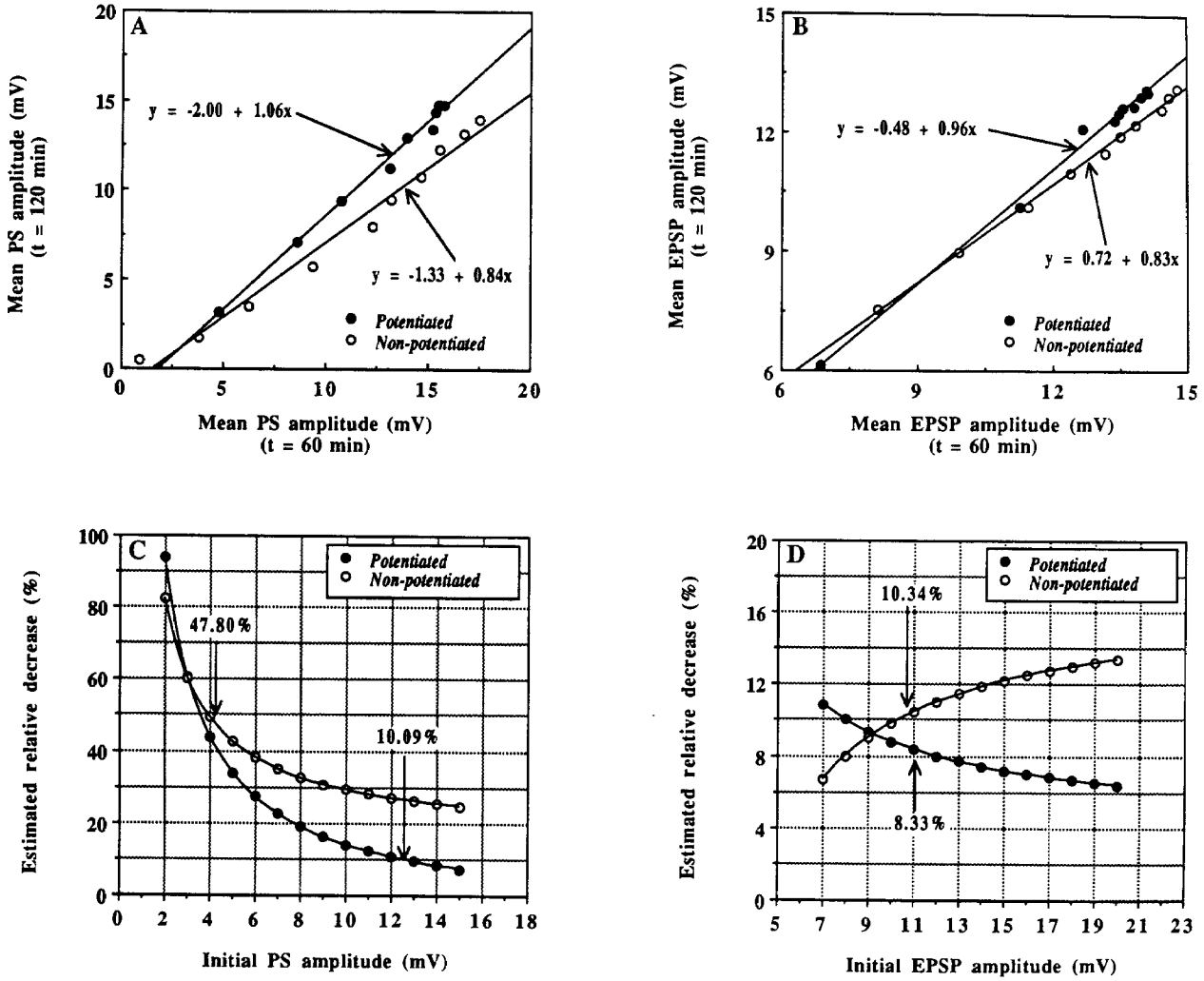

Fig. 4. **Top half**: Mean PS amplitude (A) and EPSP amplitude (B) I/O functions for potentiated and non-potentiated perforant path-evoked field potentials generated from five subjects treated with 30 mg kg^{-1} CGP 39551. Values represent the mean PS and EPSP amplitudes recorded at ten different stimulation intensities (3 sweeps per intensity) 60 (abscissa) and 120 min (ordinate) following drug injection for both potentiated (solid circles) and non-potentiated (open circles) responses. Linear regression equations for the potentiated and non-potentiated I/O data were computed and are displayed in the graphs. **Bottom half**: The linear regression equations obtained in A and B were used to construct the graphs in C and D. The graphs represent the estimated percent decrease (ordinate) given fourteen initial x values (abscissa) for PS (C) and EPSP (D) amplitudes of potentiated and non-potentiated responses, respectively. Arrows indicate the percent decrease expected for the initial PS and EPSP amplitudes observed in the subjects reported in Table II. The estimated values correspond highly with the actual values observed in Table II. Note, however, that the differential effect of CGP 39551 on the PS amplitude of potentiated and non-potentiated responses reported in Table II may have been slightly overestimated due to the discrepancy in initial magnitude of the responses. For responses of equal initial magnitude, however, the graphs predict that greater differences between potentiated and non-potentiated responses would be expected to emerge with larger initial responses (i.e., at higher stimulation intensities).

evoked potentials were both time- and dose-dependent, occurring 120 min following administration of 30 mg kg^{-1} CGP 39551. These results are in agreement with the numerous studies that have reported the prevention of LTP induction following administration of NMDA antagonists (Abraham and Mason, 1988; Coan et al., 1987; Collingridge et al., 1983; Errington et al., 1987; Gilbert and Mack, 1990; Maren et al., 1991; Morris et al., 1986; Pozza et al., 1990; Stringer and Guyenet, 1983).

Lastly, the present study revealed that CGP 39551 produced differential effects on potentiated and non-potentiated field potentials in the dentate gyrus. Both the EPSP and PS amplitude, but not the EPSP slope, of non-potentiated field potentials were attenuated by 30 mg kg^{-1} CGP 39551 120 min following drug administration. Surprisingly, potentiated field potentials were not significantly affected by the drug. These findings have important implications for the mechanisms underlying LTP expression. Most importantly, our results

indicate that the expression of LTP is not associated with increased transmitter release. If the expression of LTP were associated with increased transmitter release, the *absolute* contribution of the NMDA receptor-mediated conductance to the population synaptic potential would be expected to become larger, whereas the contribution of NMDA receptors *relative* to other synaptic conductances (i.e., AMPA receptor-mediated conductances) would be expected to remain the same. In other words, increased transmitter release would be expected to have equivalent effects on all of the synaptic conductances contributing to the field EPSP. We have found that the opposite is true. Our results indicate that following LTP induction the *absolute* contribution of NMDA receptors to the field EPSP does not change, whereas the *relative* contribution of the receptors to the synaptic potential significantly decreases. These results are in accordance with several reports indicating that the properties of NMDA receptors do not change following the induction of LTP (Kauer et al., 1988; Muller and Lynch, 1988; Muller et al., 1988; Tocco et al., 1992). Therefore, our results are most consistent with a scenario in which the induction of LTP leads to an increase in the conductance and/or number of AMPA receptors, which, in turn, reduces the relative contribution of NMDA receptors to the synaptic conductance. These findings, together with the results of Staubli et al. (1990), provide further evidence that LTP is in fact associated with a change in some properties of postsynaptic AMPA receptors in the absence of equivalent changes in NMDA receptors.

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REFERENCES

Abraham, W.C., and Mason, S.E. (1988) Effect of the NMDA receptor/channel antagonists CPP and MK801 on hippocampal field potentials and long-term potentiation in anesthetized rats. Brain Res., 462:40–46.

Bashir, Z.I., Alford, S., Davies, S. N., Randall, A.D., and Collingridge, G.L. (1991) Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. Nature, 349:156–158.

Baudry, M., and Davis, J.L. (eds.) (1991) Long-Term Potentiation: A Debate of Current Issues. MIT Press, Cambridge, MA.

Coan, E.J., Saywood, W., and Collingridge, G.L. (1987) MK-801 blocks NMDA receptor-mediated synaptic transmission and long term potentiation in rat hippocampal slices. Neurosci. Lett., 80:111–114.

Collingridge, G.L., Kehl, S.J., and McLennan, H. (1983) Excitatory amino acids in synaptic transmission in the Schaeffer-commissural pathway of the rat hippocampus. J. Physiol. (Lond.), 334:33–46.

Errington, M.L., Lynch, M.A., and Bliss, T.V.P. (1987) Long-term potentiation in the dentate gyrus: induction and increased glutamate release are blocked by D(-) aminophosphonovalerate. Neuroscience, 20:279–284.

Fagg, G.E., Olpe, H.-R., Pozza, M.F., Baud, J., Steinmann, M., Schmutz, M., Portet, C., Baumann, P., Thedinga, K., Bittiger, H., Allgeier, H., Heckendorn, R., Angst, C., Brundish, D., and Dingwall, J.G. (1990) CGP 37849 and CGP 39551: novel and potent competitive *N*-methyl-D-aspartate receptor antagonists with oral activity. Br. J. Pharmacol., 99:791–797.

Foster, T.C., and McNaughton, B.L. (1991) Long-term synaptic enhancement in CA1 is due to increased quantal size, not quantal content. Hippocampus, 1:79–91.

Gilbert, M.E., and Mack, C.M. (1990) The NMDA antagonist, MK-801, suppresses long-term potentiation, kindling, and kindling-induced potentiation in the perforant path of the unanesthetized rat. Brain Res., 519:89–96.

Hestrin, S., Nicoll, R.A., Perkel, D.J., and Sah, P. (1990a) Analysis of excitatory synaptic action in pyramidal cells using whole-cell recording from rat hippocampal slices. J. Physiol, 422:203–225.

Hestrin, S., Sah, P., and Nicoll, R.A. (1990b) Mechanisms generating the time course of dual component excitatory synaptic currents recorded in hippocampal slices. Neuron, 5:247–253.

Jung, M.W., Larson, J., and Lynch, G. (1991) Evidence that changes in spine neck resistance are not responsible for expression of LTP. Synapse, 7:216–220.

Kauer, J.A., Malenka, R.C., and Nicoll, R.A. (1988) A persistent postsynaptic modification mediates long-term potentiation in the hippocampus. Neuron, 1:911–917.

Larson, J., and Lynch, G. (1986) Induction of synaptic potentiation of hippocampus by patterned stimulation involves two events. Science, 232:985–988.

Larson, J., and Lynch, G. (1988) Role of *N*-methyl-D-aspartate receptors in the induction of synaptic potentiation by burst stimulation patterned after the hippocampal theta rhythm. Brain Res., 441:111–118.

Larson, J., and Lynch, G. (1991) A test of the spine resistance hypothesis of LTP expression. Brain Res., 538:347–350.

Lynch, G., and Baudry, M. (1991) Reevaluating the constraints on hypotheses regarding LTP expression. Hippocampus, 1:9–14.

Malinow, R., and Tsien, R.W. (1990) Presynaptic enhancement shown by whole cell recordings of long-term potentiation in hippocampal slices. Nature, 345:177–180.

Maren, S., Baudry, M., and Thompson, R.F. (1991) Differential effects of ketamine and MK-801 on the induction of long-term potentiation. NeuroReport, 2:239–242.

Massicotte, G., and Baudry, M. (1991) Triggers and substrates of hippocampal synaptic plasticity. Neurosci. Biobeh. Rev., 15:415–423.

Morris, R.G.M., Anderson, E., Lynch, G.S., and Baudry, M. (1986) Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. Nature, 319:774–776.

Mott, D.D., and Lewis, D.V. (1991) Facilitation of the induction of long-term potentiation by $GABA_B$ receptors. Science, 252:1718–1720.

Muller, D., Joly, M., and Lynch, G. (1988) Contributions of quisqualate and NMDA receptors to the induction and expression of LTP. Science, 242:1694–1697.

Muller, D., and Lynch, G. (1988) Long-term potentiation differentially affects two components of synaptic responses in hippocampus. Proc. Natl. Acad. Sci. (USA), 85:9346–9350.

Pozza, M.F., Olpe, H.-R., Brugger, F., and Fagg, G.E. (1990) Electrophysiological characterization of a novel potent and orally active NMDA receptor antagonist: CGP 37849 and its ethylester CGP 39551. Eur. J. Pharmacol., 182:91–100.

Sah, P., Hestrin, S., and Nicoll, R.A. (1989) Tonic activation of NMDA receptors by ambient glutamate enhances excitability of neurons. Science, 246:815–818.

Staubli, U., Kessler, M., and Lynch, G. (1990) Aniracetam has proportionately smaller effects on synapses expressing long-term potentiation: evidence that receptor changes subserve LTP. Psychobiol., 18:377–381.

Stringer, J.L., and Guyenet, P.G. (1983) Elimination of long-term potentiation in the hippocampus by phencyclidine and ketamine. Brain Res., 258:159–164.

Tocco, G., Devgan, K.K., Hauge, S.A., Weiss, C., Baudry, M., and Thompson, R.F. (1991) Classical conditioning selectively increases AMPA receptor binding in the rabbit hippocampus. Brain Res., 559:331–336.

Tocco, G., Maren, S., Shors, T.J., Baudry, M., and Thompson, R.F. (1992) Long-term potentiation is associated with increased ^{3}[H]-AMPA binding in rat hippocampus. Brain Res., 573:228–234.

Wilcox, R.R. (1987) New Statistical Procedures for the Social Sciences. Lawrence Erlbaum Associates, Hillsdale, NJ.