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Facilitative Effect of Carbamazepine on Previously Induced Hippocampal Long-Term Potentiation

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KUBOTA, T., I. JIBIKI, K. FUJIMOTO AND N. YAMAGUCHI. *Facilitative effect of carbamazepine on previously induced hippocampal long-term potentiation.* PHARMACOL BIOCHEM BEHAV 42(4) 843-847, 1992.—The effects of carbamazepine (CBZ) on previously induced hippocampal long-term potentiation (LTP) were examined. Acute experiments were performed on 33 adult, male rabbits. Field potentials in the dentate gyrus were elicited by single shocks to the perforant path, and LTP was induced by tetanic stimulation to the pathway without induction of seizure discharge. At a CBZ serum level of about 5 μg/ml (value ± SD = 5.40 ± 1.28 μg/ml), the previously induced LTP in population spikes (PSs) and population excitatory postsynaptic potentials (EPSPs) was facilitated. At a CBZ serum level of about 15 μg/ml (value ± SD = 14.28 ± 1.29 μg/ml), the LTP in PS alone was decreased. The effects of carbamazepine on synaptic inhibition were examined by the paired-pulse test. The inhibition was enhanced with induction of LTP. After administration of CBZ, at a CBZ serum level of about 5 μg/ml the inhibition was further enhanced, while it was attenuated at a CBZ serum level of about 15 μg/ml. These results suggest that CBZ has a facilitative effect on previously induced LTP.

Carbamazepine Hippocampus Long-term potentiation Synaptic inhibition

THE long-lasting facilitation of excitatory synaptic responses after tetanic stimulation is called long-term potentiation (LTP). LTP is most prominently induced in the hippocampal formation and is speculated to be associated with the "kindling" phenomenon (7,20) and the maintenance of memory or learning (18,21).

Carbamazepine (CBZ) is a well-known antiepileptic drug. CBZ is effective against amygdaloid-hippocampal- kindled seizures in animals (22). Furthermore, CBZ antagonizes the epileptogenic population spike (PS) in hippocampal slices (2). It is also known that CBZ has favorable effects on memory and behavior and acts as a mood stabilizer in patients with manic-depressive psychosis (1). CBZ is thus surmised to have various effects on brain functions.

Recent work has shown that phenytoin (PHT) does not block the induction of hippocampal LTP in slice preparations (4,19). In our previous studies, PHT only slightly suppressed the seizure discharges (SDs) in the hippocampus (12), but CBZ markedly shortened the hippocampal SDs (15). Our studies suggest that CBZ has a considerable effect on the electrophysiological activity of the hippocampus. In the present study, we investigated the effects of CBZ on previously induced hippocampal LTP to study CBZ effects on hippocampal electrophysiological activity. To our knowledge, CBZ effects on the previously induced LTP or on the induction of LTP have not been reported.

METHOD

Experiments were performed on 33 adult, male rabbits weighing 2.5-3.5 kg. Surgical operations were performed under halothane anesthesia and local anesthesia with lidocaine chloride. Rabbits were immobilized with tubocurarine chloride and artificially ventilated while being restrained by a painless method using a Kopf semichronic head-holder described previously (12). Based upon our earlier studies (8,9), the following methods were used. The pial surface of the right visual cortex was widely exposed. A tungsten microelectrode (tip diameter: 1-2 μm, resistance: 1-5 kΩ) for recording and a concentric electrode for stimulation (0.4 mm in diameter), placed parallel and 0.5 mm apart from each tip, were inserted into the dentate gyrus granular cell layer (3,100-3,950 μm below the pial surface) guided by laminar analysis using an oil hydraulic microdrive. The stimulation pulse for laminar

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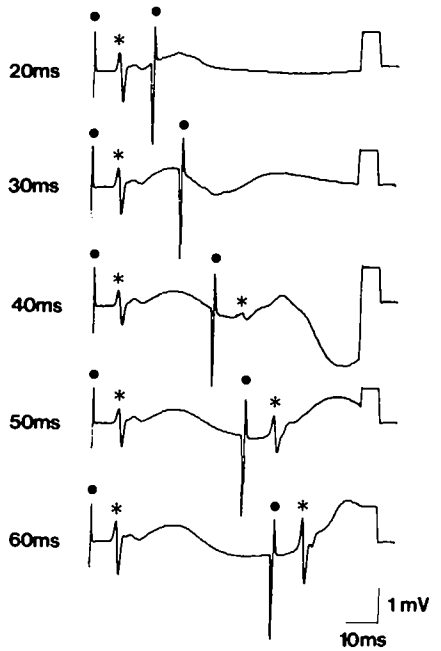

FIG. 1. Example of P-P tests. (●), first and second pulses; (*), population spike induced by each paired pulse. Numerals from 20-60 ms indicate interpulse intervals. Note that the PS amplitude evoked by the second pulse was inhibited, in comparison with the PS amplitude evoked by the first pulse, most markedly at an interpulse interval of 20 ms and progressively less inhibited at interpulse intervals of 30-50 ms and rather facilitated at an interpulse interval of 60 ms.

analysis had a strength and pulse duration of 200 μA and 200 μs, respectively, and was delivered at 10-s intervals. Another concentric electrode for stimulation (0.6mm in diameter) was inserted into the perforant path (4,100-5,000 μm below the pial surface) ipsilateral to the dentate gyrus and fixed at the point where maximal responses were elicited in the dentate gyrus by single shocks of constant intensity delivered from the stimulating electrode. The coordinates for the dentate and perforant path electrodes were, relative to bregma, 4 mm posterior, 6 mm right lateral and 4 mm posterior, 1 mm right lateral, respectively. Stainless steel screw electrodes were placed bilaterally in the skull in the motor, parietal, and visual regions for electroencephalograph (EEG) recording.

The experimental procedure was as follows. The threshold intensities of single shocks to the perforant path for inducing PSs and population excitatory postsynaptic potentials (EPSPs) in the dentate gyrus were initially examined, based upon our previous studies (8,9). As the baseline, fixed responses (FRs), which were evoked in the dentate gyrus by single shocks to the perforant path at the threshold intensity at 30-s intervals, were recorded for about 30 min and the input-output curve (I-O curve) and the paired-pulse tests (P-P test) with double shocks at a constant intensity were interposed in the FR recording. The P-P test was performed for investigation of the synaptic inhibition in the granular cell layer of the hippocampus. In the P-P test, the interpulse interval (IPI) was varied in five steps of 20, 30, 40, 50, and 60 ms (Fig. 1). The strength of the pulse stimulation applied to the perforant path varied from 200-800 μA, with a 200-μs pulse duration delivered at 30-s intervals.

After these baseline-evoked responses (FRs, I-O, curve and P-P test) were recorded, two groups of experiments were per-

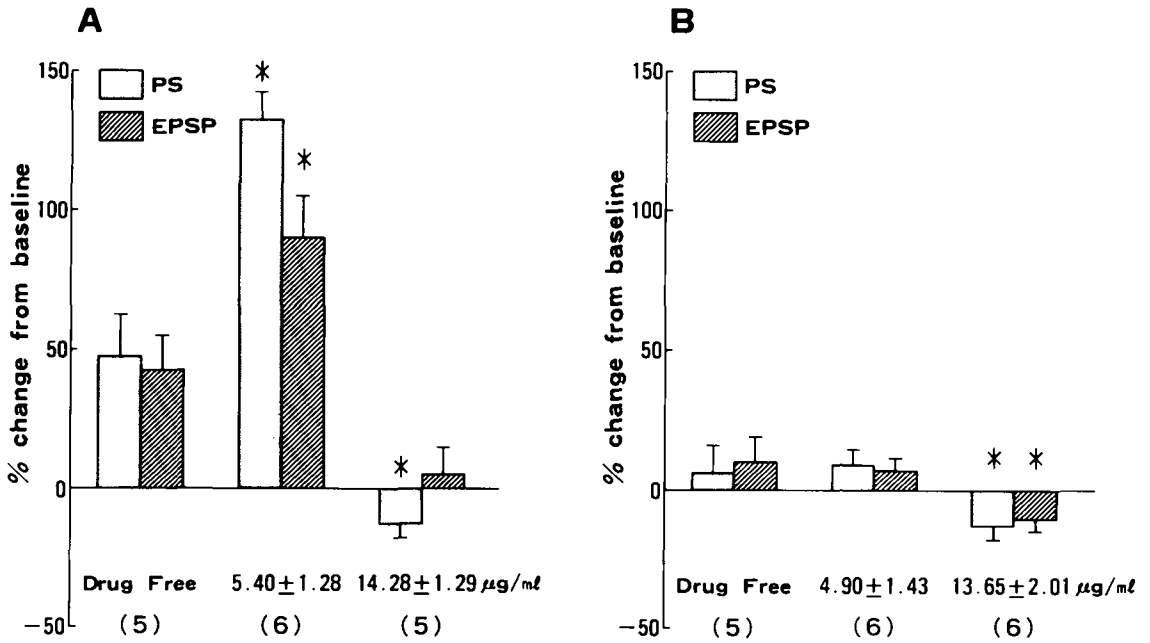

FIG. 2. (A) Effects of CBZ on LTP. % change in EPSP slope (▨) and PS amplitude (□) from baseline 30 min after CBZ administration was expressed in each of the low and high CBZ serum level and drug-free groups. CBZ serum levels (mean ± SD) and numbers of animals (parentheses) are indicated. * significant difference ($p < 0.01$) compared to the drug-free group. Note that at CBZ low serum levels, LTP in the PS and EPSP was facilitated, while PS alone was significantly decreased at high CBZ levels ($p < 0.01$). (B) Effects of CBZ on PSs and EPSPs without LTP induction, expressed as in A. Note that in all drug-free and low and high serum level groups neither the PS nor EPSPs significantly changed except for decrases at high serum levels.

formed. In one group of experiments, conducted on 16 rabbits, LTP was induced by tetanizations (200–400 μA, 2-ms duration, 200 Hz and 1 s in total duration) to the perforant path. The tetanizations consisted of three sets of stimulation spaced 5 min apart. No SDs were induced by these tetanizations. FRs after tetanization were recorded for about 30 min or more to obtain stable LTP responses, and the I-O curve and P-P test were interposed again. Next, CBZ dissolved in dimethylsulfoxide was administered intravenously. A loading dose (single injection) and infusion dose (continuous microinfusion) were used together, according to previously established theories on the pharmacokinetics of CBZ, which were applied to produce steady-state CBZ serum levels (13). CBZ serum levels were aimed at 5 μg/ml (low serum level group; $n = 6$) and 15 μg/ml (high serum level group; $n = 5$); vehicle solution alone was administered to the drug-free group ($n = 5$). Thirty minutes after CBZ administration, the FR recording, I-O curve, and P-P test were again recorded in each group. In another group of experiments, conducted on 17 rabbits, after baseline-evoked responses were recorded CBZ was soon administered as mentioned in the previous experiment, that is, low serum level group ($n = 6$), high serum level group ($n = 6$), and drug-free group ($n = 5$), with induction of LTP, and FRs were recorded for about 30 min or more with the I-O curve and P-P test. After experimental procedures were completed, a blood sample was collected from the jugular vein and the serum level of CBZ was measured.

The responses were continuously observed with a memory oscilloscope (bandpath, 0.08–3,000 Hz) and an X-Y recorder, with four sets of responses averaged with an averager.

Statistical Analysis

The PS amplitude and the EPSP slope (mV/ms) in the averaged four sets of responses were measured according to our previous study (9). The FR values were plotted serially in three sessions, that is, baseline, after LTP induction, and 30 min after CBZ administration in each group (two sessions in the group without LTP induction). These mean values of 10 averaged FRs (40 responses) after 30 min subsequent to CBZ administration were expressed as the % changes from those of baseline in each of two groups with and without LTP induction, and were statistically analyzed by one-way analysis of variance (ANOVA) followed by Scheffe's multiple comparison to determine whether the % changes significantly differed between the three groups, that is, low and high serum level and drug-free groups.

In data from the P-P test, the PS amplitude evoked by the second pulse of the pair was expressed as % of that evoked by the first pulse and was statistically analyzed by two-way ANOVA followed by Scheffe's multiple comparison to determine whether the % values at each interpulse interval significantly differed between the above-mentioned two or three sessions. In addition, with regard to the I-O curve, the PS amplitudes and EPSP slopes at varied stimulus strengths were compared between the two or three sessions.

RESULTS

Actions of CBZ on LTP

In the experiments with serum concentration goals set at 5 and 15 μg/ml, serum levels of 3.96–6.72 μg/ml (mean value ± SD = 5.40 ± 1.28 μg/ml) and 11.25–15.64 μg/ml (mean value ± SD = 14.28 ± 1.29 μg/ml), respectively, were achieved 30 min after CBZ administration. One-way ANOVA showed that there were significant differences in the % change values from baseline in both the PS amplitudes and EPSP slopes between the three groups [F values(6.38707 and 7.31678 in the PS amplitudes and EPSP slopes, respectively) > 3, 18)(0.01) = 5.092]. The following Scheffe's multiple comparison with the drug-free group showed that the low serum level group had significantly increased PS amplitudes and EPSP slopes ($p < 0.01$), and that the high serum level group had significantly decreased PS amplitudes alone ($p < 0.01$), as shown in Fig. 2A.

Actions of CBZ on Evoked Responses Without LTP

In these experiments with serum concentration goals of 5 and 15 μg/ml, serum levels of 3.23–6.49 μg/ml (mean value ± SD = 4.90 ± 1.43 μg/ml) and 11.16–15.75 μg/ml (mean value ± SD = 13.65 ± 2.02 μg/ml), respectively, were achieved 30 min after CBZ administration. One-way ANOVA showed that there were significant differences in the % change values in both the PS amplitudes and EPSP slopes among the three groups [F values(153.233 and 30.9551 in the PS amplitudes and EPSP slopes, respectively) > F(3, 22)(0.01) = 4.817]. The following Scheffe's multiple comparison with the drug-free group showed that the high serum level group alone had significantly decreased PS amplitudes and EPSP slopes ($p < 0.01$), as indicated in Fig. 2B.

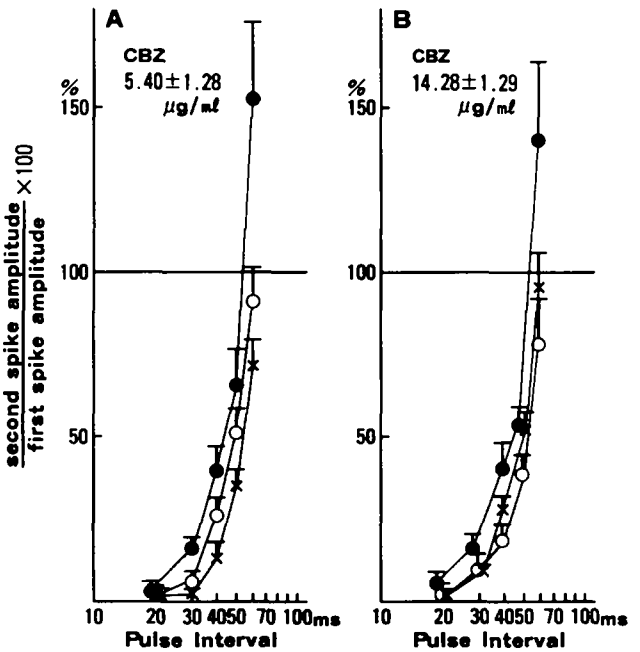

FIG. 3. Changes of synaptic inhibition in P-P tests at low and high CBZ serum levels. Abscissae indicate interpulse interval expressed as a log scale. Ordinate indicates percent of the PS amplitude evoked by the second pulse of the pair to that evoked by the first pulse. (●——●) baseline, (○——○) after induction of LTP, and (×——×) after administratin of CBZ indicate the percent changes of PS amplitudes in a series of P-P tests at different interpulse intervals. (A) Low CBZ serum level group ($n = 6$); (B) high CBZ serum level group ($n = 5$). Note that the percent of PS amplitudes at each interpulse interval decreased after LTP induction in both groups, indicating an enhancement of synaptic inhibition, and that the inhibition was further enhanced after CBZ administration in the low serum level group, while it was slightly reduced toward the baseline level in the high serum level group.

CBZ Effect on P-P Inhibition

Figure 3 indicates the three series of the P-P test, namely, prior to induction of LTP, after induction of LTP, and 30 min after administration of CBZ, at low and high serum levels. Before induction of LTP, the series of the P-P test showed clear inhibition of the PS amplitude elicited by the second pulse at 20-, 30-, and 40- or 50-ms intervals and, in contrast, enhancement at 60-ms intervals. After induction of LTP, the P-P test showed more intense inhibition of the PS amplitude elicited by the second pulse as compared with the inhibition in the baseline (Fig. 3). This inhibition was further enhanced 30 min after CBZ administration at low serum levels (Fig. 3A). However, the inhibition showed a slight reduction toward the baseline level at high CBZ serum levels (Fig. 3B). Two-way ANOVA showed that there was a significant difference in the percent values of PS amplitudes between three sessions in each of the low and high serum level groups [F value (13.8135) > $F(2, 75)(0.01) = 4.979$ in low serum level group, F value 5.13909) > $F(2, 60)(0.01) = 4.977$ in high serum level group). Next, Scheffe's multiple comparison showed that there were significant differences in the percent values of PS amplitudes between all of two sessions consisting of baseline and after LTP induction, baseline and after CBZ administration, and after LTP induction and after CBZ administration in both the low and high serum level groups ($p < 0.01$).

In addition, the I-O curves of the PS amplitudes showed that the amplitudes at each different stimulus strength were potentiated with LTP induction, still more potentiated at low CBZ serum levels, and reduced to almost the same heights as in the baseline at high CBZ serum levels, while they were slightly potentiated after the application of vehicle alone. In the groups without LTP induction, the I-O curves showed that no PS amplitude showed any appreciable change before and after CBZ or vehicle application except for a slight decrease at high CBZ serum levels.

DISCUSSION

In the present study, we examined the action of CBZ on LTP, especially on the expression or maintenance of LTP. At relatively low serum levels, CBZ facilitated LTP, whereas at relatively high serum levels CBZ suppressed it. In addition, at relatively low serum levels CBZ had no effect on the ordinary evoked responses without induction of LTP, while at relatively high serum levels CBZ suppressed the evoked responses. These results indicate that CBZ exerts a dose-dependent biphasic action on LTP and further suppresses ordinary synaptic transmission at high serum levels.

With regard to the mechanisms of action of CBZ, it is known that CBZ blocks presynaptic sodium channels and the firing of action potentials in the presynaptic terminal; this is thought to secondarily reduce voltage-dependent calcium entry into the presynaptic terminal and eventually decrease synaptic transmission (15). In the present study, the suppressive effects at high CBZ serum levels on LTP and ordinary evoked responses are probably due to the inhibition of sodium channels on the presynaptic terminals.

On the other hand, the present facilitative effect of CBZ on LTP at low serum levels is not due to a decrease in the synaptic inhibition in the granule cell layer since the synaptic inhibition was increased at low CBZ serum levels as indicated in Fig. 3A. The increase in synaptic inhibition, known to be mainly the recurrent inhibition, may result secondarily from the facilitative effect of CBZ on LTP.

It has been considered that LTP is produced by activation of NMDA receptors in the postsynaptic neurons (5,6,10) and maintained by the activation of non-NMDA type glutamate receptors (11,14,16). However, it is known that CBZ does not modify postsynaptic responses to glutamate (15). Recently, it has been strongly suggested that LTP is primarily induced by presynaptic mechanisms (3,17), namely, retrograde signals from the postsynaptic cell after tetanus stimulations cause enhanced presynaptic release of neurotransmitters (3). It is probable that the present facilitative action of CBZ is attributable to such presynaptic mechanisms.

In conclusion, a satisfactory explanation for the mechanism of the present facilitative effects of CBZ on LTP must await more detailed studies, although the present findings may shed new light on LTP.

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