Effects of T-588, a Cognitive Enhancer Compound, on Synaptic Plasticity in the Dentate Gyrus of Freely Moving Rats

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ABSTRACT

(1R)-1-benzo[b]thiophen-5-yl-2-[2-(diethylamino) ethoxy]ethan-1-ol hydrochloride (T-588) is a compound for the treatment of neurodegenerative disorders, including Alzheimer’s disease and cerebrovascular diseases. T-588 reportedly alleviates learning and memory deficits in animal models of dementia. In the present study, we investigated the effects of T-588 on the induction and decay of long-term potentiation (LTP) and on the responses to paired-pulse (pp) stimulation in freely moving rats. Perforant path-evoked field potentials were recorded in the dentate gyrus by chronically implanted electrodes. LTP was induced by high-frequency stimulation 30 min after oral administration of T-588 (0.3 or 3 mg/kg). T-588 significantly augmented the increase in population spike amplitude and field excitatory postsynaptic potential slope after LTP induction. T-588 also prolonged the decay of augmented population spike amplitude, but had no significant effect on the response to pp stimulation. These results suggest that T-588 facilitates long-term synaptic plasticity, but not short-term synaptic plasticity in the dentate gyrus of freely moving rats. The effect of T-588 on long-term synaptic plasticity may contribute to the alleviation of learning and memory dysfunction seen in animal models.

The decline of memory and other cognitive functions in patients with Alzheimer’s disease or cerebrovascular diseases is of great clinical and social concern. (1R)-1-benzo[b]thiophen-5-yl-2-[2-(diethylamino) ethoxy]ethan-1-ol hydrochloride, or T-588, was developed for treatment of these neurodegenerative diseases. T-588, characterized by high permeability through the blood-brain barrier (Ono et al., 1993), has been shown to improve learning and memory deficits in rats. For example, T-588 significantly improved learning by rats in the three-panel runway task (Ono et al., 1994) and spatial learning task (Nakada et al., 1995) following scopolamine treatment and transient forebrain ischemia, respectively. T-588 also ameliorated the impaired performance of rats in active or passive avoidance tasks following cerebral embolization and basal forebrain lesion (Ono et al., 1995b).

To evaluate T-588’s action in these findings, in vitro and in vivo studies have been performed. In a microdialysis study, single oral administration (3–30 mg/kg) of T-588 significantly increased the amount of acetylcholine (ACh) and noradrenaline (NA) in the hippocampus and neocortex (Ono et al., 1995a). T-588 was also found to increase NA release in hippocampal slice preparation (Ono et al., 1995a). The action of T-588 to increase neurotransmitter (or neuromodulator) release could alleviate the cognitive dysfunction observed in patients with neurodegenerative disorders, since several kinds of neurotransmitters in the brain, especially in the hippocampus, declined in these patients (Adolfsson et al., 1979; Whitehouse et al., 1981). Furthermore, T-588 produced slow and long-lasting depolarization, different from the mechanism of ACh, by affecting resting membrane potential and conductance in CA1 pyramidal cells of rat hippocampal slice preparation, probably mediated by suppression of potassium currents (Kimura et al., 1999). This action may increase cell excitability and may be involved in beneficial effects of T-588 on cognitive dysfunction (Ghelardini et al., 1998). Since T-588 has such effects on hippocampal slice preparation, it may influence synaptic efficacy in vivo when it is applied systemically.

Changes in synaptic efficacy have been proposed as a cellular basis for learning and memory. Long-term potentiation (LTP) is known as a long-lasting enhancement of synaptic efficacy (Bliss and Collingridge, 1993). Previous studies have shown LTP induction in several kinds of learning and mem-

ABBREVIATIONS: T-588, (1R)-1-benzo[b]thiophen-5-yl-2-[2-(diethylamino)ethoxy]ethan-1-ol hydrochloride; ACh, acetylcholine; NA, noradrenaline; LTP, long-term potentiation; DG, dentate gyrus; PS, population spike; fEPSP, field excitatory postsynaptic potential; IPI, interpulse interval; I/O, input/output; IRI, inter-recording interval; HFS, high-frequency stimulation; EPSP, excitatory postsynaptic potential; ANOVA, analysis of variance; E-S, EPSP spike; GABA, y-aminobutyric acid.
ory tasks in vivo (Laroche et al., 1989; Bramham et al., 1994; Doyère and Laroche, 1996; Rogan et al., 1997). Furthermore, blocking the N-methyl-D-aspartate receptor prevents not only LTP induction, but also spatial learning in the water maze and radial-arm maze tasks (Abraham and Mason, 1988; Butelman, 1989; Shapiro and Caramanos, 1990). On the other hand, different forms of physiological control for synaptic function related to learning and memory have been characterized, for example, paired-pulse facilitation and inhibition. Indeed, using paired-pulse stimulation, short-term synaptic efficacy is likely to change when rats are exploring a novel environment (Moser, 1996). Rapid adjustment of such temporal synaptic processing has been assumed for another form of synaptic plasticity, i.e., short-term synaptic plasticity.

As mentioned above, the increase of ACh and NA release by T-588 could be a reason for the amelioration of the learning and memory deficits observed in the previous in vivo studies. However, little is known concerning the effects of T-588 on the electrophysiological parameters for synaptic plasticity. Considering the alleviatory effect of T-588 in the previous studies, we believe it is important to test whether T-588 affects synaptic plasticity (short-term, long-term, or both) in vivo. Therefore, in the present study, we investigated the effects of T-588 on induction and maintenance of LTP as indices of long-term synaptic plasticity, and responses to paired-pulse stimulation as an index of short-term synaptic plasticity in the dentate gyrus (DG) of freely moving rats. Our results showed that T-588 did not affect the short-term synaptic plasticity, but enhanced long-term synaptic plasticity in the DG-perforant pathway. A preliminary report has already been published in abstract form (Yamaguchi et al., 1999).

**Materials and Methods**

**Subjects.** Forty-four male Fischer/344 rats (Japan SLC, Inc., Hamamatsu, Japan), weighing 211–259 g at the time of surgery, were used for the experiment. Rats were housed individually in a temperature-regulated room (24 ± 1°C) and maintained on a 12-h light/dark cycle with food and water available ad libitum. They were handled and weighed daily at around 9:00 AM.

**Surgery.** Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p., supplemented as necessary during surgery) and placed into a stereotaxic apparatus. The scalp was incised at midline and the skull was exposed. Lambda and bregma were positioned in the horizontal plane and 1.5-mm diameter holes were drilled to allow for placement of the electrodes. Recording and stimulating electrodes were constructed from pairs of twisted Teflon-coated, 125-μm diameter stainless steel wires (A-M Systems, Inc., Everett, WA). The recording electrode consisted of wires cut flat at different lengths with a 2-mm vertical distance between the tips. The bipolar stimulating electrode had 0.5-mm tip separation and was uninsulated with 0.5 mm at the tip. The recording electrode was inserted into the right-side DG (4.2 mm posterior to bregma and 2.5 mm lateral to the midline). The stimulating electrode was inserted into the perforant-path fibers on the same side as the recording electrode (7.8 mm posterior to bregma and 4.4 mm lateral to the midline). The electrodes were fixed at the location in which the population spike (PS) amplitude and field excitatory postsynaptic potential (fEPSP) slope recorded in the DG in response to the perforant-path stimulation were maximized. Two screws with a stainless steel wire were implanted into the skull over the cerebellum as the ground electrodes. All electrodes were connected to pins of a lightweight plastic minia-

**Electrophysiological Recording Apparatus.** All the recordings were performed after the rat had been transferred from the home cage to a recording box (30 × 30 × 30 cm) with woodchip bedding. The head-stage of the rat was connected to a flexible, shielded cable containing field-effect transistors (2SK389; Toshiba, Tokyo, Japan). The cable allowed the rat to move freely in the recording box. Evoked responses were collected while the rat was motionless and awake with its eyes open. The electroencephalography signal through the recording electrode was monitored on an oscilloscope (VC-10, Nihon Kohden, Tokyo, Japan). To confirm that the rat was awake, sensory stimuli (tapping sidewall of the recording box, etc.) were sometimes presented.

Evoked responses were generated by single biphasic square pulses of 100-μs duration per half-wave using a constant current isolation unit (SS202J; Nihon Kohden, Tokyo, Japan). The signals from the recording electrode were amplified (×100), filtered (bandpass from 0.1–10 kHz) and digitized at 50 kHz through an A/D converter (DAQcard-1200, National Instruments, Austin, TX). Waveforms, displayed on a PC screen (MN-370-X20; Sharp, Tokyo, Japan), were stored in the hard disk online. The amplified and filtered signals were also monitored on an oscilloscope and stored on a digital audiotape by a digital audiotape recorder (RD-125T; TEAC, Tokyo, Japan) for off-line analysis.

**Drug Treatment.** T-588 (Fig. 1) was obtained from Toyama Chemical Co., Ltd. (Tokyo, Japan) and dissolved in distilled water. T-588 solution was given orally to each animal at concentrations of 0.3 and 3 mg/kg at a volume of 5 ml/kg b.w.

**Recording Protocol: Acclimatization and Input/Output (I/O) Curve Generation.** Given that an unfamiliar environment or motor activity may affect the PS and fEPSP recordings (Hargreaves et al., 1990; Bramham et al., 1994; Kitchigina et al., 1997), rats were acclimatized to the recording box for 30 min/day for a 3-day period. Furthermore, rats were allowed ≥30 min to habituate in the recording box before data collection on each day.

On the last day of acclimatization, I/O curves were generated with the use of varying stimulating current intensities (25–1500 μA) to establish the test intensity used in the subsequent experiment. In the LTP experiment, constant test stimulus intensity was adjusted to give an fEPSP slope of 60 to 70% of the maximum response calculated by the probit (probability unit) method for each individual rat (PS amplitude was approximately 1 mV). In the paired-pulse experiment, the stimulus intensity was set at PS amplitude to produce a level 50 to 60% of the maximum calculated by the same method as for the LTP experiment. Rats were also habituated to the oral administration by giving vehicle solution during the acclimatizing period.

**LTP Experiment.** In this experiment, the rats were assigned to one of the following three experimental groups: 1) vehicle only (N = 8), 2) low-dose (0.3 mg/kg) T-588 (N = 8), and 3) high-dose (3 mg/kg) T-588 (N = 8). Response stability was monitored for 60 min, 2 days after the I/O curve measurement (day −1). The next day (day 0), evoked poten-
tials in the DG of each rat were collected for 240 min after a 30-min habituation period with a 15-min inter-recording interval (IRI) in the first 120 min and with a 30-min IRI in the following 120 min. Test solution (vehicle or T-588) was given orally 30 min after the onset of the recording. High-frequency stimulation (HFS) was applied to the perforant path 30 min after the administration of test solution because the concentration of T-588 in the brain peaks at 30 to 60 min when administered orally (Ono et al., 1993), and its time course is consistent with that of the increase in ACh and NA release in microdialysates (Ono et al., 1995a). Ten trains of 10 stimuli [interpulse interval, 2.5 ms (400 Hz); intertrain interval, 10 s] were used as HFS at the same current intensity as the test pulse. Twenty-four hours (day 1) and 48 h (day 2) later, evoked potentials were further collected for 60 min each, with a 15-min IRI after the 30-min habituation period.

As the control for the LTP series described above, non-LTP series was also performed using 15 rats (five rats each). The procedure was the same as that of the LTP series, except for the absence of HFS.  

**Paired-Pulse Experiment.** Five rats were used in this experiment. After the rats were acclimatized, an I/O curve was generated for each rat as described above, and we recorded responses to paired-pulse stimulation. Pairs of stimulus pulses were delivered at interpulse intervals (IPIs) of 20, 25, 30, 40, 50, 70, 100, 200, 300, 500, and 1000 ms. Responses to paired-pulse stimulation were recorded just before and 30 min after high-dose T-588 administration.

**Histology.** At the end of the experiments, each rat was given an overdose of sodium pentobarbital (i.p.). The electrode tip placements were marked by passing 20 μA of d.c. through the tips. Each rat was then perfused intracardially with 0.9% saline containing heparin, followed by 10% buffered formalin. The brain was removed and fixed between 20 and 80% of the first positive peak amplitude. PS onset latency (ms) was defined as the time from stimulatory artifact to first positive peak amplitude. For each time point during the experiment, average and S.E.M. were calculated from the data on eight (for LTP and basal synaptic response experiments) or five (for I/O curve recordings and paired-pulse experiments) successive evoked responses (0.033 Hz). A mean value of responses at three time points on day 0 (0, 15, and 30 min after the onset of recordings) was defined as the baseline (0%). Subsequent data were expressed as the percent change from the baseline.

Statistical analysis was performed as follows. In the LTP and basal synaptic response experiments, to confirm whether there are differences in the absolute values of each parameter at baseline and stimulus intensity for the three groups, one-way analysis of variance (ANOVA) was performed. Two-way ANOVA was used to determine changes from the baseline after HFS and statistically significant differences were evaluated further by a two-tailed Dunnett’s post hoc test. In the paired-pulse experiment, data on each IPI before and after T-588 administration were analyzed using one-way ANOVA. Statistically significant differences were evaluated at P < 0.05.

**Results**

**LTP Experiment**

**Stimulus Intensity and the Mean Absolute Value of Baseline.** Prior to evaluating the effect of T-588, we attempted to determine whether differences were present in the stimulus intensities defined by I/O responses and mean absolute values of baseline for each parameter of evoked potentials between groups (see Data Analysis). Figure 4 shows the summarized results. No significant differences in stimulus intensity were observed among the three groups either in the absence of LTP \( F(2,12) = 0.082, P > 0.05 \) or in the presence of LTP \( F(2,21) = 0.035, P > 0.05 \) (Fig. 4A). No significant differences were noted among the three groups in the mean absolute value of PS amplitude (Fig. 4B), the fEPSP slope (Fig. 4C), and PS onset latency (Fig. 4D), either in the absence or presence of LTP.

**Effect of T-588 on Basal Synaptic Response.** In the non-LTP experimental series, we tested the effect of T-588 on basal synaptic response. As shown in Fig. 6A-b, there was a significant effect of group \( F(2,12) = 3.842, P < 0.05 \) on PS amplitude. Two-tailed Dunnett’s post hoc comparison test revealed that PS amplitude in the high-dose (3 mg/kg) T-588 group was slightly but significantly higher than that of the vehicle group on day 0 \( P < 0.05 \). There was also a significant group effect for the fEPSP slope (Fig. 6B-b) on day 0 \( F(2,12) = 11.251, P < 0.05 \) and day 1 \( F(2,6) = 4.938, P <
which was then decreased to 171.3% at 90 min and 133.8% at 180 min. Field EPSP slope increased by 22.4% 15 min after LTP induction and returned to 4.9% at 180 min. PS onset latency shortened by 28.2% 15 min after HFS and 23.4% at 180 min after LTP induction. Field EPSP slope also increased by 18.5% after 15 min and 4.9% after 180 min. PS onset latency at 15 and 180 min after HFS shortened by 28.1 and 29.1%, respectively.

Figure 6A-a shows the effect of T-588 on the induction of LTP as population data (means ± S.E.M.) on day 0. T-588 significantly augmented the induction of LTP for PS amplitude in a dose-dependent manner on day 0. ANOVA indicated a significant group effect \( F(2,168) = 19.218, P < 0.05 \). Post hoc testing revealed that PS amplitude in both the low-dose \((P < 0.05)\) and high-dose \((P < 0.05)\) T-588 groups was significantly higher than that of the vehicle group.

Administration of low-dose and high-dose T-588 (0.3, 3 mg/kg p.o.) also significantly enhanced the increase in the fEPSP slope induced by HFS \( F(2,168) = 7.707, P < 0.05 \) (Fig. 6B-a), although it was not as prominent as that seen for PS amplitude. Decreases in PS onset latency induced by HFS were not different between groups \( F(2,168) = 0.868, P > 0.05 \) on day 0 (Fig. 6C-a).

**Effect of T-588 on Retention of LTP.** To determine the effect of T-588 on the maintenance of LTP, we also recorded the field-evoked potentials on the subsequent 2 days (day 1 and day 2) after LTP induction (day 0). In the vehicle group, although the increase in PS amplitude induced by HFS was 200.7 ± 59.9% at 15 min after LTP induction, it decayed to 80.1 ± 37.8% on day 1 and 7.5 ± 25.2% on day 2, which was almost the same as the pre-HFS level. In the low-dose T-588 group, the augmented PS amplitude (290.9 ± 51.0% at 15 min after HFS on day 0) decreased, following a time course identical with that observed in the vehicle group. In the high-dose T-588 group, a higher increase of PS amplitude generated by HFS was maintained until day 2, compared with the low-dose group. In the high-dose T-588 group, the augmented PS amplitude (388.8 ± 84.3%) also decreased during the subsequent 2 days (173.2 ± 40.5% on day 1 and 90.6 ± 40.2% on day 2). However, ANOVA indicated significant group differences in PS amplitude on day 1 \( F(2,105) = 10.529, P < 0.05 \) and day 2 \( F(2,105) = 8.905, P < 0.05 \). Post hoc testing revealed that PS amplitudes of the high-dose T-588 group were significantly higher than those of vehicle group on day 1 \((P < 0.05)\) and day 2 \((P < 0.05)\).

**Paired-Pulse Experiment.** Figure 7 shows typical waveforms of evoked potentials by paired-pulse stimulation with the IPI at 25 (A), 100 (B), and 300 ms (C). On the second evoked potential, a strong depression of PS amplitude was observed compared with the first evoked potential at 25-ms IPI (Fig. 7A, early paired-pulse depression). In contrast, a clear augmentation of PS amplitude was observed on the second evoked potential at 100-ms IPI (Fig. 7B, paired-pulse facilitation). Paired-pulse depression was again observed at 300-ms IPI (Fig. 7C, late paired-pulse depression). Figure 8 shows averages of data collected from the five rats for each parameter. With increases in IPIs from 20 to 1000 ms, the
A typical triphasic response pattern (early depression, facilitation, and late depression) was again observed in PS amplitude (Fig. 8A). There were no significant effects of T-588 administration on the paired-pulse stimulation for the early depression (at 25-ms IPI: preT-588, 261.1 ± 10.8%; postT-588, 253.9 ± 15.1%; P = 0.710), facilitation (at 100-ms IPI: preT-588, 56.9 ± 34.0%; postT-588, 54.9 ± 32.8%; P = 0.968) and late depression (at 300-ms IPI: preT-588, 18.9 ± 7.5%; post-T-588, 28.8 ± 7.6%; P = 0.993).

In contrast to PS amplitude, only a small depression was observed in fEPSP at shorter IPIs (20–100 ms), but facilitation of the second evoked potential did not occur (Fig. 8B). Population spike onset latency of the second evoked potential was longer than that of the first evoked responses from 20- to 300-ms IPI (Fig. 8C). Neither paired-pulse depression of the fEPSP slope nor the increase in PS onset latency in the second evoked potential was significantly changed by T-588.

**Discussion**

**Effect of T-588 on Long-Term Synaptic Plasticity:**

**Effect of T-588 on the Induction of LTP.** In the present study, we demonstrated that an oral administration of T-588 potentiated the amplitude of evoked potentials by perforant path stimulation after HFS in the DG of freely moving rats. The effective doses (0.3, 3 mg/kg) for this action were the same as the doses that produced alleviatory effects on learning and memory deficits observed in previous studies (Ono et al., 1994, 1995b; Nakada et al., 1995).

In rat brain slice preparation, T-588 enhances NA release due to suppression of NA uptake in a calcium-independent manner (Miyazaki et al., 1997; Maekawa et al., 1998). Moreover, T-588 (3–30 mg/kg p.o.) significantly increases NA and ACh content in the hippocampus of freely moving rats (Ono et al., 1995a). Anatomically, the DG receives noradrenergic and cholinergic inputs through the projection of fibers from the medial septum, diagonal band, and the nucleus locus coeruleus (Loy et al., 1980; Frotscher and Leranth, 1985). Activation of cholinergic or noradrenergic afferents enhances LTP induction at perforant-path-DG granule cell synapses (Burgard and Sarvey, 1990). Therefore, the facilitatory effect of T-588 on LTP induction observed in the present study could partly be generated by activation of NA and/or the ACh pathway.

Although we observed the enhancement of LTP both in PS amplitude and the fEPSP slope, the increase in PS amplitude was larger than that in the fEPSP slope. PS amplitude reflects the number of granule cell discharges excited by a given afferent input (Andersen et al., 1971), whereas the fEPSP slope (or amplitude) is regarded as the efficacy of the synaptic process through neurotransmitter release from presynaptic and/or postsynaptic receptor properties (Bliss and Gardner-Medwin, 1973). It has been reported that m1-m4 muscarinic ACh receptor subtypes are differentially ex-
pressed in the presynaptic and postsynaptic regions of the DG (Rouse et al., 1998). Due to the different localization of muscarinic ACh receptors at presynaptic and postsynaptic sites, ACh decreases the fEPSP amplitude by decreasing glutamate release from perforant path terminals in behaving rats (Foster and Deadwyler, 1992), whereas it increases granule cell excitability postsynaptically (Fantie and Goddard, 1982). This differential influence of ACh could be the differential effects of T-588 on PS amplitude and the fEPSP slope. However, the differential influence of ACh on presynaptic and postsynaptic (or cell excitability) components is not consistent with the present observation of an increase in basal EPSP responses to T-588. To explain this, it is necessary to postulate some other mechanism, such as a common excitatory action of T-588 on presynaptic and postsynaptic components. It is also possible that the differential action of T-588 on fEPSP and PS is specific for long-term synaptic plasticity, but not true for basal synaptic responses.

Although basal synaptic response of PS amplitude and the fEPSP slope increased slightly after T-588 oral administration, these changes were not remarkable compared with those after LTP induction. Hirata et al. (1999) have reported that synaptic vesicular endocytosis (vesicular uptake) was reduced by T-588 during tetanic stimulation in glutamatergic crayfish neuromuscular junction, whereas no apparent changes in the amplitude of single endplate potential and endplate current (i.e., single stimulation) were observed by T-588 application. These findings show that T-588 produces significant changes in responses when a neuron receives high-frequency inputs, compared with low-frequency inputs. Similar effects (i.e., a difference between single and tetanic stimulations) have been reported in studies on different compounds: agonists of the phosphatidylinositol hydrolysis-coupled receptor, such as subtypes of m1, m3 ACh receptors, or metabotropic glutamate receptors 1 and 5 do not change basal synaptic responses, but facilitate LTP induction in the rat hippocampus (Burgard and Suravey, 1990; Riedel et al., 1995). It is intriguing for us to investigate the effects of T-588 on activity-dependent synaptic efficacy, including long-term

Fig. 6. Effects of T-588 on basal synaptic responses and LTP recorded from the dentate gyrus of freely moving rats. The changes in PS amplitude (A), fEPSP slope (B), and PS onset latency (C) in LTP (a; n = 8) and basal response (b; n = 5) are shown. (see Materials and Methods). Open circles, vehicle-treated group; gray triangles, low-dose T-588 (0.3 mg/kg p.o.)-treated group; filled diamonds, high-dose T-588 (3 mg/kg p.o.)-treated group. *Significant difference between the high-dose T-588-treated group and the vehicle-treated group (P < 0.05). #Significant difference between the low-dose T-588-treated group and the vehicle-treated group (P < 0.05).

Fig. 7. Representative recordings elicited by paired-pulse stimulation of the perforant path. Interpulse intervals were 25 (A), 100 (B), and 300 ms (C). Arrows indicate stimulus artifact. Calibration marks indicate 5 ms and 5 mV.
depression and depotentiation. Further studies are necessary to address this issue.

In the present study, there were no significant differences for the decrease of PS onset latency between the T-588 and vehicle-treated groups, except for day 2. This time course was different from those of T-588 effects on PS amplitude and the fEPSP slope after HFS. At present, there are no data to explain this discrepancy; a further study is necessary to elucidate this point.

**Effects of T-588 on Maintenance of LTP.** Maintenance of LTP is known to be important for retention of associative memory (Doyère and Laroche, 1992). The cellular processes in maintenance of LTP are known to be distinct from those involved in its induction. It has two physiological components: synaptic LTP and E-S (EPSP-spike) potentiation. EPSPs are increased after LTP due to synaptic LTP and EPSPs more readily elicit postsynaptic spike firing due to E-S potentiation (Chavez-Noriega et al., 1989; Evans and Viola-McCabe, 1996).

We observed that the PS amplitude in the high-dose T-588 group was significantly higher than that in the vehicle-treated group. This indicates that T-588 contributes to the maintenance of LTP, at least through E-S potentiation. T-588 may modify LTP maintenance postsynaptically, because E-S potentiation is thought to be produced by an increase in postsynaptic excitability (Bliss and Gardner-Medwin, 1973) and/or a reduction in postsynaptic inhibition. Abraham et al. (1987) have reported that E-S potentiation is due primarily to a long-lasting GABA-mediated inhibition (Abraham et al., 1987). Enhancing GABA_A receptor function, which may disturb memory formation, reduced E-S potentiation. In addition, a contribution of the dopaminergic system in E-S potentiation is indicated (Yanagihashi and Ishikawa, 1992). Therefore, changes in the GABAergic and/or dopaminergic system could be involved in the effects of T-588 on E-S potentiation observed in the present study.

In the present study, both low-dose and high-dose T-588 treatments slightly, but significantly, enhanced the fEPSP slope on day 1, whereas vehicle treatment did not. This indicates that T-588 contributes to the maintenance of LTP through synaptic LTP as well. Ono et al. (1995a) have reported that T-588 (3–10 \( \mu \)M) potentiates cAMP accumulation when applied with isoproterenol (0.3 \( \mu \)M) to bath medium in rat hippocampal slices. Since protein kinase A signal transduction pathway activated by cAMP is reported to be critical for LTP maintenance (Nguyen and Kandel, 1996), we speculate that this amplification of cAMP accumulation is one possible mechanism for the maintenance of LTP.

**Effect of T-588 on Short-Term Synaptic Plasticity.** Consistent with the results in previous in vivo studies (Joy and Albertson, 1993; Moser, 1996), by varying IPIs from 20 to 1000 ms, we identified a typical triphasic pattern of paired-pulse early depression, facilitation, and late depression for the PS amplitude of second evoked potentials. The early depression of PS is known to be due to the activation of interneurons, which feed back on the somatic region in the DG (Halasy and Somogyi, 1993; Moser, 1996) and produce GABA-mediated inhibition (Albertson and Joy, 1987). The late depression seems to be mediated by \( \text{Ca}^{2+} \)-dependent \( \text{K}^- \) channels opening (Thalmann and Ayala, 1982). PS paired-pulse facilitation results from a selective increase in an \( \text{N}-\text{methyl-D-aspartate} \)-mediated synaptic response (Joy and Albertson, 1987). Furthermore, field EPSP reportedly depends upon the increase of neurotransmitter (glutamate) release response to second pulse stimulation (Joy and Albertson, 1993). In the present study, administration of T-588 had no significant effects on the triphasic response of PS amplitude, the inhibitory process of fEPSP, and the increase of PS onset latency in any IPIs. Therefore, the cellular and synaptic mechanisms for the short-term synaptic plasticity may be less involved in the alleviatory effects of T-588 seen in the previous behavioral experiment (Ono et al., 1993, 1994, 1995b; Nakada et al., 1995) in which the same doses of T-588...
as those in the present study were used. However, this does not rule out a possibility that T-588 has some effects on short-term synaptic plasticity. The present results only indicated that T-588 does not show significant effects on short-term synaptic plasticity at a considerably low concentration range (0.3 or 3.0 mg/kg). Indeed, Hirata and coworkers (1999) have reported that, at a higher concentration range, T-588 reduces paired-pulse and repetitive-pulse facilitation at mouse and crustacean neuromuscular junctions.

**Conclusion.** In the present study, we showed that T-588 enhanced and maintained long-term synaptic plasticity (E-S potentiation and synaptic LTP), but did not affect short-term synaptic plasticity, in the DG-perforant pathway of freely moving rats. Since maintenance of LTP is known to be important for learning and memory (Doyère and Laroche, 1992), the effects of T-588 on long-term synaptic plasticity could contribute to the alleviation of cognitive dysfunction in senile dementia.

**References**


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Effects of T-588 on Synaptic Plasticity In Vivo