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Endogenous 5-HT Tonically Inhibits Spontaneous Firing Activity of Dorsal Hippocampus CA1 Pyramidal Neurons Through Stimulation of 5-HT_{1A} Receptors in Quiet Awake Rats: *In Vivo* Electrophysiological Evidence

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The present study was performed to examine an overall effect of endogenous serotonin (5-HT) on the spontaneous firing activity of the dorsal hippocampus CA1 pyramidal neurons in quiet awake rats. A selective 5-HT_{1A} antagonist N-[2–[4–(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635: 0.03–0.2 mg/kg, s.c.) significantly increased the firing activity. A depletion of 5-HT with parachlorophenylalanine (PCPA: 500 mg/kg/day $\times$ 3 days) completely abolished this increasing effect of WAY-100635. The baseline spike frequency of the PCPA-treated rats (3.90 $\pm$ 0.39 Hz) was significantly higher than that of the vehicle-treated rats (2.09 $\pm$ 0.19 Hz). A 5-HT_{2A} antagonist ritanserin (1 mg/kg, i.p.) and a 5-$HT_{3/4}$ antagonist 2-methoxy-4-amino-5-chloro benzoic acid 2-(diethylamino) ethyl ester (SDZ-205557: 3 mg/kg, s.c.) did not modify the firing activity and the increasing effect of WAY-100635. These results suggest that, in quiet awake rats, endogenous 5-HT would tonically inhibit the spontaneous firing activity of the CA1 pyramidal neurons mainly through stimulating 5-HT_{1A} receptors.

[Neuropsychopharmacology 24:141–151, 2001]

KEY WORDS: *Endogenous serotonin (5-HT); Awake; Unrestrained; CA1 pyramidal neuron, Firing activity; Tonic inhibition, 5-HT_{1A} receptor*

It has been shown that there are at least 14 serotonin (5-HT; 5-hydroxytryptamine) receptor subtypes over seven distinct receptor subfamilies. Among these seven receptor subfamilies, only the 5-HT_1 subfamily is inhibitory whereas the others are excitatory (see Hoyer et al. 1994; Glennon and Dukat 1995 for reviews). Several *in situ* hybridization studies have demonstrated that ten of these 14 receptor subtypes are detectable over the CA1 region of the rat hippocampus (see Andrade 1998 for review).

In accordance with the distribution and exuberance of 5-HT receptors expressed in this area, several *in vitro* electrophysiological studies have demonstrated multiple actions of 5-HT on the excitability of the CA1 pyramidal neurons. These are the direct inhibitory and exci-

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Received 12 April 2000; revised 14 July 2000; accepted 19 July 2000.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/01/$–see front matter
PII S0893-133X(00)00181-0

tatory actions through stimulating 5-HT_{1A} and 5-HT_4 receptors on the pyramidal neurons, respectively. In addition, via inhibitory interneurons, 5-HT indirectly decreases and increases the excitability through stimulating 5-HT_2 and 5-HT_3, and 5-HT_{1A} receptors, respectively (see Hoyer et al. 1994; Aghajanian 1995; Andrade 1998 for reviews). Given these extremely diverse actions of 5-HT demonstrated *in vitro*, endogenous 5-HT should produce multiple actions simultaneously on the excitability of the CA1 pyramidal neurons *in vivo.*

These *in vitro* data have been obtained with the preparations isolated from various potentially confounding factors, such as the effects of activation of other receptor systems. Contrary, *in vivo* cells in the brain are exposed continuously to the various neurotransmitter, neuromodulator and hormonal stimuli. In any cell, these stimuli are integrated spatially and temporally. Furthermore, the intracellular signaling cascades are extensively interconnected. Moreover, even *in vivo*, the use of anesthesia drastically reduces the 5-HT tone (Jacobs 1985; Romero et al. 1997) as well as the spontaneous firing activity of the hippocampus pyramidal neurons (Blier et al. 1993). The reduction in the firing activity indicates that the use of anesthesia *per se* markedly decreases the excitability of hippocampal pyramidal neurons. Thus, an overall action of endogenous 5-HT on the firing activity of the pyramidal neurons in awake rats is unpredictable solely basing on the data obtained from *in vivo* studies with anesthetized animals and from *in vitro* experiments. Therefore, using awake animals is essential to scrutinize the influence of endogenous 5-HT. To our knowledge, such studies examining the net effect of endogenous 5-HT on cellular activity in the brain of awake rats have not been performed so far.

Thus, the present study was carried out to evaluate the net influence of endogenous 5-HT on the spontaneous firing activity of the dorsal hippocampus CA1 pyramidal neurons of awake, unrestrained rats. In this line, an effect of a 5-HT depletion by a 5-HT synthesis inhibitor parachlorophenylalanine (PCPA) and that of a selective 5-HT_{1A} receptor blockade with a 5-HT_{1A} antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635) (Foster et al. 1995; Fletcher et al. 1996) on the firing activity were examined. Effects of other 5-HT_{1A} antagonists WAY-100135 and (-)pindolol (Fletcher et al. 1993) were also examined. Furthermore, interactions of WAY-100635 with a 5-HT_{2A} antagonist ritanserin (Leysen et al. 1985) and with a 5-HT_4 antagonist 2-methoxy-4-amino-5-chloro benzoic acid 2-(diethylamino) ethyl ester (SDZ-205557) (Eglen et al. 1995) were tested, respectively.

MATERIALS AND METHODS

Animals

Adult male Wistar rats, weighing 250–300 g on the days of the surgery were used. Animals were kept in standard laboratory conditions (12:12 light/dark cycle with free access to food and water, at a room temperature of 23 ± 0.5°C). The experimental protocols were approved by the committee for laboratory animal welfare and ethics of Nihon University School of Medicine and met the National Institutes of Health Guide for the care and use of Laboratory animals. In general, the animals were subjected to multiple (1–4) antagonist experiments. Intervals of at least three days were allowed between the experiments. The animals used in the PCPA and its control experiments were sacrificed immediately after these experiments.

Surgical and recording procedures are essentially same as described in Tada et al. (1999). The data analyzing procedure was modified in the present study, which will be mentioned below.

Surgery

The surgery was carried out under pentobarbital anesthesia (60 mg/kg, i.p) with supplementary injections as necessary. A microelectrode bundle, consisting of two tungsten electrodes (15 μm in a tip diameter each), was permanently fixed to a microdrive. The combination of the electrodes and the microdrive was attached to a stereotaxic manipulator and then lowered to place the tips of the electrodes within the cortex above the dorsal hippocampus CA1 region (coordinate: AP; 4.0 mm anterior to lambda, LM; 2.7 mm lateral to the midline, V; 1.0 mm ventral to the cortical surface). Four stainless steel screws were threaded into the skull of bilateral frontal and temporal area for anchors and the ground. The entire apparatus was anchored to the skull with dental cement.

Recording Procedure

After the recovery period of at least seven days, animals were subjected to the recording and drug administration. All data collection was performed during the latter half of the light segment of the light/dark cycle. Animals were placed in an experimental chamber (40 × 40 × 40 cm) where all experimental trials took place. Electrical activities were oscilloscopically monitored throughout from the start of the electrode advancing procedure to the end of data collection. By using the microdrive, the electrodes were advanced in small steps (<25 μm) until stable unit activities with characteristics of pyramidal neurons were encountered (Figure 1A). Extracellular unit activities from the microelectrodes (band-pass; 0.3–10 kHz) were stored on a magnetic tape using a data recorder (MR 20; TEAC, Tokyo, Japan; Tape speed 38 cm/sec).

Simultaneously, verbal descriptions and electrical signals indicating the location and direction of the rat's body and head, and its behavioral states were recorded on the same magnetic tape. To eliminate cable movement artifacts, an operational amplifier (TL074CN;

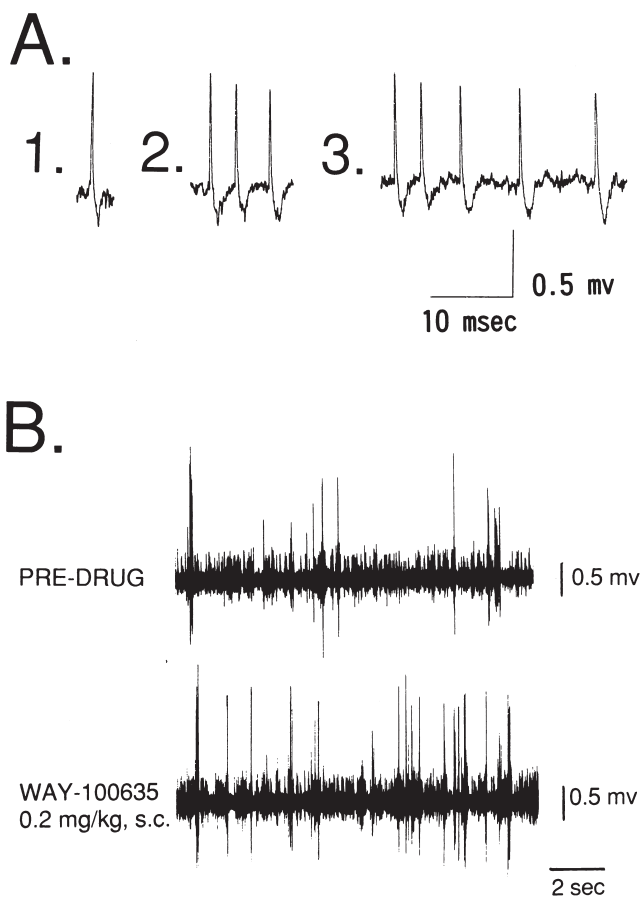

Figure 1. Typical unit recordings of hippocampal CA1 pyramidal neurons in awake, unrestrained rats. **(A)** A representative thermal recorder tracings of complex-spike cell firings obtained from the same neuron. **(B)** A response of pyramidal neurons to s.c. injection of WAY-100635 (0.2 mg/kg). WAY-100635 increased the firing activity.

Texas Instruments, Dallas, TX) was used. The unit activities were then printed out on papers by a thermal recorder (RTA 1200; Nihon Kohden Tokyo Japan). The further analysis of the activity was performed by the visual inspection of these paper tracings (time resolution: 3.16 msec/cm).

After the experiments, rats were anesthetized deeply with lethal dose of pentobarbital, and direct anodal current was passed through the recording electrode (20 μA for 30 sec). Then the rat was perfused intracardially with 10% formalin. The location of the recording site was examined histologically

Identification and Isolation of CA1 Pyramidal Neurons

Criteria for a complex-spike cell were as follows: 1) the neuron showed a single spike or a spontaneous burst of 2–9 action potentials of decreasing amplitude and increasing duration; 2) the inter-spike intervals were less than 10 msec; 3) the negative spike duration was 0.4–0.6 msec; and 4) the frequency of the firing event was less than 5 Hz. The criteria were generated on the basis of the previous studies (Fox and Ranck 1975; Suzuki and Smith 1985). Recordings with a low signal-to-noise ratio (<5:1) were not employed for data analysis. When necessary, single neuron isolation from the multiple unit recording was carried out using the paper tracings of the activities printed out by the thermal recorder. The waveforms of unit activities were traced onto semi opaque papers, then the individual neurons were distinguished each other basing on both amplitudes and shapes of spikes. When the shape and amplitude of the single and first spikes of given firing events are same (differences in the amplitudes were less than 5% of a given spike), these firing events were regarded as being originated from one pyramidal cell.

Data analysis and Estimated Items

Two or three 10.42-sec recording segments during pre- and post-drug sessions were subjected to the further data analysis, respectively. The numbers of the segments were identical between the pre- and post-antagonist sessions of each experiment. In each experiment, the pre- and post-drug data analyzed were chosen from the recordings obtained when the rat showed no observable body movement with its eyes open. Moreover, we selected the segments so as to the position and direction of the body and head of the rat in the experimental chamber were consistent across the pre- and post-drug segments. The segments were selected referring to the verbal descriptions and electrical signals indicating the specific behavioral condition of rats recorded on the same magnetic tape. This procedure was necessary because the firings of hippocampal pyramidal cells are often space-specific and dependent on behavioral states (O'Keefe and Dostrovsky 1971).

The effect of the drug was evaluated by direct comparison of absolute value of estimated items between the pre- and post-drug sessions for each neuron. The four estimated items were: 1) the number of firing events/sec (event rate: Hz); 2) the numbers of spikes/sec (spike frequency: Hz): 3) the average of the number of spikes per firing event (spikes/event); and 4) the proportion of complex spike firings among whole firing events of a neuron appeared in the selected 10-sec segments of a given session (CS%). Details of these items are mentioned below referring Figure 1A that shows typical thermal recorder tracings of the firing activities of a complex spike cell.

1. Event rate. We counted each of single-spike and complex-spike firings as one "firing event" regardless of the number of action potentials appeared in an event. Thus, each of the firing events presented in Figure 1A (1–3) was counted as one event. When

these three firing events appear in a 10-sec segment, the estimated event rate is 0.3 Hz.
2. The spike frequency represented the number of action potentials of a neuron per second. We counted the firing activities presented in Figure 1A (1–3) as one, three and five spikes, respectively. When these activities are recorded during a 10-sec segment, the spike frequency is 0.9 Hz.
3. The spike frequency divided by the event rate equaled the average number of spikes per firing event (spikes/event) for each session. Thus, in the case of Figure 1A, the estimated value is three spikes/event.
4. In Figure 1A, among the three firing events, two events are complex spike firings. Thus, CS% of this Figure 1A example is 66.7%.

When the estimated event rate of a neuron during a pre-dug session (baseline) was 0.2 Hz and lower, the neuron was excluded from the data to avoid misinterpretation of the drug effects due to under- or over- estimation of the firing frequency. If the firing frequency is low, the appearance of one firing event could cause a drastic change in the evaluation. For example, when only one event appears during each of two selected 10-sec tracings, the estimated event rate is 0.1 Hz. If another firing event had occurred several 10 msec before or after each of two tracings, the evaluation might be approximately 0.2 Hz. Thus, it seems reasonable to exclude neurons of such a low event rate in order to reduce such inadequate evaluation.

Drug-Effect Evaluation

An effect of the antagonist was evaluated by a direct comparison between the pre- and post-drug absolute values of the four items of individual neurons. The post-drug sessions for WAY-100135, (-) pindolol and WAY-100635 started 10 min after the s.c. injections and lasted up to 5 min. Effects of SDZ-205557 (s.c.) alone were examined 5, 10, and 20 min after its injection. Effects of ritanserin (i.p.) itself were evaluated 10, 20, 30, and 40 min after its injection. SDZ-205557 and WAY-100635 were simultaneously injected and their interaction was evaluated 10 min after the administration. To see the interaction between ritanserin and WAY-100635, ritanserin was injected i.p. 30 min prior to the administration of WAY-100635. Also in this case, the post-drug sessions occurred 10 min after the injection of WAY-100635. For the control of the ritanserin plus WAY-100635 experiments, an equivalent volume (1 ml/kg) of the vehicle (0.1 M tartrate) plus WAY-100635 experiments were performed on the same time schedule.

PCPA (500 mg/kg/day, i.p.) was injected for three successive days. On the fourth day, the effects of WAY-100635 were examined to see if the depletion of 5-HT could modify the effects of WAY-100635. In addition, effects of the 5-HT depletion alone on the spontaneous firing activities were evaluated by comparing the baseline values of the neurons between PCPA-treated and the vehicle-received (control: 0.5% solution of gum arabic, 5 ml/kg, i.p. for three days) rats.

Statistics

Data were expressed as means ± SEM. The effect of the 5-HT depletion with PCPA on the baseline firing activities was evaluated by use of a two-tailed unpaired Student's t-test. The effects of (-) pindolol, WAY-100135, and WAY-100635 were analyzed by use of a paired Student's t-test. Interactions of ritanserin and SDZ-205557 with WAY-100635 were assessed by using a two-way analysis of variance (ANOVA) for repeated measures followed by a least-significant-difference test. All statistical analysis was carried out by comparing the pre- and post-drug absolute values of an individual neuron. A probability value of $p < .05$ was considered as statistically significant.

Drugs

Ritanserin (Janssen, Amsterdam, The Netherlands) was dissolved in 0.1 M tartaric acid. (-) Pindolol (Novartis, Basel, Switzerland) was dissolved in a minimum amount of 1M acetic acid and made up to volume with 0.9% NaCl. WAY-100135 (Mitsubishi-Tokyo Pharmaceuticals, Tokyo, Japan) was suspended in a 5% carboxymethylcellulose solution. WAY-100635 (Wyeth-Ayerst, Princeton, NJ) and SDZ-205557 (Research Biochemicals, Natick, MA) were dissolved in 0.9% NaCl. PCPA (Tokyo Kasei, Tokyo, Japan) was injected i.p. as a fine suspension in a 0.5% solution of gum arabic with a drug concentration of 100 mg/ml. All drugs were freshly prepared.

RESULTS

All the recording sites were located in the dorsal hippocampus CA1 region.

Effect of a 5-HT Depletion by PCPA on Baseline Firing Activity of the Pyramidal Neurons

The numbers of neurons recorded per rat were similar between vehicle- and PCPA-treated rats (vehicle: 2.47 ± 0.24 neurons/rat from 17 rats, PCPA: 2.50 ± 0.22 neurons/rat from 16 rats). Figure 2 shows the distributions of the event rates and spike frequencies of the pyramidal neurons recorded from vehicle- ($n = 42$) and PCPA-treated rats ($n = 40$). These two baseline values of the 5-HT-depleted rats were significantly higher than those of control rats (event

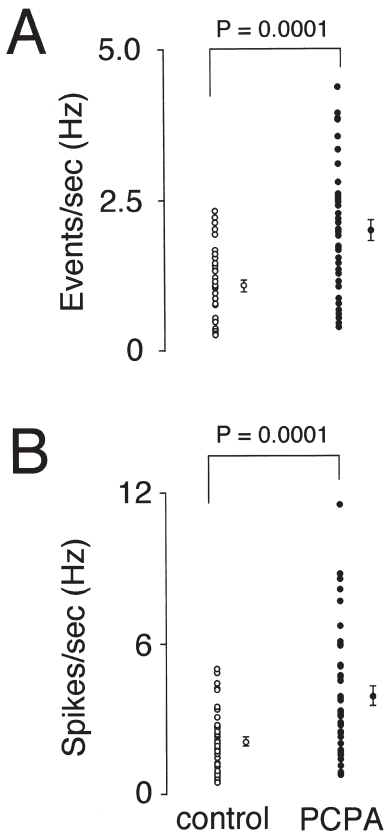

Figure 2. An effect of a 5-HT depletion with three-day PCPA treatment. Distributions of the baseline event rate **(A)** and spike frequency **(B)** of the dorsal hippocampus CA1 pyramidal neurons recorded from control (open circle: n = 42) and 5-HT-depleted rats (closed circle: n = 40). Each data point represents each value of a neuron.

rate: control 1.07 ± 0.01 Hz vs. PCPA 2.20 ± 0.22 Hz, t_{82} = 4.653, p = .0001; spike frequency: control 2.09 ± 0.19 Hz vs. PCPA 4.41 ± 0.49 Hz, t_{82} = 4.288, p = .0001).

The other parameters, spikes/event and CS%, were not different between the two groups (spikes/event: control 2.02 ± 0.05 vs. PCPA 2.00 ± 0.07, CS%: control 65.97 ± 3.54% vs. PCPA 62.90 ± 3.21%). The event rate of the control was comparable with the rates previously reported during this awake immobile behavioral state (Suzuki and Smith 1985; Tada et al. 1999).

Effects of 5-HT Antagonists on the Spontaneous Firing Activity of Dorsal Hippocampus CA1 Pyramidal Neurons

All of the 5-HT antagonists tested in the present study, WAY-100635, WAY-100135, (-)pindolol, ritanserin, and SDZ-205557, did not change the spikes/event and CS%. The spikes/event of all of the sessions ranged from 1.87 to 2.33. The CS% values were within 59.7–85.1%.

Saline (0.5 ml/kg, s.c.) did not change the event rate (109.8 ± 5.7% of the baseline) and spike frequency (106.1 ± 7.6% of the baseline), indicating the stability of the present measures (Figure 3). As illustrated in Figures 1B and 3, the subcutaneous administrations of a selective 5-HT_{1A} antagonist WAY-100635 significantly increased the event rates of CA1 pyramidal neurons to more than 130% of the respective baselines at doses of 0.03–0.2 mg/kg (0.03 mg/kg; t_{20} = 3.167, p = .0049: 0.1 mg/kg; t_{20} = 2.47, p = 0.0221: 0.2 mg/kg; t_{17} = 3.125, p = .0062). The effect of 0.01 mg/kg of this compound on the event rate did not reach statistical significance (136.9 ± 13.2% of the baseline: t_{20} = 1.787, p = .089. (Figure 3A).

Similarly, WAY-100635 significantly increased the spike frequency at doses of 0.1 and 0.2 mg/kg (0.1 mg/kg; t_{20} = 2.378, p = .0275: 0.2 mg/kg; t_{17} = 2.182, p =

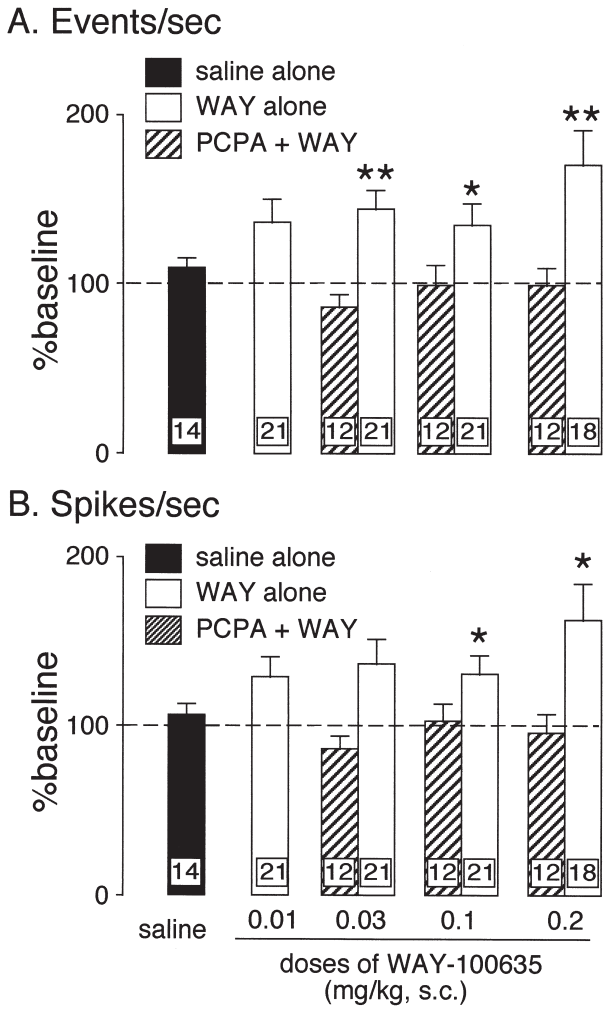

Figure 3. Influence of s.c. injections of saline and WAY-100635 on the event rate **(A)** and spike frequency **(B)**. In naive rats, saline (closed columns) did not change these values whereas WAY-100635 significantly increased the event rate and spike frequency (open columns). After the 5-HT depletion, the increasing effect of WAY-100635 was completely abolished (hatched columns). The numbers of neurons tested are given at the bottom of each column. *$p < .05$, **$p < .01$ vs. each pre-drug (baseline) value (paired Student's t-test).

.0434). The 0.01 and 0.03 mg/kg of WAY-100635 also increased the spike frequency to 129.2 ± 11.8% and 137.0 ± 14.4% of the baseline without statistical significance, respectively (0.01 mg/kg: $t_{20} = 1.663$, $p = .1119$; 0.03 mg/kg: $t_{20} = 2.044$, $p = .0544$. (Figure 3B).

After the depletion of endogenous 5-HT with the three-day PCPA pretreatment (500 mg/kg/day, i.p.), WAY-100635 did not produce any change in the four evaluated items including the event rate and firing frequency at the doses of 0.03-0.2 mg/kg (Figure 3).

WAY-100135, at doses of 5 and 20 mg/kg, did not exert any effect on the four parameters. Although this compound seemed to increase the event rate and spike frequency to 129.0 ± 23.4% and 129.7 ± 27.5% of the baselines at a dose of 5 mg/kg, respectively, there was no statistical significance ($t_9 = 0.289$, $p = .779$ and $t_9 = 0.015$, $p = .9882$) (Figure 4).

(-)Pindolol, at a dose of 1 mg/kg, did not cause any changes in the event rate and spike frequency. However, at a dose of 3 mg/kg, (-)pindolol significantly decreased the event rate and spike frequency to 71.5 ± 6.7% ($t_{16} = 4.464$, $p = .0004$) and 77.5 ± 8.1% ($t_{16} = 2.689$, $p = .0161$), respectively.

Ritanserin (1 mg/kg, i.p.) alone did not alter the event rate and spike frequency 10, 20, 30, and 40 min after the injections (data not shown). Similarly, SDZ-205557 (3 mg/kg, s.c.) itself did not change these parameters 5, 10. and 20 min after the injections (data not shown also).

Interactions of 5-HT_{2A} and 5-$HT_{3/4}$ antagonists with the increasing effect of WAY-100635

WAY-100635 (0.2 mg/kg) administered with a 5-$HT_{3/4}$ antagonist SDZ-205557 (3 mg/kg, s.c.) produced significant increases in the event rate (153.6 ± 10.9% of the baseline, $t_{14} = 5.371$, $p = .0001$) and in the spike frequency (149.8 ± 10.2% of the baseline, $t_{14} = 5.1$, $p = .0002$). The extents of the increases were slightly smaller than those caused by WAY-100635 alone (event rate and spike frequency: 170.7 ± 20.0% and 162.4 ± 21.5% of the baselines, respectively) (Figures 3 and 5). The difference in the extents was not statistically significant (Figure 5).

When WAY-100635 (0.2 mg/kg, s.c.) was injected 30 min after the 5-HT_{2A} antagonist ritanserin (1 mg/kg, i.p.), WAY-100635 produced significant increases in the event rate (158.7 ± 22.3% of the baseline, $t_7 = 3.137$, $p = .0164$) and in the spike frequency (160.4 ± 21.6% of the baseline, $t_7 = 2.855$, $p = .0245$). The degrees of these increases were identical to those obtained in the control, 0.1 M tartrate plus WAY-100635 (event rate: 155.8 ± 15.7% of the baseline, $t_{11} = 4.258$; $p = .0013$; spike frequency: 161.1 ± 11.4% of the baseline, $t_{11} = 3.511$, $p = .0049$) (Figure 5).

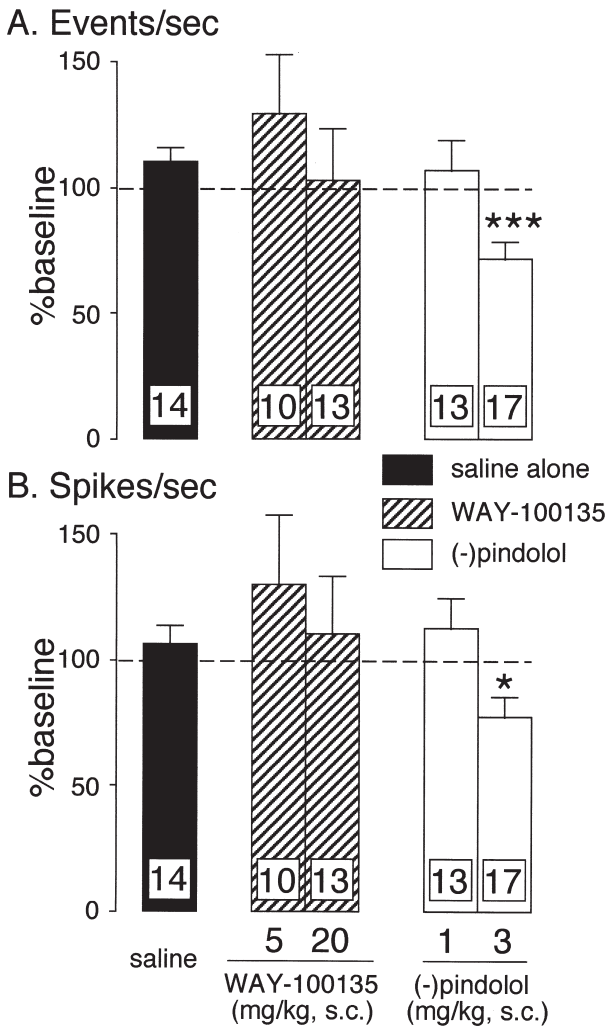

Figure 4. Influence of s.c. injections of WAY-100135 and (-)pindolol on the event rate **(A)** and spike frequency **(B)** of naive rats. WAY-100135 (hatched columns) produced small and insignificant increases in the event rate and spike frequencies at a dose of 5 mg/kg. The higher dose (20 mg/kg) of this drug did not change these parameters. (-)Pindolol (open columns) did not exert any effect on these parameters at a dose of 1 mg/kg, whereas this compound significantly decreased them at a dose of 3 mg/kg. For the reference, data on saline injection are also presented (closed columns). The numbers of neurons tested are given at the bottom of each column. $*p < .05$, $***p < .001$ vs. each pre-drug value (paired Student's t-test).

DISCUSSION

The present *in vivo* electrophysiological experiments using unanesthetized rats revealed that the baseline event rate and spike frequency of the CA1 pyramidal neurons after the three-day PCPA treatment (500 mg/kg/day, i.p.) were significantly higher when compared to those after the vehicle-administration (Figure 2). It was also shown that the numbers of neurons recorded from one rat were similar between PCPA- and vehicle-treated

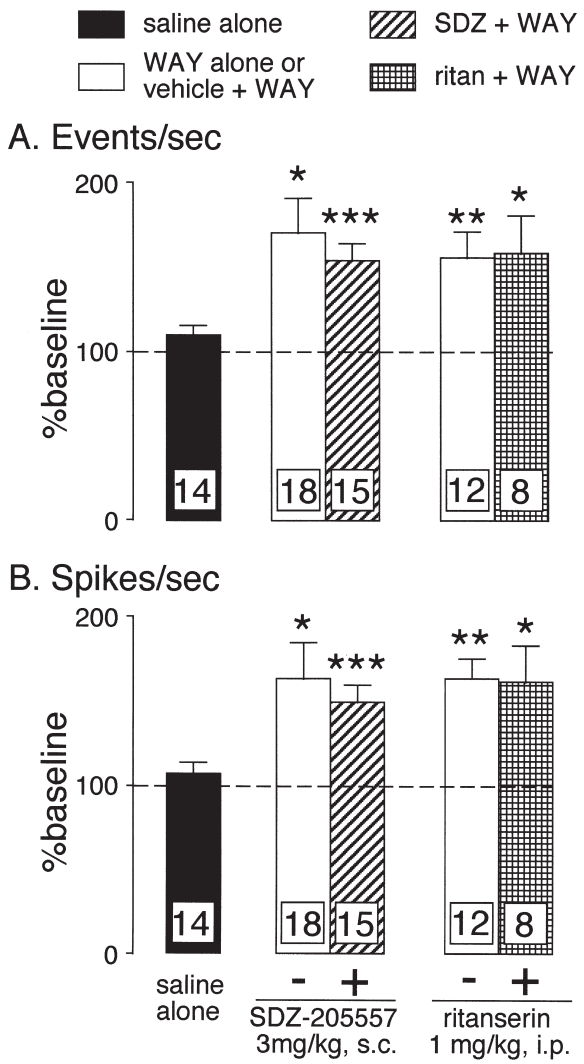

Figure 5. Influence of SDZ-205557 and ritanserin on the increasing effect of WAY-100635. The data, the event rates **(A)** and spike frequencies **(B)**, are expressed as the percentages of pre-WAY-100635 values (baselines). For the reference, data on saline injection are also presented (closed columns). WAY-100635 (0.2 mg/kg) administered with a 5-$HT_{3/4}$ antagonist SDZ-205557 (3 mg/kg, s.c.; hatched columns) produced significant increases in the event rate **(A)** and the spike frequency **(B)**. The extents of the increases were slightly smaller than those caused by WAY-100635 alone (open columns of the middle part). However, the difference was not statistically significant (a two-way repeated measure ANOVA followed by a least-significant-difference test). A 5-HT_{2A} antagonist ritanserin (1 mg/kg, i.p.; meshed columns) did not modify the increasing effects of WAY-100635 (0.2 mg/kg) on the event rate **(A)** and in the spike frequency **(B)** as indicated by the identical degree of the increase with the control, 0.1 M tartrate plus WAY-100635 (open columns of the right part). The numbers of neurons tested are given at the bottom of each column. $^{*}p < .05$, $^{**}p < .01$ vs. each pre-WAY-100635 value (paired Student's t-test).

groups, indicating the PCPA treatment caused an increase in the activity of individual neurons rather than that in the number of active neurons during the recordings. Inconsistently, we have recently claimed that the baseline event rate of the PCPA-treated rats was not significantly different from that of naive rats (Tada et al. 1999). Such inconsistency between our present and previous results might be due to the limited number of neurons tested in the previous study.

The previous value after the PCPA treatment (1.65 ± 0.45 Hz, $n = 14$) was lower than the present value (1.98 ± 0.17 Hz, $n = 40$). The present variability was much smaller than the previous one as indicated by the SEMs. In the present study, the conclusion was derived from comparisons of the values between vehicle- and PCPA-treated groups. However, the previous study lacked the control. Accordingly, the present data reaching a statistical significance seem more reliable than the previous ones. Several studies have demonstrated that such PCPA treatment depletes 5-HT in the hippocampus to less than 5% of the control level (Vanderwolf and Baker 1986; Kasamo et al. 1994). Thus, it was feasible that the 5-HT depletion with PCPA increased the firing activity of the pyramidal neurons as indicated by the significantly higher values of the event rate and spike frequency of the PCPA group. Therefore, it was likely that an overall effect of endogenous 5-HT on the firing activity of the pyramidal neurons of quiet awake rats might be a tonic inhibition. This interpretation is further supported by the other present observations that the selective 5-HT_{1A} receptor blockade with WAY-100635 increased the spontaneous firing activity and that the PCPA pretreatment abolished this increasing effect (Figures 1 and 3). Taken together, these present results suggested that endogenous 5-HT exerts the tonic inhibition through stimulating 5-HT_{1A} receptors.

Recently, using chloral hydrate-anesthetized rats, Haddjeri et al (1998) have observed that acute intravenous administration of WAY-100635 (0.1 mg/kg) does not change the firing activity of dorsal hippocampus CA3 pyramidal neurons in control rats whereas it increases the firing activity in rats that had received a chronic antidepressant treatment. It has been well documented that the several classes of the long-term antidepressant treatments consistently enhance the 5-HT neurotransmission (Blier and de Montigny 1994). Given the claims that the use of anesthesia drastically reduces the 5-HT tone (Jacobs 1985; Romero et al. 1997), the observations by Haddjeri et al. (1998) suggest that the increasing effect of WAY-100635 may appear only when the 5-HT neurotransmission is at least modestly active. This interpretation is in the almost complete agreement with the present observations that the depletion of endogenous 5-HT abolished the increasing effect of WAY-100635 in awake rats (Figure 3). Furthermore, Haddjeri et al. (1998) have demonstrated that inactivation of hip-

pocampal $G_{i/o}$-coupled 5-HT_{1A} receptors with the intrahippocampal injection of pertussis toxin completely abolishes the increasing effect of WAY-100635, indicating the site of the increasing action was the intrahippocampal 5-HT_{1A} receptors of the antidepressant-treated, 5-HT active, anesthetized rats. Although the precise site of the increasing action of WAY-100635 in the present study is still unclear since the interactions among regions, neurons and neurotransmitters should be more complicated in awake animals, the observation by Haddjeri et al. (1998) would suggest that, in the present study, WAY-100635 exerted the increasing action mainly through blocking a tonic inhibitory influence of endogenous 5-HT via postsynaptic 5-HT_{1A} receptors probably located in the hippocampus.

Since the discharge activity of central 5-HT neurons is regulated by a local negative feedback mechanism through somatodendritic 5-HT_{1A} autoreceptors (Aghajanian 1978; Blier et al. 1989; Blier and de Montigny 1994), systemic administration of WAY-100635 to awake rats can increase extracellular 5-HT levels by blocking this negative feedback loop. Given the excitatory nature of the 5-HT receptors other than the 5-HT_1 subfamily (Hoyer et al. 1994; Glennon and Dukat 1995), such a presumed increase in the availability of 5-HT might facilitate the excitability of the CA1 pyramidal neurons through enhancing the activation of the excitatory 5-HT receptors. However, several microdialysis experiments using awake rats have shown that WAY-100635 does not change the extracellular 5-HT levels in various forebrain regions including the at doses up to 1 mg/kg, s.c. and up to 10 mg/kg, i.p. (Bosker et al. 1996; Matos et al. 1996; Romero et al. 1996; Romero and Artigas 1997; Trillat et al. 1998). These doses are remarkably higher than the present ones that increased the firing activity (0.03 – 0.2 mg/kg) (Figures 1B and 3). Thus, it seems unlikely that the increasing effect of WAY-100635 was produced indirectly through such enhancements of the stimulation of the excitatory 5-HT receptors. Therefore, it is conceivable that the increasing effect was produced by the blockade of 5-HT_{1A} receptors *per se.*

Among excitatory 5-HT receptors, 5-HT_4 receptors are densely distributed in the rat hippocampus including the pyramidal cell layer of the CA1 field (Waeber et al. 1994; Eglen et al. 1995). Previous *in vitro* electrophysiological studies have shown that applications of 5-HT onto the CA1 pyramidal neurons produce both hyperpolarization and deporalization through the activation of 5-HT_{1A} and 5-HT_4 receptors, respectively (Chaput et al. 1990; Andrade and Chaput 1991; Roychowdhury et al. 1994; Torres et al. 1995). It has been also demonstrated that, in the presence of WAY-100635, the 5-HT applications solely facilitate the firing activity through stimulating 5-HT_4 receptors, *in vitro* (Corradetti et al. 1996; Pugliese et al. 1998). Such a concomitant excitatory effect of endogenous 5-HT would be masked by a tonic inhibition through 5-HT_{1A} receptors. Therefore, in the present study, it seems reasonable to assume that the selective and "silent" 5-HT_{1A} receptor antagonist WAY-100635 (Foster et al. 1995; Fletcher et al. 1996) increased the firing activity through unmasking the endogenous 5-HT-produced activation of the excitatory 5-HT receptors including the 5-HT_4 subtypes. In addition, it has been shown that 5-HT indirectly decreases the excitability of the CA1 pyramidal neurons through stimulating 5-HT_2 receptors on inhibitory interneurons (see Hoyer et al. 1994; Aghajanian 1995; Andrade 1998 for reviews). Thus, the involvement of 5-HT receptors other than the 5-HT_{1A} subtype in the increasing effect should be also taken into account.

However, a selective 5-HT_{2A} antagonist ritanserin (1 mg/kg, i.p.) (Leysen et al. 1985) did not modify the increasing effect of WAY-100635 (Figure 5). This is a supra-sufficient dose of ritanserin to block a 5-HT_{2A} receptor-mediated behavior of rats (Costall and Naylor 1995; Imeri et al. 1999). Thus, the involvement of 5-HT_{2A} receptors could be ruled out whichever this excitatory 5-HT receptor facilitates or diminishes the excitability of the pyramidal neurons. It has been shown that the present dose of a 5-$HT_{3/4}$ antagonist SDZ-205557 (3 mg/kg, s.c.) (Eglen et al. 1995) is the supra-maximal dose to block the behavior induced by a 5-HT_4 receptor-stimulation in rats (McMahon and Cunningham 1999). This antagonist slightly attenuated the increasing effect of WAY-100635 (Figure 5). Although there was not statistical significance, this attenuating effect of SDZ-205557 suggested that the increasing effect of WAY-100635 could be, in part, ascribed to the stimulation of 5-HT_4 receptors by endogenous 5-HT.

Previously, we have shown that a 5-HT_{1A} antagonist NAN-190 does not change the firing activity (Tada et al. 1999). Similarly, WAY-100135 did not change the firing activity in the present study (Figure 4). Previous microdialysis studies have shown that NAN-190 and WAY-100135 act as partial agonists at somatodendritic 5-HT_{1A} receptors and decrease *in vivo* 5-HT release (Hjorth and Sharp 1990; Assié and Koek 1996; see Fletcher et al. 1993 for review). In keeping with the effect of these antagonists on the 5-HT output, it is suggested that NAN-190 and WAY-100135 decreased the extracellular 5-HT levels in our previous and present studies (Tada et al. 1999). When the 5-HT level decreased, stimulation of 5-HT receptors other than 5-HT_{1A} class by endogenous 5-HT might be reduced in the presence of these 5-HT_{1A} antagonists. Therefore, the lack of the increasing effect of NAN-190 and WAY-100135 on the firing activity would be compatible with the assumption that WAY-100635 increased the firing activity through unveiling the activation of the excitatory 5-HT receptors including the 5-HT_4 subtype.

Interestingly, another 5-HT_{1A} antagonist (-)pindolol decreased the firing activity (Figure 4). It has been dem-

onstrated that (-)pindolol increases extracellular 5-HT in the cat brain (Fornal et al. 1999) and that this drug does not antagonize postsynaptic 5-HT_{1A} receptors in the rat hippocampus (Artigas et al. 1996). These observations indicate the decrease in the firing activity could be produced by the (-)pindolol-induced increase in the extracellular 5-HT. This interpretation is in agreement with the abovementioned assumption that an overall effect of endogenous 5-HT is inhibitory since (-)pindolol might not exert any antagonistic activity at postsynaptic 5-HT receptors of the rat brain *in vivo* (Artigas et al. 1996). However, Sprouse et al. (1998) have shown that pindolol, an isomer of (-)pindolol possessing a less affinity for 5-HT_{1A} receptors and a greater affinity for *beta*-adrenoceptors, decreases the firing activity of CA3 pyramidal neurons of chloral hydrate-anesthetized rats. Since the decreasing effect of pindolol was not antagonized by WAY-100635, the authors suggested the non-5-HT_{1A} multiple receptor mechanism with the obvious possibility for the blockade of *beta*-adrenoceptors (Sprouse et al. 1998). Given the similarity between this previous (Sprouse et al. 1998) and the present studies in terms of the experimental animals, the targeted area and the evaluated activity, the present (-)pindolol-induced decrease in the firing activity could be attributable to the non-5-HT_{1A} multiple receptor mechanisms, especially to the blockade of *beta*-adrenoceptors. However, at present, it is unclear whether the decreasing effect of (-)pindolol was produced by its 5-HT_{1A} antagonistic, *beta*-blocking, or other known and unknown pharmacological properties. Further studies are required to elucidate it.

The drugs examined in the present study could change behavioral states of the animals. Previous studies have demonstrated that PCPA and NAN-190 decrease slow wave sleep and increase wakefulness (Li and Satinoff 1992; Neckelmann et al. 1996), and that ritanserin increases slow wave sleep and decreases waking (Bjorvatn and Ursin 1990; Silhol et al. 1992). Suzuki and Smith (1985) have shown that the pyramidal neurons are most active during slow wave sleep, slightly less active during a quiet awake state, and least active during an active awake condition and paradoxical sleep. Thus, one might assume that the present observations reflect indirect influences of the tested drugs through their direct effects on the states of the animals. Given the correlation between the behavioral states and the firing activity (Suzuki and Smith 1985), ritanserin is expected to increase the firing activity since this drug could increase slow wave sleep and decrease wakefulness (Bjorvatn and Ursin 1990; Silhol et al. 1992). However, ritanserin did not change the firing activity (data not shown). Similarly, PCPA and NAN-190 are expected to reduce the firing activity through increasing wakefulness (Li and Satinoff 1992; Neckelmann et al. 1996) whereas PCPA increased and NAN-190 unchanged the firing activity (Figure 2) (Tada et al. 1999). Moreover, it has been demonstrated that a selective 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) increases the wakefulness (Monti and Hantos 1994) and decreases the firing activity (Tada et al. 1999), which is consistent with the behavioral correlates (Suzuki and Smith 1985). Taken together, the actual influences of the drugs on the firing activity were extremely different from the expectable indirect influences through the effects on the behavioral states. Thus, it is likely that the changes in the firing activity reflect the direct drug effects rather than the indirect influence via effects on the behavioral states.

In summary, the present data suggested that endogenous 5-HT, in the sum total, tonically inhibits the spontaneous firing activity of the dorsal hippocampus CA1 pyramidal neurons through stimulating postsynaptic 5-HT_{1A} receptors probably located in the hippocampus. However, the site of action was not precisely elucidated.

ACKNOWLEDGMENTS

This work was supported by grants from the Japanese Ministry of Education, Science, Sports and Culture (#09671012) to KK and the Pharmacopsychiatry Research Foundation to TK. The authors thank Ms. Naomi Anzai for her secretarial assistance.

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