ELSEVIER

European Journal of Pharmacology 398 (2000) 239–246

ejp

www.elsevier.nl/locate/ejphar

The effect of the acute administration of various selective 5-HT receptor antagonists on focal hippocampal seizures in freely-moving rats

Kei-ichiro Watanabe [a,e], Charles R. Ashby Jr. [b], Hiroshi Katsumori [c,e], Yoshio Minabe [d,e,*]

[a] *Department of Neuropsychiatry, Faculty of Medicine, University of Tokyo, Tokyo 113-8655, Japan*
[b] *PHS Department, St. John's University, 8000 Utopia Parkway, Jamaica, NY 11439, USA*
[c] *Neurology Service, Veterans Affairs Medical Center, Sepulveda, CA 91343-2099, USA*
[d] *Department of Psychiatry, Takamatsu Prefectural Hospital, Takamatsu, Ishikawa 929-1293, Japan*
[e] *National Institute of Neuroscience, Kodaira, Tokyo 0187-8502, Japan*

Received 16 December 1999; received in revised form 3 March 2000; accepted 24 March 2000

Abstract

In this study, we assessed the effects of the acute administration of various 5-HT receptor antagonists on hippocampal partial seizures generated by low-frequency electrical stimulation in male Wistar rats. The seizure threshold and severity were determined by measuring the pulse number threshold and primary and secondary afterdischarges, respectively, and the latency of secondary discharge was also determined. The administration of either the selective 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazineyl]ethyl]-*N*-(pyridinyl)-cyclohexanecarboximimde 3 HCl (WAY 100635, 0.1–1 mg/kg i.p.), the selective 5-HT_3 receptor antagonist granisetron (0.3–3 mg/kg i.p.), the selective 5-HT_{2A} receptor antagonist *R*-(+)-*a*-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl) ethyl]-4-piperidine-methanol (MDL 100907, 0.3–3 mg/kg i.p.) or the 5-$HT_{2B,C}$ receptor antagonist antagonist *N*-(1-methyl-5-indolyl)-*N*′-(3-pyridyl) urea HCl (SKB 200646A, 5–50 mg/kg i.p.) did not alter the pulse number threshold compared to vehicle-treated animals. However, the acute administration of WAY 100635 (0.3 mg/kg) and M100907 (1 mg/kg) significantly increased, whereas granisetron (1 mg/kg) decreased, the primary afterdischarge duration compared to vehicle-treated animals. The latency of secondary after discharge was significantly decreased by WAY 100635 (1 mg/kg) and granisetron (3 mg/kg) compared to vehicle-treated animals. These results suggest that in this model, the antagonism of 5-HT_{1A}, 5-HT_{2A}, 5-HT_3 or 5-$HT_{2B,C}$ receptors do not lower or raise seizure threshold. However, the antagonism of 5-HT_{1A} receptors may increase or augment seizure severity.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Kindling; Hippocampus; Seizure

1. Introduction

There is an accumulating evidence indicating that experimental manipulations that attenuate serotonergic neurotransmission can induce and/or augment epileptic seizures. Indeed, the depletion of brain 5-hydroxytryptamine (5-HT) levels by *p*-chlorophenylalanine and the serotonergic neurotoxin 5,7-dihydroxytrpytamine appears to exacerbate seizures that are chemically-induced and seizures in genetic models of epilepsy (Browning et al., 1978; Jobe et al., 1973; Racine and Coscina, 1979; Statnik et al., 1996). Furthermore, it has been postulated that the etiology of seizures experienced by a certain subset of epileptic patients may be related to a deficit in serotonergic neurotransmission (Giroud et al., 1990; Pranzatelli et al., 1995; Shaywitz et al., 1975; Verma et al., 1984). In contrast, manipulations that augment serotonin function, including fluoxetine, 5-HTP, certain anticonvulsants (Dailey et al., 1996; De La Torre et al., 1970; Laird and Jobe, 1987; Leander, 1992; Wada et al., 1993a,b; Yan et al., 1992, 1994) and dorsal raphe (Kovacs and Zoll, 1974; Lazarova et al., 1983, 1984) stimulation generally attenuate or suppress epileptic seizures in animals.

Evidence obtained from pharmacological, binding and molecular biological studies have demonstrated the presence of multiple receptors for 5-HT in the mammalian brain (Hoyer et al., 1994; Zifa and Fillion, 1992) and a number of selective 5-HT receptor subtype antagonists have been synthesized and pharmacologically characterized. However, at this time, no studies have examined the effect of selective 5-HT receptor antagonists on seizure threshold and/or severity in various animal models of

* Corresponding author. Tel.: +81-76-281-1125; fax: +81-76-282-5351.

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00258-2

epilepsy. Therefore, in this study, we examined the effect of the acute systemic administration of 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazineyl]-ethyl]-*N*-(pyridinyl)-cyclohexanecarboximimde 3 HCl (WAY 100635; Forster et al., 1995), the 5-HT_{2A} receptor antagonist *R*-(+)-*a*-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl) ethyl]-4-piperidine-methanol (M100907, Kehne et al., 1996; Schmidt et al., 1995), the 5-$HT_{2B,C}$ receptor antagonist *N*-(1-methyl-5-indolyl)-*N*′-(3-pyridyl) urea HCl (SKB 200646A, Forbes et al., 1993) and the 5-HT_3 receptor antagonist granisetron (Sanger and Nelson, 1989) on hippocampally-generated partial seizures, elicited by low frequency stimulation in freely moving male Wistar rats. The seizure threshold and severity of the seizures was determined by measuring the pulse number threshold and the afterdischarge duration (primary and secondary), respectively. This model was chosen since (1) it has behavioral similarities to temporal lobe epilepsy in humans; (2) the hippocampus is believed to be one of the sites of origin of partial complex seizures in humans; (3) the hippocampus has a moderate to high density of 5-HT_{1A} (Pompeiano et al., 1992; Pazos and Palacios, 1985), 5-HT_{2A} (Pompeiano et al., 1994; Pazos et al., 1985) and 5-HT_{2C} (Mengod et al., 1990) and has functional 5-HT_3 receptors; and (4) we have shown that in our model, the pulse number threshold is raised by carbamazepine (Watanabe et al., 1997; Katsumori et al., 1998), a first-line drug in the treatment of partial complex seizures in humans (McNamara, 1996).

2. Materials and methods

2.1. Animals

Male Wistar rats (Clea, Tokyo, Japan; $n = 82$), weighing 280 g at the time of surgery, were used in all experiments. The animals were housed in plastic cages with wood chip bedding in the animal care facility under constant temperature (23–25°C) and humidity (50–60%) on a 12 h light/12 h dark cycle (lights on at 0800 h). The animals were permitted access to food and water ad libitum. All experiments were conducted between 0900 and 1100 h.

2.2. Implantation of electrodes

The animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and placed in a stereotaxic frame under an electronically controlled heating pad to maintain the body temperature at 37–38°C. Tripolar electrodes (three twisted, 0.2 mm diameter polyurethane-coated stainless steel lines) were implanted bilaterally, with the tips placed in the dentate gyrus of the dorsal hippocampus (posterior 3.5 mm from bregma, lateral 2.0 mm from the sagittal sinus and ventral 4.0 mm from the cortical surface, according to the atlas of Paxinos and Watson, 1986). Stainless steel screws were placed in the skull and served as anchors and the reference electrodes. The electrodes were attached to the skull by dental acrylic cement and connected with a socket.

2.3. Stimulation procedure

After a post-operative period of 1 week, the animals received electrical stimulation pulses of 2 Hz for 12.5 s (biphasic square-wave pulses, 1 ms in duration at 500–800 μA, base-to-peak), using an automated, computer-assisted stimulation system (Nihon Koden, Sen-7103) and constant current units to elicit seizures. The stimulation frequency of 2 Hz was used because frequencies of 3 Hz or greater did not always permit us to determine the definitive onset of the afterdischarge due to the presence of stimulation artifacts on the electroencephalogram (EEG) recordings. All rats were stimulated once daily for 10 consecutive days without drug treatment. After determining that the seizure parameters were stable, the pharmacological experiments were conducted.

It is known that dentate gyrus kindling requires a greater number of electrical stimulations to progress than amygdaloid kindling and the behavioral seizure stage displays instability (Grace et al., 1990). However, we have previously shown (Katsumori et al., 1996, 1998) that with our stimulation parameters, the seizure parameters, such as afterdischarge duration and pulse number threshold, were stable during the period showing stage-1 seizures (i.e., immobility, facial grooming, wet-dog shake behavior or locomotion without a clonic component, based on the classification of Racine (1972)). Therefore, in this study, we only used animals that showed stage-1 partial seizures.

The afterdischarge duration, which is indicative of seizure severity, was determined by measuring the total time of epileptic discharges (with an amplitude that is at least equivalent to the height of the pre-stimulation background on the EEG), i.e., the primary and secondary afterdischarges, present on the EEG (see Fig. 1). The interval between the end of the primary afterdischarge and the beginning of the secondary afterdischarge is the latency of secondary afterdischarge. The pulse number threshold, defined as the number of stimulating pulses, required to elicit an afterdischarge, i.e., seizure threshold, was also measured (see Fig. 1). The behavior of the animals was videotaped using an EEG-VTR system (Nihon Koden, VY-440A).

2.4. Evaluation of drug treatments

Animals received intraperitoneal injections of either vehicle (40% w/v of 2-hydroxypropyl-betacyclodextrin, 1 ml/kg) WAY 100635 (0.1, 0.3 or 1 mg/kg), M100907 (0.3, 1 or 3 mg/kg), SKB 200646A (5, 15 or 50 mg/kg) or granisetron (0.3, 1 or 3 mg/kg) intraperitoneally 1 h

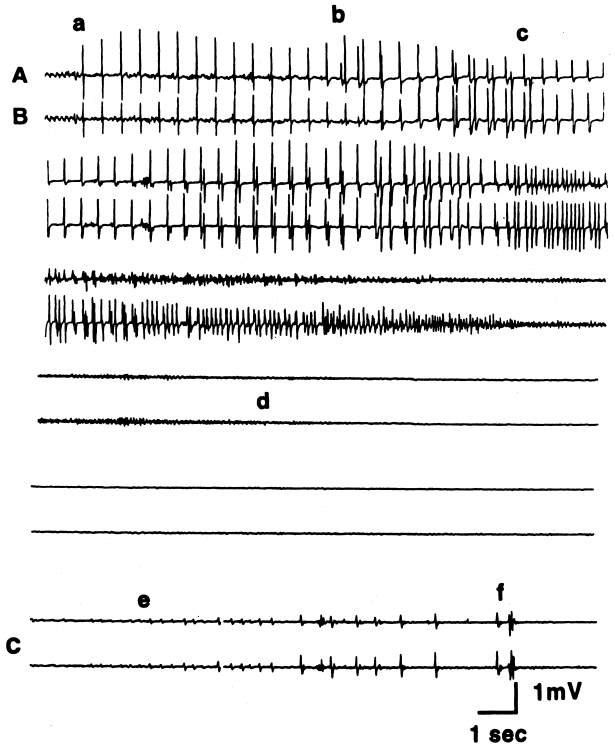

Fig. 1. (A), (B) Bilateral, intracranial EEG recording from the dentate gyrus during the 2-Hz kindling stimulation. Consecutive tracings are shown from top to bottom. (A) Ipsilateral recording of stimulated side; (B) contralateral recording; (a) beginning of kindling stimulation; (b) triggering of primary afterdischarge (primary AD); (c) termination of stimulation; (d), (e) termination of primary AD.
In this recording, the pulse number threshold is 14 (the number of stimulating pulses from (a) to (b)). The afterdischarge duration is the interval from (b) to (d).
(C) Bilateral, interictal EEG recording from the dentate gyrus. (e) Triggering of secondary afterdischarge (secondary AD); (f) termination of secondary afterdischarge.
In this recording, the secondary afterdischarge duration is the interval between (e) and (f). The interval between the end of the primary afterdischarge (d) and the beginning of the secondary afterdischarge (e) is the latency of secondary afterdischarge.

before each stimulation. The doses of WAY 100635, MDL 100907, SKB 200646A and granisetron used were based on previous studies indicating that the doses chosen will significantly antagonize the central effects of agonists at the 8-hydroxy-2-(di-*n*-propylamino)tetralin or 8-OH-DPAT (5-HT_{1A}; Fletcher et al., 1996; Cryan et al., 1999), [±]-1-(2,5-dimethoxy)-4-iodophenyl-2-aminopropane or DOI (5-HT_{2A}, a 5-$HT_{2A,C}$ agonist; Kehne et al., 1996), *meta*-chlorophenylpiperazine (*m*CPP)-induced hypophagia (5-HT_{2C}; Forbes et al., 1993, 1995), and 2-methylserotonin (5-HT_3 receptors; Ashby et al., 1991), respectively. The control recordings were done 2 days before (pre-drug controls) and 2 days after each drug treatment (post-drug controls). The effect of each treatment was evaluated by measuring the pulse number threshold, primary and secondary afterdischarge duration and the latency of secondary afterdischarge. If an afterdischarge was not identified after a low-frequency stimulation, twenty-five 10-Hz stimulations were delivered to trigger an afterdischarge. In these cases, only the duration and latency were measured and the pulse number threshold was recorded as 25, i.e., the maximum number of stimulating pulses. If the same rat was used repeatedly in this experiment, at least 7 days were allowed to elapse between the injection of different drugs in order to avoid problems related to drug accumulation or drug–drug interactions.

2.5. Statistics

All of the seizure parameter data were expressed as the mean ± S.E.M. The data from the pre-drug controls was compared to the post-drug controls using Wilcoxon's sign rank test for paired replicates.

2.6. Drugs

The compounds granisetron and SKB 200646A were obtained from SmithKline and Beecham Pharmaceuticals (Ware, UK), WAY 100635 from Wyeth–Ayerst (Princeton, NJ) and MDL 100907 from Hoechst Marion Roussel (Cincinnati, OH).

3. Results

The injection of vehicle did not significantly alter any of the seizure parameters (data not shown). Furthermore, there were no significant differences between the pre-drug and post-drug controls following the 48-h wash-out or withdrawal period.

3.1. The effects of WAY 100635 on seizure threshold and severity

The intraperitoneal administration of either 0.1, 0.3 or 1 mg/kg of WAY 100635 did not significantly alter the pulse number threshold compared to the pre-drug controls (Table 1). However, the 0.3 mg/kg dose produced a significant increase in the primary afterdischarge duration, whereas the 1.0 mg/kg dose significantly decreased and increased the latency of the secondary afterdischarge and the secondary afterdischarge duration, respectively, compared to pre-drug controls (Table 1).

3.2. The effects of MDL 100907 on seizure threshold and severity

The intraperitoneal administration of either 0.3, 1 or 3 mg/kg of MDL 100907 did not alter pulse number threshold, latency of secondary afterdischarge or secondary afterdischarge duration compared to the pre-drug controls (Table 1). In contrast, the acute administration of 1 mg/kg of

Table 1
The effects of 5-HT receptor antagonists and PCPA on seizure parameters of hippocampus-generating partial seizures in rats
Each value represents the mean ± S.E.M.

Compound	Dose (mg/kg)	N	Pulse-number threshold			Primary afterdischarge duration (s)			Latency of secondary afterdischarge duration (s)			Secondary afterdischarge (s)		
			Pre-drug control	Drug	Post-drug control	Pre-drug control	Drug	Post-drug control	Pre-drug control	Drug	Post-drug control	Pre-drug control	Drug	Post-drug control
WAY-1A	0.03	10	12.0±0.7	12.1±1.0	12.3±0.9	47.5±2.9	42.5±5.1	46.4±3.2	39.2±4.6	41.7±4.6	39.9±5.0	12.6±2.9	12.2±3.6	12.2±2.9
	0.1	9	11.6±0.6	12.1±0.9	12.2±0.7	51.9±5.8	80.0±17.3*	48.8±5.7	34.4±4.3	36.1±5.5	30.1±3.6	16.8±3.2	13.0±3.6	17.3±2.9
	0.3	10	12.0±0.7	13.6±1.2	12.7±0.8	49.4±6.9	55.5±7.1	54.8±6.9	40.7±3.7	28.7±4.7*	39.0±4.4	14.4±3.1	21.0±3.4**	14.4±2.5
MDL 100907	0.3	8	11.5±0.7	11.6±0.8	12.0±0.5	62.0±6.7	70.6±8.8	59.1±6.3	35.0±2.6	31.9±4.5	30.5±3.0	19.4±2.3	16.6±3.2	18.7±3.0
	1.0	9	12.1±0.7	12.0±0.7	11.9±0.8	50.6±4.9	60.4±6.7*	57.0±7.0	45.2±3.0	41.1±5.4	43.4±3.8	12.1±1.8	13.6±2.7	11.8±2.9
	3.0	8	12.4±0.7	12.4±0.8	12.3±0.7	59.2±8.5	65.9±12.4	54.8±7.4	29.5±4.1	31.5±5.0	30.6±4.5	18.0±2.9	17.0±4.0	16.1±3.5
SKB 200646A	5	9	11.9±0.8	11.9±0.7	11.7±0.6	55.2±7.0	59.0±5.8	62.3±7.2	40.0±3.6	43.2±4.4	34.1±4.3	12.7±2.9	11.9±2.6	13.8±2.9
	15	7	12.6±0.6	13.1±1.2	12.9±0.7	49.7±4.3	55.4±4.1	53.3±8.9	50.5±3.0	53.4±3.7	51.6±5.2	16.1±3.6	16.6±3.5	12.4±3.5
	50	8	12.0±0.4	11.4±0.4	12.0±0.5	45.0±2.8	53.3±4.5	48.0±4.3	50.3±4.8	50.1±4.8	50.1±4.9	17.8±1.3	15.3±1.8	14.9±2.0
Granisetron	0.3	8	11.1±0.4	11.4±0.5	11.9±0.5	51.6±4.8	49.9±6.7	54.5±6.2	36.8±3.5	38.9±2.5	36.8±3.1	17.4±1.7	22.9±3.3	18.8±1.9
	1.0	9	11.8±0.8	12.1±0.8	12.1±0.7	57.1±8.2	48.1±6.4*	53.0±5.3	36.7±3.4	37.3±4.1	38.2±3.2	16.2±1.9	18.7±2.9	13.7±2.1
	3.0	9	12.9±0.9	11.6±0.9	11.8±0.8	56.9±8.1	52.3±7.0	57.1±8.2	36.7±3.5	33.2±3.2*	36.7±3.4	15.9±2.5	19.7±3.2	16.4±1.9

* Significantly different from pre-drug control, $p < 0.05$, Wilcoxon signed-rank test.
** Significantly different from pre-drug control, $p < 0.01$, Wilcoxon signed-rank test.

MDL 100907 significantly increased the primary afterdischarge duration compared to the pre-drug controls (Table 1).

3.3. The effects of SKB 200646A on seizure threshold and severity

The administration of 5, 15 or 50 mg/kg i.p. of SKB 200646A did not alter pulse number threshold, primary or secondary afterdischarges, or the latency of the secondary afterdischarge compared to pre-drug controls (Table 1).

3.4. The effects of granisetron on seizure threshold and severity

The administration of 0.3, 1 or 3 mg/kg i.p. of granisetron did not alter the pulse number threshold or secondary afterdischarge duration compared to pre-drug controls. Interestingly, at 1 mg/kg i.p., granisetron significantly decreased the primary afterdischarge duration compared to pre-drug controls. However, the 3 mg/kg dose of granisetron significantly decreased the latency of secondary afterdischarge compared to pre-drug controls (Table 1).

4. Discussion

4.1. SKB 200646A

The results of this study indicate that the acute administration of 5, 15 or 50 mg/kg i.p. of the 5-$HT_{2B,C}$ receptor antagonist SKB 200646A did not alter the pulse number threshold, primary or secondary afterdischarge duration or latency of secondary afterdischarge. This suggests that SKB 200646A in partial hippocampally kindled male Wistar rats, does not alter seizure threshold or severity. To our knowledge, this is the first study to examine the effect of SB 200646A on seizures in any animal model of epilepsy, including kindling. It is possible that the lack of effect of SB 200646A on seizure threshold and severity was due to the administration of an inadequate dose. This is unlikely since the ED_{50} dose for SB 200646A to block the *m*CPP-induced hypolocomtion, a behavior that is believed to result from 5-HT_{2C} receptor stimulation, is 19.2 mg/kg (Kennett et al., 1994), a dose lower than the highest dose used in our study. In contrast, mice lacking 5-HT_{2C} receptors express spontaneous seizures, display lower seizure threshold, are prone to sound-induced seizures and are more sensitive to lethality produced by the proconvulsant agent pentylenetetrazole administration (Applegate and Tecott, 1996; Brennan et al., 1997; Tecott et al., 1995), suggesting that 5-HT_{2C} receptors are required to maintain neuronal activity within normal limits. However, it may be difficult to compare our results to the 5-HT_{2C} receptor knockout mice as it is possible that compensatory changes,

as opposed to the missing 5-HT_{2C} receptors themselves, may have produced the observed seizures. Finally, it should be pointed out that SB 200646A has high affinity for 5-HT_{2B} receptors (Forbes et al., 1995), and one cannot rule out the possibility that the antagonism of 5-HT_{2B} receptors counterbalances any detrimental actions produced by 5-HT_{2C} receptor antagonism. However, this can only be ascertained by examining the effect of a 5-HT_{2B} receptor antagonist on seizure threshold and severity in our model.

4.2. M100907

The acute administration of 0.3, 1 or 3 mg/kg i.p. of M100907, a selective 5-HT_{2A} receptor antagonist, did not significantly alter the pulse number threshold, secondary afterdischarge duration or latency of secondary afterdischarge. However, the 1 mg/kg dose of M100907 significantly increased the primary afterdischarge duration, suggesting that at this dose, M100907 increased seizure severity in our model. Nonetheless, this finding should be considered with the fact that the 3 mg/kg dose did not alter seizure severity, suggesting that M100907's action is not dose-dependent. Furthermore, even though the absolute values for the afterdischarge duration following 0.3 (70.6 s) and 3 mg/kg of M100907 (65.9 s) were greater than that for the 1 mg/kg dose (60.4 s), the pre-drug control value for this dose was lower. Thus, had a different control group been examined, the 1 mg/kg data may not have been significant.

Currently, the exact role of the 5-HT_{2A} receptor in the generation of seizures remains to be elucidated. To our knowledge, no studies have been published regarding the effect of selective 5-HT_{2A} receptor antagonists alone on seizure threshold and severity in kindled rats. It has been shown that electroshock-induced seizures in female Wistar rats are attenuated by 5-hydroxytryptophan and this effect is blocked by the non-selective 5-HT_{2A} receptor antagonist ketanserin, suggesting that 5-HT_{2A} receptors may be involved in the increase in seizure threshold (Löscher and Czuczwar, 1985). However, it is difficult to compare our results to those obtained in the aforementioned study as (1) ketanserin, in addition to binding to 5-HT_{2A} receptors, also has appreciable affinity for dopamine D_2, receptors, α_1-adrenoceptor and H_1 histaminergic receptors (Leysen et al., 1981; Van Wijngaarden et al., 1990) and amine release sites (Schotte and Leysen, 1988, 1989); (2) our method for inducing seizures was different; and (3) we used male as opposed to female rats. Interestingly, it has been shown that the acute administration of the 5-$HT_{2A,C}$ receptor agonist DOI (1 mg/kg) significantly decreased, whereas ketanserin increased the latency to generalized tonic-clonic seizures in female cats with hippocampal-kindled seizures (Wada et al., 1992). We have previously shown that the acute administration of 3 mg/kg of DOI decreased the latency of the secondary afterdischarge in male Wistar rats compared to the pre-drug controls (Watanabe et al., 1997). Thus, it appears that the administration of either a 5-HT_{2A} receptor antagonist, such as M100907, or a 5-HT_{2A} receptor agonist, produces similar actions, which seems confounding. However, it has been shown that DOI is a partial agonist at 5-HT_{2C} receptors (Hoyer et al., 1989), which makes it difficult to interpret data obtained with DOI.

4.3. Granisetron

The acute administration of 0.3, 1 or 3 mg/kg i.p. of the selective 5-HT_3 receptor antagonist granisetron (Sanger and Nelson, 1989) did not significantly alter the pulse number threshold, suggesting that antagonism of 5-HT_3 receptors does not affect seizure threshold in partial seizures in freely-moving Wistar rats. However, compared to pre-drug controls, the 1 mg/kg dose of granisetron decreased the primary afterdischarge duration, suggesting that at this dose, granisetron decreased seizure severity. In contrast, the 3 mg/kg dose of granisetron significantly decreased the latency of secondary afterdischarge. Currently, it is difficult to interpret our results as the role of the 5-HT_3 receptor in the etiology of seizures is unknown and our study is the first to examine the effect of a selective 5-HT_3 receptor antagonist on partial hippocampal seizures. We have previously reported that using the same seizure model as this study, the acute administration (0.3–3 mg/kg i.p.) of the selective 5-HT_3 receptor agonist SR 57227A (Bachy et al., 1993) did not alter seizure threshold or severity (Watanabe et al., 1997). In contrast, the duration of the afterdischarge of generalized seizures was increased after the intracranial administration of the 5-HT_3 receptor agonist 1-(*m*-chlorophenyl)-biguanide (*m*-CPBG) in amygdaloid-kindled male Wistar rats (Wada et al., 1997). However, it may be difficult to compare our results to the aforementioned ones as our animals only displayed stage-1 seizures and we used another 5-HT_3 receptor agonist that was given by a different route. Clearly, additional studies are needed to clarify the role of the 5-HT_3 receptor in the etiology of partial seizures.

4.4. WAY 100635

The acute administration of 0.1, 0.3 or 1 mg/kg i.p. of WAY 100635 did not alter the pulse number threshold in our model, suggesting that WAY 100635 does not alter the seizure threshold. However, the 0.1 mg/kg dose significantly increased the primary afterdischarge duration, whereas the 0.3 and 1 mg/kg doses significantly increased and decreased the latency of secondary discharge and the secondary afterdischarge duration, respectively. Tentatively, these results suggest that seizure severity may be increased in partial hippocampally kindled male Wistar rats. However, in normal Wistar rats, doses as high as 10 mg/kg i.p. of WAY 100635 do not produce seizures (unpublished data). The exact explanation for WAY 100635's action in our model is unknown. The search for

an explanation is complicated by the fact that the exact role of the 5-HT_{1A} receptor in the etiology of seizures in various animal models or in human is unknown. For example, we have previously shown that the systemic administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.1–1 mg/kg i.p.) did not alter the pulse number threshold, primary or secondary afterdischarges or latency of secondary afterdischarge compared to pre-drug controls in male Wistar rats using the same model (Watanabe et al., 1997, 1998), indicating that 8-OH-DPAT did not alter the threshold or the severity of seizures. Similarly, it has been reported that in female Wistar rats, the administration of 0.5 or 1 mg/kg s.c. of 8-OH-DPAT did not alter the maximal electric shock threshold or amygdaloid-kindled seizures induced by electrical stimulation, respectively (Löscher and Czuczwar, 1985). Furthermore, 8-OH-DPAT (0.5 or 1 mg/kg s.c.) did not alter seizure threshold in gerbils (Löscher and Czuczwar, 1985). In contrast, there is an evidence that 8-OH-DPAT produces a significant decrease in seizures generated by various experimental manipulations (Gariboldi et al., 1996; Wada et al., 1992, 1993c). Overall, it is difficult to compare the aforementioned studies as the discrepant results could be due to the differences in experimental design such as the model of epilepsy, species used and route of drug administration.

Because WAY 100635 was given systemically, it is impossible to determine whether it is producing its effects by acting on pre- or postsynaptic 5-HT_{1A} receptors. Furthermore, since there is a high density of 5-HT_{1A} receptors in a number of brain areas, including the hippocampus (Pazos and Palacios, 1985; Pompeiano et al., 1992), the site(s) of action in the brain remains to be elucidated. Previously, it has been shown that the iontophoresis of 8-OH-DPAT can suppress neuronal activity in the hippocampus and medial prefrontal cortex (Ashby et al., 1991; Klancnik et al., 1991; Sprouse and Aghajanian, 1988). Therefore, it is possible that the stimulation of 5-HT_{1A} receptors could inhibit epileptiform activity generated by a number of experimental manipulations. Indeed, 8-OH-DPAT produces a decrease in epileptiform activity in hippocampal brain slices induced by bicuculline (Salgado and Alkadhi, 1995). Furthermore, the systemic and intrahippocampal administration of 8-OH-DPAT inhibits the number of seizures and delays the latency of the onset of seizures generated by kainic acid in male Sprague–Dawley rats, and this effect is antagonized by intrahippocampal administration of WAY 100635 (Gariboldi et al., 1996). Therefore, it is possible that in kindled animals, serotonergic tone may be reduced and the administration of WAY 100635 may further diminish 5-HT tone. However, this hypothesis must be verified by further experiments such as microinjection studies into specific brain areas.

In conclusion, the results of this study obtained in rats with stage 1 kindled seizures suggest that the antagonism of 5-HT_{1A}, 5-HT_{2A}, 5-HT_3 or 5-$HT_{2B,C}$ receptors do not lower or raise seizure threshold. However, the antagonism of 5-HT_{1A} receptors may increase or augment seizure severity. In addition, the lack of significant drug effects on seizure threshold and severity may have been related to using animals with stage 1 (mild) seizures, although this remains to be verified.

Acknowledgements

The authors wish to thank Wyeth–Ayerst, SmithKline and Beecham and Hoechst Marion Roussel for their donations of drugs. This work was supported by grants from the Japanese Ministry of Education and Culture 08671125 and the Ministry of Health and Welfare to Y.M., and NIMH R2955155 to C.R.A., Jr.

References

Applegate, C.D., Tecott, L., 1996. Global changes in seizure susceptibility in mice lacking 5-HT_{2C} serotonin receptors. Abstr. Soc. Neurosci. 22, 2086.

Ashby, C.R. Jr., Minabe, Y., Edwards, E., Wang, R.Y., 1991. Electrophysiological characterization of 5-HT_3 receptors in the rat medial prefrontal cortex. Brain Res. 550, 161–171.

Bachy, A., Heaulme, M., Giudice, A., Michaud, J.-C., Lefevre, I.A., Soulilhac, J., Manara, L., Emerit, M.B., Gozlan, H., Hamon, M., Keane, P.E., Soubrie, P., Le Fur, G., 1993. SR 57227A: a potent and selective agonist at central and peripheral 5-HT_3 receptors in vitro and in vivo. Eur. J. Pharmacol. 237, 299–309.

Brennan, T.J., Seely, W.W., Kilgard, M., Schreiner, C.E., Tecott, L.H., 1997. Sound-induced seizures in serotonin 5-HT_{2C} receptor. Nat. Genet. 16, 387–390.

Browning, R.A., Hoffman, W.E., Simonton, R.L., 1978. Changes in seizure susceptibility after intracerebral treatment with 5,7-dihydroxytryptamine: the role of serotonergic neurons. Ann. N. Y. Acad. Sci. 305, 437–456.

Cryan, J.F., Kelliher, P., Kelly, J.P., Leonard, B.E., 1999. Comparative effects of serotonergic agonists with varying efficacy at the 5-HT(1A) receptor on core body temperature: modification by the selective 5-HT(1A) receptor antagonist WAY 100635. J. Psychopharmacol. 13, 278–283.

Dailey, J.W., Yan, Q.-S., Adams-Curtis, L.E., Ryu, J.R., Ko, K.H., Mishra, P.K., Jobe, J.C., 1996. Neurochemical correlates of antiepileptic drugs in the genetically epilepsy-prone rat. Life Sci. 58, 259–266.

De La Torre, J.C., Kawanaga, H.M., Mullan, S., 1970. Seizure susceptibility after manipulation of brain serotonin. Arch. Int. Pharmacodyn. Ther. 188, 293–304.

Fletcher, A., Forster, E.A., Bill, D.J., Brown, G., Cliffe, I.A., Hartley, J.E., Jones, D.E., McLenachan, A., Stanhope, K.J., Critchley, D.J., Childs, K.J., Middlefell, V.C., Laufumey, L., Corradetti, R., Laporte, A.M., Gozlan, H., Hamon, M., Dourish, C.T., 1996. Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT1A receptor antagonist. Behav. Brain Res. 73, 337–353.

Forbes, I.T., Kennett, G.A., Gadre, A., Ham, P., Hayward, C.J., Martin, R.T., Thompson, M., Wood, M.D., Baxter, G.S., Glen, A., Murphy, O.E., Stewart, B., Blackburn, T.P., 1993. *N*-(1-methyl-5-indolyl)-*N*′-(3-pyridyl)urea hydrochloride: the first selective 5-HT_{1C} receptor antagonist. J. Med. Chem. 36, 1104–1107.

Forbes, I.T., Ham, P., Booth, D.H., Martin, R.T., Thompson, M., Baxter, G.S., Blackburn, T.P., Glen, A., Kennett, G.A., Wood, M.D., 1995.

5-Methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole: a novel 5-HT_{2C} /5-HT_{2B} receptor antagonists with improved affinity, selectivity, and oral activity. J. Med. Chem. 38, 2524–2530.

Forster, E.A., Cliffe, I.A., Bill, D.J., Dover, G.M., Jones, D., Reilly, Y., Fletcher, A., 1995. A pharmacological profile of the selective silent 5-HT1A receptor antagonist, WAY-100635. Eur. J. Pharmacol. 28, 81–88.

Gariboldi, M., Tutka, P., Samanin, R., Vezzani, A., 1996. Stimulation of 5-HT_{1A} receptors in the dorsal hippocampus and inhibition of limbic seizures induced by kainic acid in rats. Br. J. Pharmacol. 119, 813–818.

Giroud, M., Dumas, R., Dauvergne, M., D'Athis, P., Rochette, L., Beley, A., Bralet, J., 1990. 5-Hydroxyindoleacetic acid and homovanillic acid in cerebrospinal fluid of children with febrile convulsions. Epilepsia 31, 178–181.

Grace, G.M., Corcoran, M.E., Skeleton, R.W., 1990. Kindling with stimulation of dentate gyrus: Part I. Characterization of electrographic and behavioral events. Brain Res. 509, 249–256.

Hoyer, D., Waeber, C., Schoeffter, P., Palacios, J.M., Dravid, A., 1989. 5-HT1C receptor-mediated stimulation of inositol phosphate production in pig choroid plexus. A pharmacological characterization. Naunyn-Schmiedeberg's Arch. Pharmacol. 339, 252–258.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Myelcharane, E.J., Saxena, P.R., Humphrey, P.P.A., 1994. VII. International union of pharmacology of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46, 157–203.

Jobe, P.C., Picchioni, A.L., Chin, L., 1973. Role of brain 5-hydroxytryptamine in audiogenic seizure in the rat. Life Sci. 13, 1–13.

Katsumori, H., Ito, Y., Higashida, H., Minabe, Y., 1996. Anti- and proconvulsive actions of levcromakalim, an opener of ATP-sensitive K^+ channel, in the model of hippocampus-generating partial seizures in rats. Eur. J. Pharmacolacol. 311, 37–44.

Katsumori, H., Minabe, Y., Osawa, M., Ashby, C.R. Jr., 1998. Acute effects of various GABA receptor agonists and glutamate antagonists on focal hippocampal seizures in freely moving rats elicited by low-frequency stimulation. Synapse 28, 103–109.

Kehne, J.H., Baron, B.M., Carr, A.A., Chaney, S.F., Elands, J., Feldman, D.J., Frank, R.a., van Giersbergen, P.L., McCloskey, T.C., Johnson, M.P., McCarty, D.R., Poirot, M., Senyah, Y., Siegel, B.W., Waldmeier, C., 1996. Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100,907 as a potent 5-HT_{2A} antagonist with a favorable CNS safety profile. J. Pharmacol. Exp. Ther. 277, 968–981.

Kennett, G.A., Wood, M.D., Glen, A., Grewal, S., Forbes, I., Gadre, A., Blackburn, T.P., 1994. In vivo properties of SB 200646A, a 5-$HT_{2B/2C}$ receptor antagonist. Br. J. Pharmacol. 111, 797–802.

Klancnik, J.M., Obenaus, A., Phillips, A.G., Baimbridge, K.G., 1991. The effects of serotonergic compounds on evoked responses in the dentate gyrus and CA1 region of the hippocampal formation of the rat. Neuropharmacology 30, 1201–1209.

Kovacs, D.A., Zoll, J.G., 1974. Seizure inhibition by median raphe nucleus stimulation in rat. Brain Res. 70, 165–169.

Laird, H.E., Jobe, P.C., 1987. The genetically epilepsy-prone rat. In: Jobe, P.C., Laird, H.E. (Eds.), Neurotransmitters and Epilepsy. Humana Press, Clifton, NJ, pp. 57–94.

Lazarova, M., Bendotti, C., Samanin, R., 1983. Studies on the role of serotonin in different regions of the rat central nervous system on pentylenetetrazol-induced seizures and the effect of di-*n*-propylacetate. Naunyn Schmiedeberg's Arch. Pharmacol. 322, 147.

Lazarova, M., Bendotti, C., Samanin, R., 1984. Evidence that the dorsal raphe area is involved in the effect of clonidine against pentylenetetrazol-induced seizures in rats. Naunyn Schmiedeberg's Arch. Pharmacol. 325, 12–16.

Leander, J.D., 1992. Fluoxetine, a selective serotonin-uptake inhibitor, enhances the anticonvulsant effects of phenytoin, carbamazepine and ameltolide (LY201116). Epilepsia 33, 573–576.

Löscher, W., Czuczwar, S.J., 1985. Evaluation of the 5-hydroxytryptamine receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin in different rodent models of epilepsy. Neurosci. Lett. 60, 201–206.

Leysen, J.E., Awouters, F., Kennis, L., Laduron, P.M., Vandenberkin, J., Janssen, P.A., 1981. Receptor binding profile of R 41468, a novel antagonist at 5-HT_2 receptors. Life Sci. 28, 1015–1022.

McNamara, J.O., 1996. Drugs effective in the therapy of the epilepsies. In: Molinoff, P.B., Ruddon, R.E. (Eds.), The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, pp. 461–486.

Mengod, G., Nguyen, H., Le, H., Waeber, C., Lubbert, H., Palacios, J.M., 1990. The distribution and cellular localization of the serotonin 1C receptor mRNA in the rodent brain examined by in situ hybridization histochemistry. Comparison with receptor binding studies. Neuroscience 35, 577–591.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Pazos, A., Palacios, J.M., 1985. Quantitative autoradiographic mapping of serotonin receptors in the rat brain: Part II. Serotonin-1 receptors. Brain Res. 346, 205–230.

Pazos, A., Cortes, R., Palacios, J.M., 1985. Quantitative autoradiographic mapping of serotonin receptors in the rat brain: Part II. Serotonin-2 receptors. Brain Res. 346, 231–249.

Pompeiano, M., Palacios, J.M., Mengod, G., 1992. Distribution and cellular location of mRNA coding for 5-HT_{1A} receptor in the rat brain: correlation with receptor binding. J. Neurosci. 12, 440–453.

Pompeiano, M., Palacios, J.M., Mengod, G., 1994. Distribution of the serotonin 5-HT_2 receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2B} receptors. Mol. Brain Res. 23, 163–178.

Pranzatelli, M.R., Tate, E., Huang, Y., Haas, R.H., Bodensteiner, J., Ashwal, S., Franz, D., 1995. Neuropharmacology of progressive myoclonis epilepsy: response to 5-hydroxy-L-tryptophan. Epilepsia 36, 783–791.

Racine, R.J., 1972. Modification of seizure activity by electrical stimulation: motor seizures. Electroencephalogr. Clin. Neurophysiol. 32, 281–294.

Racine, R., Coscina, D.V., 1979. Effects of midbrain raphe lesions or systemic *p*-chlorophenylalanine on the development of kindled seizures in rats. Brain Res. Bull. 4, 1–7.

Salgado, D., Alkadhi, K.A., 1995. Inhibition of epileptiform activity by serotonin in rat CA1 neurons. Brain Res. 669, 176–182.

Sanger, G.J., Nelson, D.R., 1989. Selective and functional 5-hydroxytryptamine$_3$ receptor antagonism by BRL 43694 (granisetron). Eur. J. Pharmacol. 159, 113–124.

Schmidt, C.J., Sorenson, S.M., Kehne, J.H., Carr, A.A., Palfreyman, M.G., 1995. The role of 5-HT_{2A} receptors in antipsychotic activity. Life Sci. 56, 2209–2222.

Shaywitz, B.A., Cohen, D.J., Bowers, M.B., 1975. Reduced cerebrospinal fluid 5-hydroxyindoleacetic acid and homovanillic acid in children with epilepsy. Neurology 25, 72–79.

Schotte, A., Leysen, J.E., 1988. Distinct autoradiographic labeling of serotonin 5-HT_2 receptors, α_1-adrenoceptors and histamine-H_1 receptors and of tetrabenazine-replaceable ketaserine binding sites in rodent brain with [^{125}I]7-amino-8-iodo-ketanserine. Eur. J. Pharmacol. 145, 213–216.

Schotte, A., Leysen, J.E., 1989. Identification of serotonin 5-HT_2 receptors, a1-adrenoceptors and amine release sites in rat brain by autoradiography with [^{125}I]7-amino-8-iodo-ketanserine. Eur. J. Pharmacol. 172, 99–106.

Sprouse, J.S., Aghajanian, G.K., 1988. Responses of hippocampal pyramidal cells to putative 5-HT_{1A} and 5-HT_{1B} agonists: a comparative study with dorsal raphe neurons. Neuropharmacology 27, 707–715.

Statnik, M.A., Maring-Smith, M.-L., Clough, R.W., Wang, C., Dailey, J.W., Jobe, P.C., Browning, R.A., 1996. Effect of 5,7-dihydroxytryptamine on audiogenic seizures in genetically epilepsy-prone rats. Life Sci. 59, 1763–1771.

Tecott, L.H., Sun, L.M., Akana, S.F., Strack, A.M., Lowenstein, D.H., Dallman, M.F., Julius, D., 1995. Eating disorder and epilepsy in mice lacking 5-HT_{2C} serotonin receptors. Nature 374, 542–546.

Van Wijngaarden, I., Tulp, M.Th.M., Soudijn, W., 1990. The concept of selectivity in 5-HT receptor research. Eur. J. Pharmacol., Mol. Pharmacol. Sect. 188, 301–312.

Verma, A.K., Gupta, G.K., Maheshwari, M.C., 1984. 5-HIAA in cerebrospinal fluid of patients with status epilepticus. Epilepsia 25, 499–501.

Wada, Y., Nakamura, M., Hasegawa, H., Yamaguchi, N., 1992. Role of serotonin receptor subtype in seizures kindled from the feline hippocampus. Neurosci. Lett. 141, 21–24.

Wada, Y., Nakamura, M., Hasegawa, H., Yamaguchi, N., 1993a. Microinjection of the serotonin uptake inhibitor fluoxetine elevates hippocampal seizure threshold in rats. Neurosci. Res. Commun. 13, 143–148.

Wada, Y., Nakamura, M., Hasegawa, H., Yamaguchi, N., 1993b. Effect of serotonin uptake inhibiting antidepressants on hippocampal kindled seizures in cats. Neurosci. Res. Commun. 12, 119–124.

Wada, Y., Nakamura, M., Hasegawa, H., Yamaguchi, N., 1993c. Intrahippocampal injection of 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) inhibits partial and generalized seizures induced by kindling stimulation in cats. Neurosci. Lett. 159, 179–182.

Wada, Y., Shiraishi, J., Nakamura, M., Koshino, Y., 1997. Effects of the 5-HT_3 receptor agonist 1-(*m*-chlorophenyl)-biguanide in the rat kindling model of epilepsy. Brain Res. 759, 313–316.

Watanabe, K., Minabe, Y., Katsumori, H., Ashby, C.R. Jr., Narita, N., 1997. Serotonin receptors and rat hippocampal seizures. Soc. Neurosci. Abstr. 23, 973.

Watanabe, K., Minabe, Y., Ashby, C.R. Jr., Katsumori, H., 1998. Effect of acute administration of various 5-HT receptor agonists on focal lippocampal seizures in freely moving rats. Eur. J. Pharmacol. 350, 181–188.

Yan, Q.S., Mishra, P.K., Burger, R.L., Bettendorf, A.E., Jobe, P.C., Dailey, J.W., 1992. Evidence that carbamazepine and antiepilepsirine may produce a component of anticonvulsant effects by activating serotonergic neurons in genetically epilepsy-prone rats. J. Pharmacol. Exp. Ther. 261, 652–659.

Yan, Q.S., Jobe, P.C., Cheong, J.C., Koe, K.H., Dailey, J.W., 1994. Role of serotonin in the anticonvulsant effect of fluoxetine in genetically epilepsy-prone rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 350, 149–152.

Zifa, E., Fillion, G., 1992. 5-Hydroxytryptamine receptors. Pharmacol. Rev. 44, 401–458.