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Anticonvulsive Properties of YM-14673, a New TRH Analogue, in Amygdaloid-Kindled Rats

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YATSUGI, S. AND M. YAMAMOTO. *Anticonvulsive properties of YM-14673, a new TRH analogue, in amygdaloid-kindled rats.* PHARMACOL BIOCHEM BEHAV **38**(3) 669–672, 1991.—Effects of YM-14673 (N^{α}-[{(S)-4-oxo-2-azetidinyl}-carbonyl]-L-prolinamide dihydrate), a new TRH analogue, on the development of kindling and the duration of afterdischarge (AD) were observed in amygdaloid-kindled rats in comparison with those of TRH. The right medial amygdaloid nucleus was electrically stimulated once a day for establishment of kindling. YM-14673 and TRH were administered intraperitoneally 15 and 30 min prior to electrical stimulation from 2 days after the first stimulation, respectively, and the number of stimulations required for the development of generalized seizures was measured. YM-14673 (1 mg/kg) showed tendency to suppress the development of kindling ($p<0.1$), but TRH even at high dose (10 mg/kg) showed little effect on it. In addition, the experiment for the AD duration was conducted in full-kindled rats from one day after at least 3 reproducible generalized seizures were elicited by electrical stimulation of the medial amygdaloid nucleus once a day. Both YM-14673 (1 mg/kg) and TRH (10 mg/kg) administered 15 and 30 min prior to electrical stimulation of the amygdala, respectively, significantly shortened the AD duration in full-kindled rats. At these doses, both drugs desynchronized spontaneous cortical electroencephalogram (EEG) in normal rats. These results indicate that YM-14673 seems to possess anticonvulsive property in rats.

YM-14673 TRH Amygdaloid kindling Afterdischarge Rat

THYROTROPIN-RELEASING hormone (TRH) is well known as a hypothalamic hormone which releases thyrotropin (TSH) (3) and prolactin (6) from the pituitary. In addition to these hormonal actions, it has been reported that TRH had central nervous system arousal properties in both animals and men (11,14), and accelerated release and/or turnover of neurotransmitters such as acetylcholine and monoamines (8). So some attempts have been made to evaluate the clinical efficacy of TRH on spinocerebellar degeneration (18), depression (11), schizophrenia (5), disturbance of consciousness (14) and epilepsy (9). Sato et al. (15,16) reported that TRH and γ-butyrolactone-γ-calbonyl-L-histidyl-L-prolinamide citrate (DN-1417), TRH analogue, attenuated the development of kindling and seizures in amygdaloid-kindled animals. Furthermore, it has been reported that DN-1417 had beneficial effects on symptoms in patients with myoclonus epilepsy and Lennox syndrome (4,19).

Since TRH is known to be rapidly metabolized in the body, we have searched for TRH analogues that are more potent and longer-lasting than TRH. Recently, we have reported that N^{α}-[{(S)-4-oxo-2-azetidinyl}carbonyl]-L-histidyl-L-prolinamide dihydrate (YM-14673) (22) was much more potent and longer-lasting than TRH in antagonizing pentobarbital sleep in mice. The present study describes about the anticonvulsive properties of YM-14673 and TRH.

METHOD

Animals

Male Wistar rats (Japan SLC, Shizuoka, Japan) weighing 300–350 g were housed in group under 12-hour light-dark conditions, and given laboratory chow and water ad lib.

Spontaneous Electroencephalogram in Rats

Under anesthesia with sodium pentobarbital (55 mg/kg IP), screw electrodes were implanted into the skull on the surface of the cerebral cortex. The spontaneous electroencephalogram (EEG) was recorded every 10 min after intraperitoneal administration of the test drugs and analyzed for theta band (4 to 7.75 Hz) using a data analytic apparatus (ATAC-450, Nihon Koden, Tokyo). The effects of the test drugs were evaluated by comparing the appearance rate of theta band for 10 min in the drug-treated groups with that in the saline-treated group.

Development of Amygdaloid Kindling and Afterdischarge

Rats were anesthetized with sodium pentobarbital (55 mg/kg IP) and the bipolar electrodes consisted of two twisted Teflon-coated 0.2 mm diameter stainless steel wires separated by 0.5 mm at the tip were implanted in the right medial amygdaloid nu-

cleus (A 6.4, L 3.3, H 1.8) according to the coordinates of Paxinos and Watson (10) and cerebral cortex. After a postoperative period of at least 10 days, the right amygdala was stimulated according to following procedures. The initial stimulus intensity, regulated by a constant-current unit, was set at 2.0 V, and was increased by 0.2-V steps every 10 min until an afterdischarge (AD) was generated. The stimulus intensity that first produced AD was designated as the AD threshold and it was used for producing subsequent kindling. When AD was generated at 2.0 V on initial day, the intensity was reduced in 0.2-V steps from second day to the day when AD was no longer elicited, and the minimum intensity stimulus which produced AD was designated as the AD threshold. Stimulation at the AD threshold was applied once daily in 1-s trains of 50 Hz until the rats elicited a generalized convulsion every day for 3 days. TRH and YM-14673 were given 15 and 30 min prior to each daily stimulation during the drug sessions (from 2 to 24 days), respectively. Assessment of amygdaloid seizure stage was conducted according to following 6 classes reported by Racine (12): Stage 0, no behavioral response to amygdaloid stimulation; Stage 1, mouth movement; Stage 2, head nodding; Stage 3, forepaw up and/or forelimb clonus; Stage 4, rearing; Stage 5, generalized convulsive seizure with falling.

The AD duration was measured from EEG record and defined as the period of synchronous bursting activity. In nondrug treatment rats, after at least 3 reproducible stage 5 seizures were elicited, TRH and YM-14673 were given 15 and 30 min prior to stimulation on the next day, respectively. Then, change in the AD duration was observed. After these pharmacological studies, the rats were anesthetized with sodium pentobarbital (55 mg/kg IP) and their brains were perfused with physiological saline and 10% formalin. Histological examination was conducted in order to verify the position of the electrode in some cases.

Drugs

YM-14673 was synthesized in our laboratories and dissolved in 0.9% saline solution. The following drugs were commercially obtained: TRH (Peptide Institute, Inc., Japan), sodium pentobarbital (Tanabe, Japan). The volume for injection was 0.1 ml/100 g body weight.

Statistics

The statistics significance of the results in the present study was calculated using the Student's *t*-test.

RESULTS

Spontaneous Electroencephalogram

YM-14673 (0.1 and 1 mg/kg IP) increased the appearance rate of theta wave (Fig. 1A), suggesting that the drug desynchronized the spontaneous EEG. The EEG was desynchronized for about 90 min and over 120 min by administration of YM-14673 in doses of 0.1 and 1 mg/kg, respectively. TRH (10 mg/kg IP) showed the similar EEG actions as YM-14673, however, pharmacological action of TRH in a dose of 10 mg/kg continued for about 60 min (Fig. 1B).

Effect on the Development of Kindling

The number of the amygdaloid stimulations required for the development of stage 5 generalized convulsion was not affected by administration of YM-14673 (0.1 mg/kg IP) and TRH (10 mg/kg IP) (Fig. 2, Table 1). YM-14673 (1 mg/kg IP) showed tendency to extend the number of stimulations ($p<0.1$) (Fig. 2, Table 1). On the other hand, the number of stimulations required for the development of other stages (1–4) was not significantly affected by administration of both drugs (Table 1).

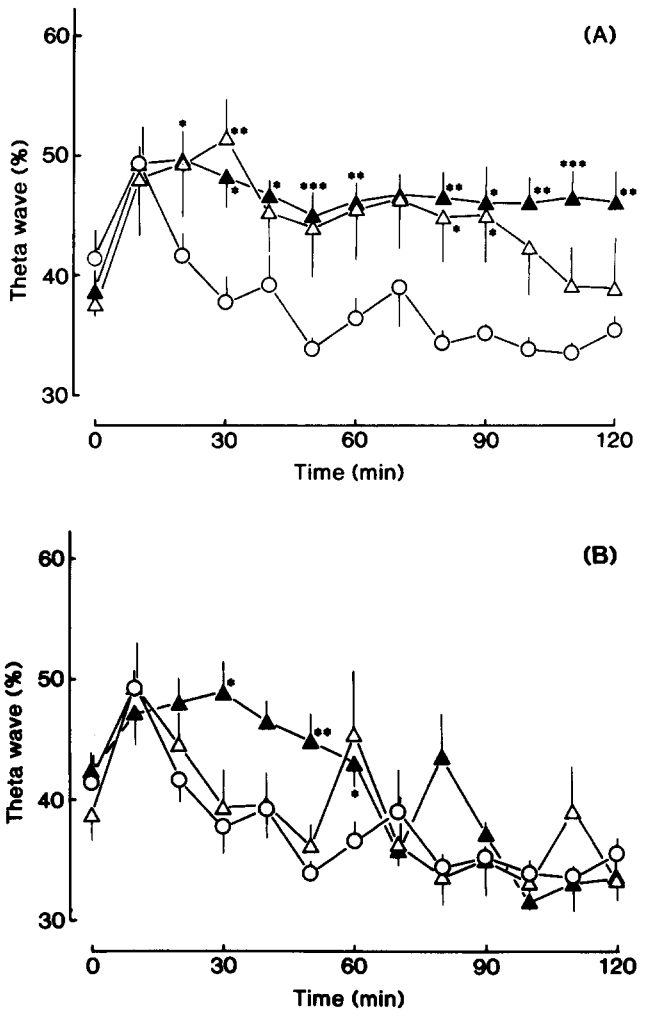

FIG. 1. Effects of YM-14673 (A) and TRH (B) on the appearance rate of the theta-wave component in rats. Each point represents the mean ± S.E.M. from 4–5 rats. (A): ○—○; saline (IP), △—△; YM-14673 (0.1 mg/kg IP), ▲—▲; YM-14673 (1 mg/kg IP) (B): ○—○; saline (IP), △—△; TRH (1 mg/kg IP), ▲—▲; TRH (10 mg/kg IP). Significantly different from the value for saline-treated group: $*p<0.05$, $**p<0.01$, $***p<0.001$ (Student's *t*-test).

Effect on the AD Duration

YM-14673 (1 mg/kg IP) significantly shortened the AD duration in full-kindled rats ($p<0.001$) (Fig. 3). At this dose, the seizure stages in full-kindled rats were also reduced; in two out of eight rats, the behavioral seizures regressed from stage 5 to stage 2, and in others, those kept stage 5 seizures. TRH (10 mg/kg IP) also significantly shortened the AD duration in full-kindled rats ($p<0.05$) (Fig. 3); in three out of twelve rats, the behavioral seizures regressed from stage 5 to stage 0, 1 or 3, and in others, those kept stage 5 seizures.

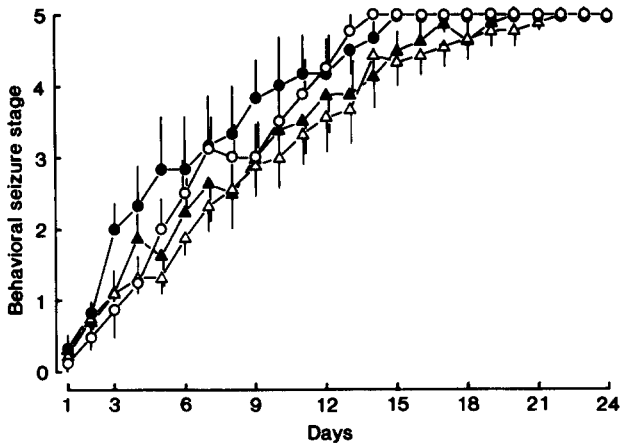

FIG. 2. Effects of YM-14673 and TRH on the development of kindling in amygdaloid-kindled rats. Each point represents the mean ± S.E.M. from 6–9 rats. ○—○: saline (IP), ●—●: YM-14673 (0.1 mg/kg IP), △—△: YM-14673 (1 mg/kg IP), ▲—▲: TRH (10 mg/kg IP).

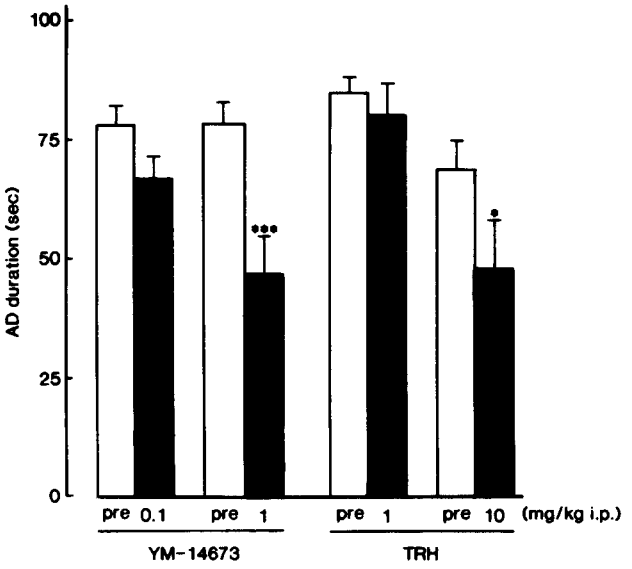

FIG. 3. Effects of YM-14673 and TRH on the AD duration in the full-kindled rats. Each column shows mean ± S.E.M. from 7–12 rats. Pre value represents the average of AD duration on both 1 and 2 days before drug administration. Significantly different from the pre value: $*p<0.05$, $***p<0.001$ (Student's t-test).

DISCUSSION

Kindling, an animal model of complex partial epilepsy, refers to the phenomenon in which periodic focal application of initially subconvulsive electrical stimuli leads to the progressive intensification of seizures, culminating in limbic and clonic motor seizures (2). It has been reported that pharmacological efficacy of antiepileptic drugs in kindling model is correlated with clinical efficacy in patients with epilepsy (20,21). The present study demonstrated that both YM-14673 (1 mg/kg IP) and TRH (10 mg/kg IP) significantly shortened the AD duration in fully kindled rats. YM-14673 (1 mg/kg IP) showed tendency to suppress the behavioral manifestations of development of kindling. The appearance rate of the theta wave component was increased by administration by YM-14673 (1 mg/kg IP) and TRH (10 mg/kg IP) suggesting that both drugs facilitated the spontaneous EEG. Sadanaga (13) reported that electrical stimulation of the mesencephalic reticular formation desynchronized the spontaneous EEG without affecting duration of the hippocampal afterdischarge in rabbits. Therefore, anticonvulsive properties of YM-14673 and TRH may not be induced by change in spontaneous EEG activity. The pharmacological potent action of YM-14673 may be ascribable to the stability of YM-14673 with regard to enzymatic degradation in comparison with that of TRH (Imazaki, 1988, personal communication).

Sato et al. (16) have already reported that TRH and DN-1417 (IV) suppressed both the development of kindling and AD duration in fully kindled cats. However, these pharmacological actions of both drugs were not dose-dependent. Furthermore, DN-1417 administered intraventricularly showed the anticonvulsive actions dose dependently in rats (15). In the present study, intraperitoneal administration of YM-14673 showed tendency to suppress the development of kindling, and TRH even at high dose (10 mg/kg IP) showed little effect on it. The difference between present and other's results (15,16) may be due to differences of administration route and/or animal species.

Increases in the TRH content and the number of TRH receptor were observed in the amygdala and pyriform cortex for 48 hours, and in the striatum for 3 weeks after the last seizure in kindled rats (7). But the relationship between changes in TRH transmission and the suppressive effects of TRH on kindling is not clear now.

Involvement of the central catecholaminergic system is proposed in the development of kindling and seizure in fully kindled animals. Reserpine, which depletes monoamines in brain, and 6-hydroxydopamine (6-OHDA), which selectively destroys catecholaminergic neurons, enhanced kindling in rats (1). On the other hand, cocaine and methamphetamine chronically administered for inducing supersensitivity of dopaminergic receptors, suppressed kindling (18). YM-14673 antagonized hypothermia in reserpinized mice (23) and increased monoamine contents in the brain of rats (Okada, 1990, unpublished), indicating that this drug possesses facilitating effects on central monoaminergic system. The present observations that YM-14673 showed tendency to suppress the development of kindling and seizure in fully kindled rats may be due to, in part, accelerating release and/or turnover of catecholamines.

TABLE 1

EFFECTS OF YM-14673 AND TRH ON THE DEVELOPMENT OF KINDLING IN AMYGDALOID-KINDLED RATS (n = 6–9)

Stage	Drug Dose (mg/kg IP)	Saline	YM-14673 0.1	YM-14673 1	TRH 10
1		2.9 ± 0.4	1.8 ± 0.3	2.0 ± 0.2	2.3 ± 0.4
2		5.1 ± 0.5	5.0 ± 1.4	6.8 ± 1.0	6.4 ± 1.1
3		8.1 ± 0.9	6.8 ± 1.6	9.7 ± 1.2	8.6 ± 1.6
4		10.4 ± 1.1	8.8 ± 1.9	13.1 ± 1.6	12.0 ± 1.5
5		10.9 ± 1.0	9.7 ± 1.7	14.3 ± 1.5*	13.9 ± 1.6

[1]Number of stimulation.

*$p<0.1$ vs. saline-treated group (Student's t-test).

In conclusion, YM-14673 shortened the AD duration and showed tendency to suppress the development of kindling in amygdaloid-kindled rats. Thus we found that YM-14673 seemed to possess anticonvulsive property in rats.

REFERENCES

1. Arnold, P. S.; Racine, R. J.; Wise, R. A. Effects of atropine, reserpine, 6-hydroxydopamine, and handling on seizure development in the rat. Exp. Neurol. 40:457–470; 1973.
2. Goddard, G. V.; McIntyre, D.; Leech, C. K. A permanent change in brain function resulting from daily electrical stimulation. Exp. Neurol. 25:295–330; 1969.
3. Hollander, C. S.; Mitsuma, T.; Shenkman, L.; Woolf, P.; Gershengorn, M. C. Thyrotropin-releasing hormone: Evidence for thyroid response to intravenous injection in man. Science 175:209–210; 1972.
4. Ianaga, K.; Inoue, Y. Effects of a thyrotropin releasing hormone analog in a patient with myoclonus epilepsy. Kurume Med. J. 28:201–210; 1981.
5. Ianaga, K.; Nakano, K.; Nagata, T.; Tanaka, M.; Ogawa, N. Behavioral effects of protirelin in schizophrenia. Arch. Gen. Psychiatry 35:1011–1014; 1978.
6. Jacobs, L. S.; Snyder, P. J.; Utiger, R. D.; Wiber, J. F.; Daughaday, W. H. Increased serum prolactin after administration of synthetic thyrotropin releasing hormone (TRH) in man. J. Clin. Endocrinol. Metab. 33:996–998; 1971.
7. Kajita, S.; Ogawa, N.; Sato, M. Long-term increase in striatal thyrotropin-releasing hormone receptor binding caused by amygdaloid kindling. Epilepsia 28:228–233; 1987.
8. Metcalf, G. Regulatory peptides as a source of new drugs. The clinical prospects for analogues of TRH which are resistant to metabolic degradation. Brain Res. Rev. 4:389–408; 1982.
9. Nemeroff, C. B.; Prange, A. J.; Bissette, G. J.; Breese, G. R.; Lipton, M. A. Thyrotropin-releasing hormone (TRH) and its β-alanine analogue potentiation of the anticonvulsant potency of phenobarbital in mice. Psychopharmacol. Commun. 1:305–307; 1975.
10. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
11. Prange, A. J.; Wilson, I. C.; Lara, P. P.; Alltop, L. B.; Breese, G. R. Effects of thyrotropin-releasing hormone in depression. Lancet II: 999–1002; 1972.
12. Racine, R. J. Modification of seizure activity by electric stimulation. II. Motor Seizure Electroencephalogr. Clin. 32:281–294; 1972.
13. Sadanaga, Y. Mode of action of antipsychotic drugs in a hippocampal afterdischarge. Folia Pharmacol. Japon. 61:337–352; 1965.
14. Sano, K. Clinical studies on thyrotropin releasing hormone tartrate for the treatment of disturbance of consciousness. Adv. Neurol. Sci. 23:184–210; 1979.
15. Sato, M.; Kajita, S.; Okamoto, M.; Morimoto, K.; Ogawa, T.; Otsuki, S.; Ogawa, N.; Nagai, Y.; Narumi, S. Anticonvulsant action of thyrotropin-releasing hormone analog (DN-1417) and changes in brain monoamines. Seishin Shinkeigaku Zasshi 87:176–185; 1985.
16. Sato, M.; Morimoto, K.; Wada, J. A. Antiepileptic effects of thyrotropin-releasing hormone and its new derivative, DN-1417, examined in feline amygdaloid kindling preparation. Epilepsia 25:537–544; 1984.
17. Sato, M.; Tomoda, T.; Hikasa, N.; Otsuki, S. Inhibition of amygdaloid kindling by chronic pretreatment with cocaine or methamphetamine. Epilepsia 21:497–507; 1980.
18. Sobue, I.; Yamamoto, H.; Kanagaya, M.; Iida, M.; Takayanagi, T. Effects of thyrotropin releasing hormone on ataxia of spinocerebellar degeneration. Lancet I:418–419; 1980.
19. Ueda, S.; Nakamura, J.; Inanaga, K. Clinical effects of TRH analog (DN-1417) on the Lennox Syndrome. J. Jpn. Epilepsy Soc. 1:31–39; 1983.
20. Wada, J. A.; Ozawa, T.; Sato, M.; Wake, A.; Corcoran, M. E.; Troupin, A. S. Acute anticonvulsant effects of diphenylhydantoin, phenobarbital and carbamazepin: A combined electroclinical and serum level study in amygdaloid-kindled cats and baboons. Epilepsia 17:77–88; 1976.
21. Wada, J. A.; Sato, M.; Wake, A.; Green, J. R.; Troupin, A. S. Prophylactic effects of phenytoin, phenobarbital and carbamazepin in kindling cat preparations. Arch. Neurol. 33:426–434; 1976.
22. Yamamoto, M.; Shimizu, M. Effects of a new TRH analogue, YM-14673 on central nervous system. Naunyn Schmiedebergs Arch. Pharmacol. 336:561–565; 1987.
23. Yamamoto, M.; Shimizu, M.; Iwai, A. Antagonizing effects of YM-14673, a new TRH derivative, on behavioral and electroencephalographic changes in reserpinized animals. Psychopharmacology (Berlin) 95:162–166; 1988.