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Effect of Thyrotropin-releasing Hormone on Pentobarbitone-induced Sleep in Rats: Continuous Treatment with a Sustained Release Injectable Formulation

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Abstract—The mode of action and the time course of the effects of continuous thyrotropin-releasing hormone (TRH) treatment using a two-week sustained release injectable formulation of TRH-containing copoly((±)-lactic/glycolic acid) microspheres (TRH-SR) on pentobarbitone-induced sleeping time were studied in rats. Subcutaneous treatment with TRH-SR at doses corresponding to 0·05 and 0·2 mg of TRH kg^{-1} day^{-1} caused a dose-related shortening of pentobarbitone-induced sleeping time with a minimum effective dose (MED) of 0·05 mg kg^{-1} day^{-1}, without affecting the body weight gain. On the other hand, the MED of TRH when given as a bolus subcutaneous injection was 40 mg kg^{-1}. The effect of TRH-SR treatment was blocked by intraperitoneal scopolamine (0·1 mg kg^{-1}) and mecamylamine (2 mg kg^{-1}) but not by scopolamine methyl bromide (0·1 mg kg^{-1}). The results indicate that continuous TRH treatment using TRH-SR causes shortening of pentobarbitone-induced sleeping time at doses lower than those required using bolus injection and probably by a mechanism involving the central cholinergic system.

Thyrotropin-releasing hormone (TRH) was originally identified as the substance which causes the liberation of thyrotropin and prolactin from the pituitary gland (Bøler et al 1969). TRH, besides its endocrine action, has many actions on the central nervous system (CNS) (Horita et al 1986) and many clinical attempts to use TRH and its derivatives for various CNS disorders have been made. Their effectiveness against depression (Kastin et al 1972), prolonged disturbance of consciousness (Manaka et al 1977), schizophrenia (Inanaga et al 1978), epilepsy (Inanaga & Inoue 1981; Matsumoto et al 1987), amyotrophic lateral sclerosis (Engel et al 1983), spinocerebellar ataxia (Sobue et al 1986), and spinal cord injury (Aii et al 1986) has been demonstrated.

The plasma half-life of TRH, however, is so short (Nagai et al 1980) that repeated or continuous infusion of TRH is necessary to obtain clinical effects. Advances in pharmaceutical engineering have resulted in the introduction of new pharmaceuticals which enable treatment using drugs with short biological half-lives at much less frequent intervals (Wakiyama et al 1981; Redding et al 1984; Tice & Cowsar 1984; Wise 1984; Sanders et al 1986; Ogawa et al 1989; Okada et al 1989). Injectable microspheres prepared with a biodegradable polymer, copoly((±)-lactic/glycolic acid) (PLGA), and used to produce a prolonged controlled injectable dosage form of TRH (TRH-SR) have been recently developed in the Pharmaceutical Research Laboratories of Takeda Chemical Ind. Ltd (Heya et al 1991a,b). In addition, TRH-SR which is designed to release the drug over 2 weeks has been shown to provide almost constant plasma TRH levels from 1 to more than 10 days after subcutaneous injection in rats, although it causes a transient increase in the TRH level in the first few hours (Nagai et al, unpublished observation).

In the present report, we examined the time course and mechanism of the pharmacological action of TRH after administration of TRH-SR. The antagonism of pentobarbitone-induced sleep was used as the test to evaluate the pharmacological action since it is most reproducible among tests assessing the CNS action of TRH and the involvement of a cholinergic mechanism has been well established (Prange et al 1974; Breese et al 1975; Miyamoto et al 1982).

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Materials and Methods

Animals

Male Wistar rats 7 weeks old at the first pentobarbitone injection, were kept under constant environmental conditions (24 ± 1°C and a regular 12-h light/dark cycle, lights on 0730 h), with free access to food and water, and housed 5 rats to a cage.

Measurement of sleeping time

The sleeping time was measured by an investigator who was unaware of each individual animal's treatment. Rats were given sodium pentobarbitone (30 mg kg^{-1}) intravenously in the afternoon (1300–1500 h). Sleeping time (min) was taken as the period of the loss of the righting reflex. Percent shortening was calculated as [1 − (sleeping time in the drug-treated group/sleeping time in the control group)] × 100.

Drugs and drug injection

The following drugs were used: thyrotropin-releasing hormone (TRH, L-pyroglutamyl-L-histidyl-L-prolinamide L-tartrate monohydrate, Takeda, Japan), TRH (free base)-containing PLGA microspheres (TRH-SR, two-week sustained release injectable type containing 5·89% of TRH, Takeda, Japan), sodium pentobarbitone (Nacalai Tesque,

Japan), scopolamine hydrochloride (Sigma, USA), scopolamine methyl bromide (Tokyo Kasei, Japan), and mecamylamine hydrochloride (Sigma). In the present study, the doses of TRH-SR are expressed as the amount of TRH released per day, and those of TRH are expressed as the weight of free base. TRH-SR was suspended in vehicle (50 mg of D-mannitol, 5 mg of carboxymethylcellulose sodium and 1 mg of Tween 80 dissolved in 1 mL distilled water) and injected subcutaneously in a volume of 2 mL kg^{-1}. Sodium pentobarbitone was dissolved in physiological saline and injected intravenously into the tail vein in a volume of 1 mL kg^{-1}. TRH was dissolved in physiological saline and injected subcutaneously 5 min before pentobarbitone injection in a volume of 2 mL kg^{-1}. Scopolamine (0·1 mg kg^{-1} as the salt), scopolamine methyl bromide (0·1 mg kg^{-1}) and mecamylamine (2 mg kg^{-1} as the salt) were also dissolved in physiological saline and injected intraperitoneally in a volume of 2 mL kg^{-1}, 30 min before the pentobarbitone injection.

Analysis of data

The time courses of the shortening of the pentobarbitone-induced sleeping time and the change in body weight following TRH-SR injection were analysed by a two-way analysis of variance. Differences between the control group and the TRH-SR or the TRH multiple dose-treated group were compared utilizing Duncan's multiple range test (two-tailed) following a one-way analysis of variance. Other differences between groups were compared utilizing Student's *t*-test (two-tailed).

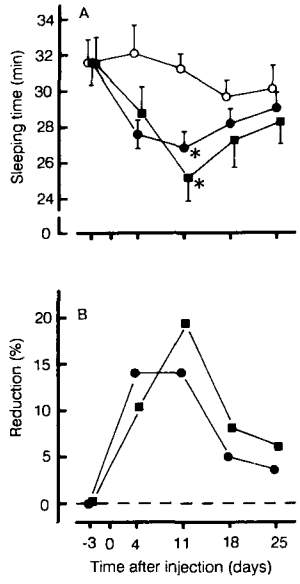

FIG. 1. Time course of the antagonism of pentobarbitone-induced sleep caused by continuous TRH treatment using two-week sustained release injectable TRH-containing PLGA microspheres (TRH-SR) in rats. A, sleeping time; and B, percent shortening in sleeping time. ○ Vehicle-treated control group, ● TRH-SR 0·05 mg kg^{-1} day^{-1}, ■ 0·2 mg kg^{-1} day^{-1}. The doses of TRH-SR are expressed as the amount of TRH released per day. Sleep was induced by intravenous injection of sodium pentobarbitone (30 mg kg^{-1}). The data shows the mean ± s.e.m. of 14 rats in the control group and 12 rats in each of the other groups. *$P<0·01$ compared with the control group (Duncan's multiple range test, two-tailed).

Results

Effect of TRH-SR treatment on pentobarbitone-induced sleeping time and body weight

The time course of the antagonism of pentobarbitone-induced sleep by TRH-SR treatment is shown in Fig. 1. The mean sleeping time in the vehicle-treated control group was almost constant, ranging from 29·6 to 32·1 min, for the 5 measurement points during the experiment. On the other hand, that in the TRH-SR-treated groups was consistently shorter than that in the control group. Although no significant effect was observed 4 days after TRH-SR injection ($F(2,35)=3·10$, $P>0·05$), the effect of TRH-SR treatment was significant 11 days after injection ($F(2,35)=9·79$, $P<0·001$). At that time, the shortening effect was larger in both TRH-SR-treated groups (percent shortening, 0·05 mg kg^{-1} day^{-1}, 14%, and 0·2 mg kg^{-1} day^{-1}, 19·4%), and there were significant differences between the vehicle-treated control group and both the TRH-SR 0·05 mg kg^{-1} day^{-1}- and 0·2 mg kg^{-1} day^{-1}-treated groups ($T=4·38$, $P<0·01$, and $T=6·05$, $P<0·01$, respectively). Following the 14-day TRH-releasing period, a significant effect of TRH-SR treatment was no longer observed (on day 18 post-injection, $F(2,35)=1·21$, $P>0·1$, and on day 25 post-injection, $F(2,35)=0·635$, $P>0·5$). The two-way analysis of variance of the pentobarbitone-induced sleeping time at 2 points (on day 4 and 11 post-injection) revealed that both the dose of TRH-SR and the time course of treatment are significant factors ($F(2,35)=6·97$, $P<0·01$ and $F(1,35)=5·49$, $P<0·05$, respectively). However, the interaction term of the analysis was not significant ($F(2,35)=1·61$, $P>0·20$). Table 1 shows that the body weight gain in all three groups was smooth, indicating that TRH-SR treatment has no influence on the change in body weight ($F(2,35)=1·99$, $P>0·2$).

Table 1. Effect of TRH-SR treatment on the percent change in body weight in rats.

Dose (mg kg^{-1} day^{-1})	Days after TRH-SR injection					
	−3	0	4	11	18	25
Vehicle	−14·3±0·3	0	10·4±0·3	30·5±0·7	44·9±1·2	56·5±1·6
TRH-SR						
0·05	−14·9±0·4	0	9·4±0·7	32·7±0·9	46·9±1·5	58·0±1·7
0·2	−14·5±0·5	0	8·4±0·4	27·4±1·2	43·5±1·8	54·8±2·0

Data are expressed as the mean ± s.e.m. The doses of two-week sustained release injectable TRH-containing PLGA microspheres (TRH-SR) are expressed as the amount of TRH released per day. The number of animals in the vehicle-treated control group was 14 and the other groups had 12 animals each. The mean body weights (± s.e.m.) in the vehicle-, TRH-SR 0·05 mg kg^{-1} day^{-1}- and 0·2 mg kg^{-1} day^{-1}-treated group at the time of injection were 186·7±1·8, 183·9±2·7, and 181·4±2·1 g, respectively.

Effect of bolus injection of TRH on the pentobarbitone-induced sleep

Two weeks after the pre-test measurement, the effect of bolus subcutaneous injection of TRH on pentobarbitone-induced sleep was studied. As shown in Table 2, treatment with TRH 5 min before the pentobarbitone injection caused a dose-related reduction in sleeping time ($F(3,96)=4{\cdot}06$, $P<0{\cdot}01$). The minimum effective dose was 40 mg kg^{-1}, and the sleeping time was shortened by 13·6%.

Effects of anticholinergic drugs on the shortening of the pentobarbitone-induced sleep by TRH-SR treatment

Eleven days after vehicle or TRH-SR (0·2 mg kg^{-1} day^{-1}) injection (2 weeks after pre-test), the effects of scopolamine (0·1 mg kg^{-1}), scopolamine methyl bromide (0·1 mg kg^{-1}) and mecamylamine (2 mg kg^{-1}) on the shortening of pentobarbitone-induced sleep by TRH-SR treatment were studied. As shown in Table 3, although scopolamine and mecamylamine had no significant influence on pentobarbitone-induced sleep itself, they almost completely blocked the shortening effect of TRH-SR treatment (both $P<0{\cdot}05$). On the other hand, scopolamine methyl bromide did not affect the pentobarbitone-induced sleep either in the vehicle- or in the TRH-SR-treated group.

Table 2. Effect of bolus injection of TRH on pentobarbitone-induced sleeping time in rats.

Drug	Dose (mg kg^{-1})	n	Sleeping time (min)		Shortening (%)
			Pre-test	Drug test	
Saline		28	31·6±1·0	34·8±1·0	—
TRH	10	24	31·6±1·2	36·0±1·6	−3·4
	20	24	31·6±1·2	33·5±1·3	3·7
	40	24	31·7±0·9	30·0±1·1[a]	13·6

Data are expressed as the mean±s.e.m. Drug test was performed two weeks after the pre-test. Saline or TRH was injected subcutaneously 5 min before sodium pentobarbitone (30 mg kg^{-1}, intravenously). n: number of animals in the group. [a]$P<0{\cdot}05$ compared with the saline-treated control group (Duncan's multiple range test, two-tailed).

Table 3. Effects of anticholinergic drugs on the pentobarbitone sleep shortening effect of TRH-SR treatment in rats.

Treatment	n	Sleeping time (min)		Shortening (%)
		Pre-test	Drug test	
Vehicle+saline	18	35·8±1·4	38·9±1·4	—
Vehicle+SCO	16	35·9±1·6	38·1±2·3	2·1
TRH-SR+saline	18	35·9±1·1	31·6±1·8[a]	18·7
TRH-SR+SCO	16	35·9±0·9	38·0±1·8[b]	2·3
Vehicle+saline	18	33·7±1·2	40·3±1·6	—
Vehicle+SMB	16	33·6±1·0	41·7±2·9	−3·5
TRH-SR+saline	17	34·4±0·8	29·7±1·0[c]	26·4
TRH-SR+SMB	16	33·7±1·0	32·8±1·7[d]	18·7
Vehicle+saline	18	34·1±1·3	39·2±1·8	—
Vehicle+MEC	16	34·0±1·3	37·7±0·9	3·8
TRH-SR+saline	18	34·6±1·4	29·7±0·9[c]	24·3
TRH-SR+MEC	16	34·0±0·9	34·1±1·7[b]	13·0

Data are expressed as the mean±s.e.m. The pre-test was performed 3 days before and the drug test 11 days after subcutaneous injection of vehicle or TRH-SR. Saline or one of the anticholinergic drugs was injected intraperitoneally 30 min before intravenous sodium pentobarbitone (30 mg kg^{-1}). TRH-SR: two-week sustained release injectable TRH-containing PLGA microspheres at a dose corresponding to TRH 0·2 mg kg^{-1} day^{-1}; SCO: scopolamine 0·1 mg kg^{-1} (hydrochloride salt); SMB: scopolamine methyl bromide 0·1 mg kg^{-1}; and MEC: mecamylamine 2 mg kg^{-1} (hydrochloride salt). [a]$P<0{\cdot}01$ and [c]$P<0{\cdot}001$ compared with the vehicle-saline-treated control group, [b]$P<0{\cdot}05$ compared with the TRH-SR-saline-treated group, and [d]$P<0{\cdot}05$ compared with the vehicle-SMB-treated group (Student's t-test, two-tailed).

Discussion

Consistent with previous reports (Prange et al 1974; Breese et al 1975; Miyamoto et al 1982), bolus injection of TRH caused a dose-related reduction in pentobarbitone-induced sleeping time in rats. When TRH was given continuously by means of TRH-SR, a similar effect was produced. The minimum effective dose (MED) for subcutaneous TRH treatment by bolus injection and for TRH-SR was 40 mg kg^{-1} and 0·05 mg kg^{-1} day^{-1}, respectively, and the percent by which the sleeping time was shortened at the MED was 13·6 and 14·8%, respectively. Thus, it is indicated that continuous treatment with TRH results in the extreme reduction of the MED to about one thousandth for pentobarbitone-induced sleep antagonism in rats.

Preliminary determination of the plasma TRH level in rats revealed that the basal level ranges from 3 to 27 pg mL^{-1}, and the level 1, 3, 10 and 21 days after injection of TRH-SR at a dose of 0·2 mg kg^{-1} day^{-1} was 210±42, 194±38, 198±64 and 8±2 pg mL^{-1} (mean±s.e.m., n=5), respectively (Nagai et al, unpublished observation). However, the sleep-shortening effect of TRH-SR treatment on day 11 post-injection was larger than that on day 4 post-injection, even though the plasma TRH level has been shown to be almost constant from one day to more than ten days after TRH-SR injection. Thus, this time course and the marked reduction in the MED caused by continuous TRH treatment with TRH-SR suggest possible secondary changes in the sites related to the effect of TRH, occurring during long-term exposure to TRH.

We have observed a reduction in the MED for the neurologic recovery-accelerating effect in rats with spinal cord injury when using more frequent treatment with TRH and suggested that a TRH administration method that exposes the animals to TRH more frequently produces better improvement in the neurologic state after spinal cord injury (Hashimoto & Fukuda 1990). Thus, it is conceivable that the reduction in the effective dose of TRH when it is used repeatedly or continuously may be a common phenomenon for all the central nervous actions of TRH. In addition, Yamamoto et al (1991) reported that although injecting TRH once daily had little effect on neurologic recovery in middle cerebral artery-occluded rats, multiple daily injections of TRH to maintain the TRH level in plasma resulted in significant acceleration of neurologic recovery in those animals. Therefore, TRH-SR treatment should be expected to have sufficient therapeutic effects in patients with TRH-sensitive illnesses even at doses considered low, compared with those required using bolus injection.

Studies of ingestive behaviour revealed that TRH given intraperitoneally or intracerebroventricularly suppressed food and water intake (Vijayan & McCann 1977; Vogel et al 1979). In addition, TRH applied electro-osmotically activates the glucoreceptor neurons in the ventromedial nucleus of the hypothalamus which has been considered as the satiety centre (Ishibashi et al 1979). In the present study, however,

continuous treatment with TRH did not affect the body weight gain. Thus, it is suggested that continuous treatment with TRH at the present doses used does not have any influence on food intake.

It is conceivable that metabolites of TRH are involved in the effect of TRH-SR. However, the putative main metabolites of TRH, histidyl-proline diketopiperazine (cyclo His-Pro) and acid TRH, are considered ineffective in reducing pentobarbitone-induced sleeping time in mice (Prasad et al 1977), although cyclo His-Pro but not acid TRH is reported to be more effective than TRH in antagonizing ethanol-induced sleep in mice (Prasad et al 1977). In addition, as is the case with bolus injection (Breese et al 1975; Miyamoto et al 1982), the shortening effect of TRH-SR treatment on pentobarbitone-induced sleeping time was blocked by intraperitoneal treatment with both muscarinic and nicotinic cholinergic antagonists, scopolamine and mecamylamine, both of which can penetrate the blood-brain barrier, while scopolamine methyl bromide, a muscarinic cholinergic antagonist which is unable to penetrate the blood-brain barrier, could not reverse the shortening effect of TRH-SR treatment, indicating that cholinergic mechanisms, probably in the central nervous system, may be involved in the effect of TRH-SR treatment. Thus, it is suggested that TRH itself and not its metabolites is involved in this effect of TRH-SR-treatment.

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