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Lack of behavioral tolerance by repeated treatment with taltirelin hydrate, a thyrotropin-releasing hormone analog, in rats

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Abstract

In order to determine whether acute tolerance develops by taltirelin hydrate ((−)-*N*-[(*S*)-hexahydro-1-methyl-2,6-dioxo-4-pyrimidinylcarbonyl]-L-histidyl-L-prolinamide tetrahydrate; taltirelin), a thyrotropin-releasing hormone (TRH) analog, we examined the motor behavior, TRH receptors and dopamine D_2 receptors following 2 weeks treatment in rats. Taltirelin selectively bound to TRH receptors and increased the spontaneous motor activity by a single administration, suggesting that the motor effect of taltirelin is mediated by TRH receptors. Following repeated treatment with TRH, there was a significant reduction in the increment of spontaneous motor activity. In contrast, after repeated treatment with taltirelin at a dose that increased the motor activity to a similar extent to TRH by a single administration, there was no apparent change in its motor effect. In accord with the motor activity, we found a significant reduction in the [^{3}H]methyl-TRH binding to TRH receptors in the brain following repeated treatment with TRH but not taltirelin. However, the [^{3}H]spiperone binding to dopamine D_2 receptors in the corpus striatum did not change by repeated taltirelin and TRH treatments. Thus, the down-regulation of TRH receptors would be a main cause of the behavioral tolerance. These results suggest that taltirelin hardly develops the behavioral tolerance due to the lack of down-regulation of TRH receptors.

Keywords: TRH (thyrotropin-releasing hormone); Taltirelin; Behavior tolerance; Motor activity; Down regulation; TRH receptor

1. Introduction

Thyrotropin-releasing hormone (TRH) is a neuropeptide that is widely distributed in the mammalian central nervous system (CNS) (Brownstein et al., 1974; Oliver et al., 1974). Apart from the thyroid stimulating hormone (TSH) releasing action, the CNS stimulant actions of TRH have been shown, including an increase in locomotor activity and antagonism to pentobarbital-induced sleep (Griffiths, 1985; Horita et al., 1986). Furthermore, pharmacological studies revealed the amelioration of motor dysfunction and disturbances in consciousness by TRH in animal models of CNS diseases (Manaka et al., 1977; Kurihara et al., 1985; Horita et al., 1986). Clinical studies have proven the therapeutic effect of TRH tartrate in patients with spinocerebellar degeneration (SCD) and prolonged impaired consciousness (Sobue et al., 1983; Sano, 1988). However, clinical use of TRH for SCD has been limited because repeated treatment with TRH causes a decrease in its therapeutic effects (Ogawa et al., 1983a). The tolerance to the action of TRH seems to be due to the down-regulation of its receptors (Ogawa et al., 1983b; Simasko and Horita, 1985).

Taltirelin hydrate ((−)-*N*-[(*S*)-hexahydro-1-methyl-2,6-dioxo-4-pyrimidinyl-carbonyl]-L-histidyl-L-prolinamide tetrahydrate; taltirelin) is a novel TRH analog which exerts more potent and longer-lasting CNS activity but lower endocrine activity than TRH (Suzuki et al., 1990; Yamamura et al., 1990, 1991a,b). Preclinical studies revealed anti-ataxic actions of taltirelin in experimental motor dysfunction models (Kinoshita et al., 1995). Furthermore, recent clinical studies have demonstrated the arrest of the progression and amelioration of ataxia by taltirelin in patients with SCD (Hirayama et al., 1997; Kanazawa et al., 1997). In contrast to TRH, taltirelin has been used for SCD without the withdrawal and intermittent treatments because taltirelin exerted therapeutic effects following 28 weeks of treatment (Kanazawa et al., 1997).

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Taltirelin binds to TRH receptors in the brain (Kinoshita et al., 1997; Asai et al., 1999), and the activation of TRH receptors induces the motor behavior (Yamamura et al., 1991a). However, the involvement of other molecules in the motor effect of taltirelin is not clear. Therefore, we examined the binding affinities of taltirelin for various molecules. It also remains unclear whether the behavioral tolerance actually develops by repeated treatment with taltirelin. Therefore, we examined the motor behavior and TRH receptors following repeated treatment with taltirelin and TRH. Moreover, brain dopaminergic systems are stimulated by the activation of TRH receptors, and primarily mediate the motor effect of taltirelin (Yamamura et al., 1991a). Therefore, we also examined the dopamine receptors following repeated treatment with taltirelin and TRH.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) were kept in an air-conditioned room with controlled temperature (23±1 °C), humidity (55±5%) and 24-h lighting (lights on 7:00 through 19:00). The animals were allowed free access to a standard pellet diet (CE-1; Clea Japan, Inc., Tokyo, Japan) and tap water. This study followed guiding principles for the care and use of laboratory animals (approved by the Japanese Pharmacological Society), and was approved by the Ethical Committee of Tanabe Seiyaku Co., Ltd.

2.2. Chemicals

Taltirelin and TRH tartrate were synthesized at Tanabe Seiyaku Co., Ltd. (Saitama, Japan). [^{3}H] methyl-TRH (MeTRH) (82.5 Ci/mmol) (NEN Life Science Products, Inc., Boston, MA), [^{3}H]spiperone (98.0 Ci/mmol) (Amersham Pharmacia Biotech, Ltd., Buckinghamshire, England), bovine serum albumin, sulpiride (Sigma Chemical Co., St. Louis, MO) and ketanserin tartrate (RBI, Natick, MA) were purchased from commercial sources.

2.3. Receptor and ion channel binding

In order to examine the selectivity, the binding affinities of taltirelin (6.3 μM) were determined with 31 receptors and ion channels by MDS Pharma Services (Taipei, Taiwan) according to their established proprietary protocols. Radioligands and tissue preparations are summarized in Table 1.

2.4. Spontaneous motor activity after single treatment with taltirelin and TRH

The animals (10 weeks old) were individually housed in stainless-steel wire mesh cages (14 W × 24 D × 16 H cm). Spontaneous motor activity was measured with a SCANET (MV-10, Toyo Sangyo Co., LTD., Toyama, Japan). After the familiarization for 60 min in the acrylic cage (45 W × 45 D × 35 H cm), spontaneous motor activity was measured for 60 min after taltirelin (0.3–3 mg/kg), TRH (10, 30 mg/kg) and saline were intraperitoneally administered.

2.5. Spontaneous motor activity and TRH receptor binding after repeated treatment with taltirelin and TRH

The animals (8 weeks old) were individually housed in stainless-steel wire mesh cages. Taltirelin (1 mg/kg/day), TRH (30 mg/kg/day) and saline were intraperitoneally administered for 14 days. The measurement of spontaneous motor activity for 60 min was performed after i.p. administration of taltirelin (1 mg/kg), TRH (30 mg/kg) and saline on the 15th day.

After the measurement of spontaneous motor activity, the brains except the cerebellum were dissected from the animals treated with saline, taltirelin or TRH for 15 consecutive days, and used for the TRH receptor binding assay according to the method described (Asai et al., 1999). The crude synaptic membranes from 15 mg of the brain were incubated with 2 nM [^{3}H]MeTRH for 24 h on ice in 1 ml of 50 mM Tris–HCl buffer (pH 7.4, 25 °C) containing 50 μg/ml bacitracin and 0.1% bovine serum albumin (BSA). Nonspecific binding was determined in the presence of 100 μM TRH. The incubation was terminated by vacuum filtration through Whatman GF/B glass fiber filters, followed by 3 rinses with 4 ml ice-cold buffer. The filters were dissolved with a scintillator and the radioactivity on the filter was measured with a liquid scintillation spectrometer (TRI-CARB 4640; Packard Instrument Co., Meriden, CT). All assays were done in triplicate. Protein contents of membrane preparations were determined by the method of Lowry et al. (1951), with BSA as the standard.

2.6. Dopamine D_2 receptor binding after repeated treatment with taltirelin and TRH

The animals (8 weeks old) were intraperitoneally administered with saline, taltirelin (1 mg/kg/day) or TRH (30 mg/kg/day) for 14 days. Twenty-four hours after the last administration, the animals were sacrificed by decapitation and the corpus striatum was quickly excised on ice.

The crude synaptic membranes were prepared from 1 mg of the corpus striatum and incubated with 1 nM [^{3}H]spiperone for 20 min at 37 °C in 1 ml of 50 mM Tris–HCl buffer (pH 7.4, 25 °C) containing 50 nM ketanserin tartrate, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. Nonspecific binding was determined in the presence of 10 μM sulpiride. The incubation was terminated by vacuum filtration through Whatman GF/B glass fiber filters, followed by 3 rinses of 4 ml ice-cold buffer. The filters were dissolved with a scintillator and the radioactivity on the filter was measured with a liquid scintillation spectrometer. All assays were done in triplicate. Protein contents of membrane preparations were determined by the method of Lowry et al. (1951), with BSA as the standard.

Table 1
Effect of taltirelin in the receptor and ion channel binding assays

Binding site	Radioligand	Tissue	% Inhibition
Adrenergic-α_1	[^{3}H]Prazosin	Rat brain	−11
Adrenergic-α_2	[^{3}H]Rauwolscine	Rat cortex	3
Adrenergic-β	[^{3}H]DHA	Rat brain	−8
Ca^{2+} channel type L			
-benzodiazepine site	[^{3}H]Diltiazem	Rat cortex	18
-dihydropyridine site	[^{3}H]Nitrendipine	Rat cortex	−2
Ca^{2+} channel type N	[^{125}I]ω-Conotoxin	Rat frontal lobe	0
Dopamine D_1	[^{3}H]SCH23390	Human recombinant	7
Dopamine D_{2L}	[^{3}H]Spiperone	Human recombinant	−7
Dopamine D_{2S}	[^{3}H]Spiperone	Human recombinant	9
Dopamine D_3	[^{3}H]Spiperone	Human recombinant	−3
Dopamine $D_{4.2}$	[^{3}H]Spiperone	Human recombinant	−4
$GABA_A$-agonist site	[^{3}H]Muscimol	Rat brain	−10
-benzodiazepine site	[^{3}H]Flunitrazepam	Rat brain	−14
Glutamate AMPA	[^{3}H]AMPA	Rat cortex	7
Glutamate kainate	[^{3}H]Kainate	Rat brain	6
Glutamate NMDA			
-agonist site	[^{3}H]CGS19755	Rat cortex	15
-phencyclidine site	[^{3}H]TCP	Rat cortex	−3
Glycine			
-strychnine sensitive site	[^{3}H]Strychnine	Rat spinal cord	23
Histamine H_1	[^{3}H]Pyrilamine	Guinea-pig brain	5
Histamine H_3	[^{3}H]NAMH	Rat brain	6
Muscarinic acetylcholine	[^{3}H]QNB	Rat cortex	19
Na^{2+} channel-site 2	[^{3}H]Batrachotoxinin	Rat brain	−3
Neurokinin NK_1	[^{3}H]Substance P	Guinea-pig submaxillary gland	−12
Neuropeptide Y_2	[^{3}H]Neuropeptide Y	Rabbit kidney medulla	9
Nicotinic acetylcholine	[^{3}H]Cytisine	Rat cortex	8
Opiate	[^{3}H]Naloxone	Rat brain	−9
Serotonin 5-HT_1	[^{3}H]5-HT	Rat cortex	−5
Serotonin 5-HT_2	[^{3}H]Ketanserin	Rat brain	21
Sigma	[^{3}H]DTG	Guinea-pig brain	12
TRH	[^{3}H]Me-TRH	Rat brain	80
Vasopressin V_1	[^{3}H]Arg-vasopressin	Rat liver	9

DHA: dihydroalprenolol; TCP: thienyl-cyclohexyl-piperidine; NAMH: *n*-α-methylhistamine; QNB: quinuclidiny benzilate; DTG: di-tolyl-quanidine.

2.7. Statistical analysis

The data are means and S.E.M. The significance of difference was assessed by one-way analysis of variance, followed by Dunnett's or Bonferroni's tests.

3. Results

3.1. Receptor and ion channel binding

We examined the affinities of taltirelin for the receptors and ion channels (Table 1). Taltirelin (6.3 μM) inhibited 80% of [^{3}H]MeTRH binding to TRH receptors in rat brain synaptic membranes. In contrast, there were less than 23% inhibitions of respective radioligand binding for 30 other receptors and ion channels at this concentration of taltirelin.

3.2. Spontaneous motor activity after single treatment with taltirelin and TRH

Taltirelin (0.3–3 mg/kg) and TRH (10 and 30 mg/kg) dose-dependently increased the spontaneous motor activity for 60 min after a single i.p. administration (Fig. 1). The increase of spontaneous motor activity by taltirelin at a dose of 1 mg/kg was almost similar to that of TRH at a dose of

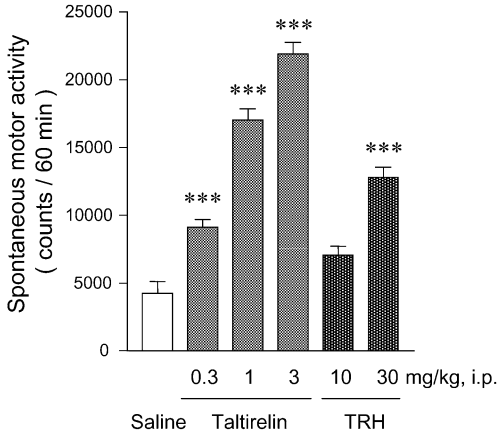

Fig. 1. Effects of single treatment with taltirelin and TRH on spontaneous motor activity in rats ($n=8$). Spontaneous motor activity was measured for 60 min after i.p. drug administration. Data are mean±S.E.M. ***$P<0.001$ vs. saline (Dunnett's multiple comparison test).

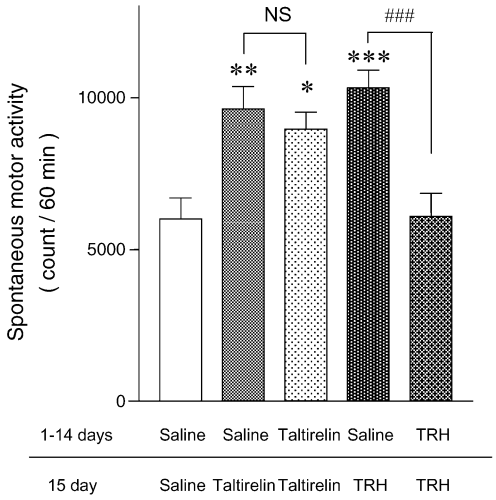

Fig. 2. Effects of repeated treatment with taltirelin (1 mg/kg) and TRH (30 mg/kg) on spontaneous motor activity in rats ($n=12$). Drugs were intraperitoneally administered for 14 days and spontaneous motor activity was measured for 60 min after the last administration at the 15th day. Data are mean±S.E.M. $*P<0.05$, $**P<0.01$, $***P<0.001$ vs. treatment with saline for 15 days, $\#\#\#P<0.001$ (Bonferroni's multiple comparison test). NS: not significant.

30 mg/kg. Therefore, in the subsequent experiments, we repeatedly administered taltirelin and TRH at doses of 1 and 30 mg/kg, respectively, to determine whether the tolerance developed following repeated treatment with taltirelin and TRH.

3.3. Spontaneous motor activity and TRH receptor binding after repeated treatment with taltirelin and TRH

Taltirelin (1 mg/kg) and TRH (30 mg/kg) increased the spontaneous motor activity to a similar extent after repeated saline administration for 14 days (Fig. 2). TRH (30 mg/kg) did not increase the spontaneous motor activity after repeated TRH administration (30 mg/kg/day) (Fig. 2). In contrast, there was no difference in the increment of spontaneous motor activity by taltirelin (1 mg/kg) after repeated taltirelin (1 mg/kg/day) and saline administrations for 2 weeks. To investigate the influences of repeated treatment with TRH and taltirelin on TRH receptors, we examined specific [^{3}H]MeTRH binding in the brain. The specific [^{3}H]MeTRH binding was significantly decreased by repeated treatment with TRH but not taltirelin (Fig. 3).

3.4. Dopamine D_2 receptor binding after repeated treatment with taltirelin and TRH

We also evaluated the effect of repeated treatment with taltirelin and TRH on dopamine D_2 receptors. Neither repeated taltirelin (1 mg/kg/day) nor TRH (30 mg/kg/day) treatment for 2 weeks affected the specific [^{3}H]spiperone binding in the corpus striatum. The values of the specific binding in each group ranged from 276.4±5.1 to 298.6±4.8 fmol/mg protein (mean±S.E.M.; $n=8$).

4. Discussion

The present study demonstrated that taltirelin exerts high affinity for TRH receptors but low affinities for other various receptors and ion channels. Two weeks of treatment with TRH reduced both the motor activity and the specific [^{3}H]MeTRH binding to TRH receptors. In contrast, repeated treatment with taltirelin did not show apparent changes in either the motor activity or the specific [^{3}H]MeTRH binding to TRH receptors. Neither repeated taltirelin nor TRH treatment affected the specific [^{3}H]spiperone binding to dopamine D_2 receptors. These observations indicate that taltirelin selectively binds to TRH receptors and increases the motor activity, and that repeated treatment with taltirelin in contrast to TRH does not induce the behavioral tolerance and selective down-regulation of TRH receptors.

Taltirelin has a high affinity for TRH receptors with K_i values of 70–170 nM in rat brain regions (Asai et al., 1999). The present study demonstrated that taltirelin displays more than micromolar affinities for various receptors and ion channels, excluding TRH receptors. Therefore, taltirelin selectively binds to TRH receptors. Although taltirelin is about 20 times less potent than TRH in displacing [^{3}H]MeTRH binding in brain TRH receptors (Asai et al., 1999), it was about 30 times more potent than TRH in increasing the spontaneous motor activity by a single administration. This potent motor effect of taltirelin can be explained by the higher resistance to enzymatic degradation of taltirelin than of TRH (Kodama et al., 1997). However, two subtypes of TRH receptors in the rat CNS have been identified: TRH-R1 and TRH-R2 receptors; while TRH binds with equal affinities (Cao et al., 1998; Itadani et al., 1998). Taken together, the motor effect of taltirelin is likely to be mediated by TRH receptors, but the relevant subtypes are to be determined.

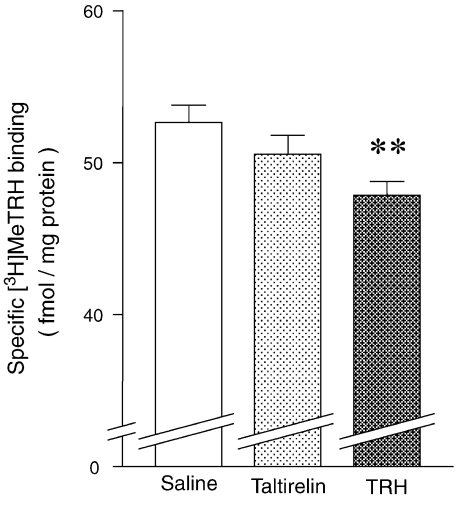

Fig. 3. Effects of repeated treatment with taltirelin (1 mg/kg) and TRH (30 mg/kg) on specific [^{3}H]MeTRH binding in rats ($n=12$). Drugs were intraperitoneally administered for 15 days and TRH receptor binding assay was performed after the measurement of spontaneous motor activity. Data are mean±S.E.M. $**P<0.01$ vs. saline (Dunnett's multiple comparison test).

Sub-acute tolerance develops in the CNS effects of TRH following repeated treatment. These include the reduced motor activity in animal studies as well as the tolerance to the therapeutic effect in patients with SCD (Ogawa et al., 1983a; Pranzatelli et al., 1988). Therefore, withdrawal and intermittent TRH treatments are needed to avoid the tolerance to behavioral and therapeutic effects (Ogawa et al., 1983a, 1984). In the present study, we observed both the development of tolerance in the behavioral effect and the reduction in the specific binding of [^{3}H]MeTRH following repeated treatment with TRH at a dose of 30 mg/kg, i.p. It has been reported that repeated treatment with TRH and its analog MK-771 reduces B_{max} but not K_d of [^{3}H]TRH binding in rat brain (Ogawa et al., 1983b; Simasko and Horita, 1985). Moreover, our preliminary study has confirmed the down-regulation of TRH receptors in the same fashion following repeated treatment with TRH at a dose of 10 mg/kg, i.p. (data not shown). Thus the down-regulation of TRH receptors in the present study would be due to the reduction in the number but not the affinity of its receptors. In contrast to TRH, there was no tolerance in the behavioral effect of taltirelin following the repeated treatment at a dose that increased the motor activity to a similar extent to TRH by a single administration. This difference in the development of behavioral tolerance between TRH and taltirelin is consistent with clinical observations following repeated treatment with them (Ogawa et al., 1983a; Kanazawa et al., 1997). In accord with the behavioral effect, there was no significant reduction in the specific binding of [^{3}H]MeTRH by repeated taltirelin treatment. These results of TRH and taltirelin indicate that the down-regulation of TRH receptors following repeated treatment would be a main cause of the development of behavioral tolerance. Taken together, it is suggested that the repeated taltirelin treatment hardly develops the behavioral tolerance due to the lack of down-regulation of TRH receptors.

With regard to the down-regulation of the G-protein coupled receptors (GPCRs), binding properties of agonists may be related to inducing the down-regulation of receptors. It has been hypothesized that the affinity of agonists is critical for inducing the down-regulation of TRH receptors (Hawkins et al., 1986). High rather than low affinity TRH agonist may easily induce the down-regulation of TRH receptors. Regarding intrinsic activity, it is reported that a full but not partial agonist tends to induce the down-regulation of GPCRs, including adrenaline and benzodiazepine receptors (Kowalski et al., 1990; Zanotti et al., 1996). As for kinetics, internalized TRH receptors restore responses after ligand dissociation (Yu and Hinkle, 1998). Taken together, weak but sufficient stimulation of GPCRs by agonists seems to underlie the lack of the down-regulation of receptors. Taltirelin has about 20 times lower affinity than TRH in terms of displacement of [^{3}H]MeTRH from the TRH receptors (Asai et al., 1999). This lower affinity of taltirelin compared to TRH would be one cause of the difficulty developing the down-regulation of receptors in vivo. However, to elucidate the mechanism that taltirelin hardly develops the down-regulation of TRH receptors, the intrinsic activity and dissociation rate of taltirelin should be determined in future experiments.

On the other hand, it was reported that TRH-degrading enzymes were elevated by repeated treatment with L-3,5,3′-triiodothyronime (T3) (Suen and Wilk, 1989). TRH releases T3 from the thyroid gland via the release of TSH. Therefore, pharmacokinetic profiles of TRH, but not enzymatically stable taltirelin, at the end of repeated treatment might be different from those at the beginning of repeated treatment. This alteration in the pharmacokinetic profiles of TRH might result in the lack of motor response to TRH following repeated treatment. Thus, it is possible that the elevation of TRH-degrading enzymes is also involved in the development of behavioral tolerance by repeated treatment with TRH. Therefore, further studies are needed to conclude the mechanism of behavioral tolerance by TRH.

Locomotor stimulating action of taltirelin and TRH is mediated primarily via the dopaminergic system, because it is inhibited by a dopamine D_2 receptor antagonist and a tyrosine hydroxylase inhibitor (Yamamura et al., 1991a). Although there is no direct stimulation of the dopamine receptors by taltirelin and TRH, these drugs enhance both dopamine synthesis and release in rat corpus striatum (Miyamoto et al., 1979; Fukuchi et al., 1998). It is known that amphetamine increases the locomotor activity by releasing dopamine (Benwell and Balfour, 1992), and induces the down-regulation of dopamine D_2 receptors following repeated treatment (Nielsen et al., 1983; Ginovart et al., 1999). In the present study, regardless of whether the down-regulation of TRH receptors was present, neither taltirelin nor TRH reduced the [^{3}H]spiperone binding to dopamine D_2 receptors after repeated treatments. Taken together, although dopaminergic systems are involved in an increase in locomotor activity by TRH agonists, dopamine D_2 receptors are not affected by repeated treatment with TRH agonists. Dopamine release by taltirelin and TRH is smaller than that by methamphetamine, a derivate of amphetamine (Fukuchi et al., 1998). Thus, this small amount of dopamine release would be one of the causes for the lack of the down-regulation of dopamine D_2 receptors following repeated treatment with TRH agonists.

In conclusion, the present study demonstrated that (1) taltirelin selectively binds to TRH receptors and increases the motor activity, and (2) repeated treatment with taltirelin in contrast to TRH does not induce the behavioral tolerance and selective down-regulation of TRH receptors. These results suggest that the motor effect of taltirelin is mediated by TRH receptors, and that taltirelin compared to TRH hardly develops the behavioral tolerance due to the lack of down-regulation of TRH receptors. Therefore, it is suggested that taltirelin is available for long-term use without a decrease in its therapeutic effects.

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