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TRH receptor agonists ameliorate 3-acetylpyridine-induced ataxia through NMDA receptors in rats

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Abstract

The effects of thyrotropin-releasing hormone (TRH) receptor agonists were examined on 3-acetylpyridine-induced cerebellar ataxia in rats. 3-acetylpyridine markedly decreased the maximal height of vertical jump, accompanied by motor incoordination. Both TA-0910 ((−)-*N*-[(*S*)-hexahydro-1-methyl-2,6-dioxo-4-pyrimidinylcarbonyl]-L-histidyl-L-prolinamide tetrahydrate; 0.3–3 mg/kg), a novel TRH analog, and TRH (10 and 30 mg/kg) significantly increased the suppressed maximal height of vertical jump after single intraperitoneal administration. The effects of these drugs reached a maximum at 1 h and disappeared 24 h after administration. Both the TA-0910 (1 mg/kg)- and TRH (10 mg/kg)-induced increases in the maximal height of vertical jump were completely counteracted by pretreatment with i.p. injected MK-801 (10,11-dihydro-5-methyl-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate; 0.1 mg/kg), an NMDA receptor antagonist. Neither bicuculline, muscimol, baclofen, cyproheptadine nor prazosin affected the effect of the TRH receptor agonists. In conclusion, TA-0910 is more potent than TRH in ameliorating cerebellar functional disorders. The anti-ataxic effects of these TRH receptor agonists may be mediated by NMDA receptors in 3-acetylpyridine-treated rats. © 1998 Elsevier Science B.V.

Keywords: TRH (thyrotropin-releasing hormone); TA-0910; 3-Acetylpyridine; Anti-ataxic effect; NMDA receptor

1. Introduction

TRH not only stimulates the release of thyroid-stimulating hormone (TSH) but also exerts a stimulant action on the central nervous system (CNS) (O'Leary and O'Connor, 1995; Griffiths, 1986; Horita et al., 1986). The CNS stimulating action of TRH is independent of its hormonal action but is likely to be mediated by TRH receptors located on CNS neurons (Breese et al., 1975; Sharif, 1985). Recently, TA-0910 ((−)-*N*-[(*S*)-hexahydro-1-methyl-2, 6-dioxo-4-pyrimidinylcarbonyl]-L-histidyl-L-prolinamide tetrahydrate; Fig. 1), a TRH analog, was found to be a potent CNS stimulator with a low potency for causing TSH release (Yamamura et al., 1990; Kinoshita et al., 1994, 1995a; Moriyasu et al., 1996). These TRH receptor agonists counteract motor dysfunction in animal models and humans (Sobue et al., 1986; Kinoshita et al., 1995a,b; Pitts et al., 1995).

3-Acetylpyridine, a metabolic antagonist of niacinamide, induces characteristic motor incoordination and ataxia in rats (Denk et al., 1968; Desclin and Escubi, 1974; Llinas et al., 1975). Rats treated with 3-acetylpyridine are characterized by selective degeneration of neurons in the inferior olive nucleus and the olivo-cerebellar tract (Denk et al., 1968; Desclin and Escubi, 1974). Similar motor dysfunction is often seen in patients with olivo-ponto-cerebellar atrophy (Koeppen and Barron, 1984). In order to elucidate the potential of TRH receptor agonists as treatment for cerebellar dysfunction, we evaluated the effects of TA-0910 and TRH on 3-acetylpyridine induced motor dysfunction in rats.

2. Materials and methods

2.1. Animals

Five hundred and six male Wistar rats (6 weeks old, 164–210 g, Charles River Japan, Yokohama, Japan) were used. They were housed in groups of 5–6 in stainless-steel

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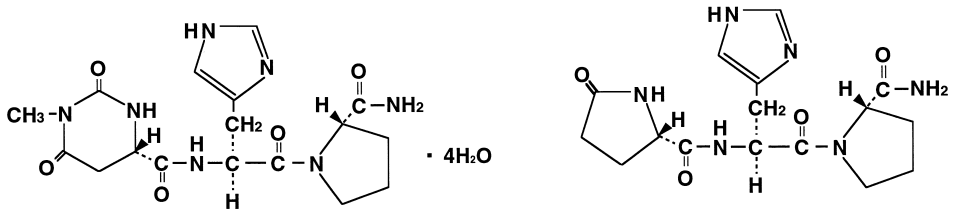

Fig. 1. Chemical structures of TA-0910 and TRH.

wire mesh cages (42W × 26D × 15H cm). The animals were kept in an air-conditioned room with controlled temperature (25 ± 0.5°C), humidity (55 ± 5%) and 12 h lighting (lights on 23.00 through 11.00) and were allowed free access to a standard pellet diet (CE-1, Clea Japan, Tokyo, Japan) and tap water.

2.2. Drugs and treatment

TA-0910, TRH tartrate and MK-801 (10,11-dihydro-5-methyl-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate) were synthesized in Tanabe Seiyaku Co. (Osaka, Japan). Other drugs used were 3-acetylpyridine (Aldrich Chemical Company, Milwaukee, WI), niacinamide (Aldrich), cyproheptadine hydrochloride (Nacalai Tesque, Kyoto, Japan), prazosin (Sigma Chemical Co., St. Louis, MO), muscimol (Sigma), baclofen (Sigma) and bicuculline methobromide (Sigma). All drugs were dissolved in physiological saline (0.9% NaCl sol., Otsuka Pharmaceutical, Co., Tokyo, Japan) immediately before use and i.p. administered in a volume of 2 ml/kg body weight.

2.3. Evaluation of motor ability

The experiment was performed according to Watanabe's method (Watanabe et al., 1997). Rats were placed in a plastic cylinder (inner diameter: 14.5 cm; height: adjustable between 20–52 cm) standing upright on a stainless-steel grid. After a foot-shock (1 mA, for 3 s) is applied through the grid by the shock generator-scrambler (NS-SG01, Neuroscience, Tokyo, Japan), rats jump on to the edge of the cylinder. After three to four days of training (ten to twenty trials a day), motor function was evaluated by measuring the maximal height that the rat could jump (maximal height of vertical jump).

2.4. Experimental schedule

After the training period, 3-acetylpyridine (75 mg/kg) was injected into the rats. 4 h after the treatment with 3-acetylpyridine, niacinamide (300 mg/kg) was injected to inhibit progressive degeneration. In our previous histological study, this combination of two drugs caused selective

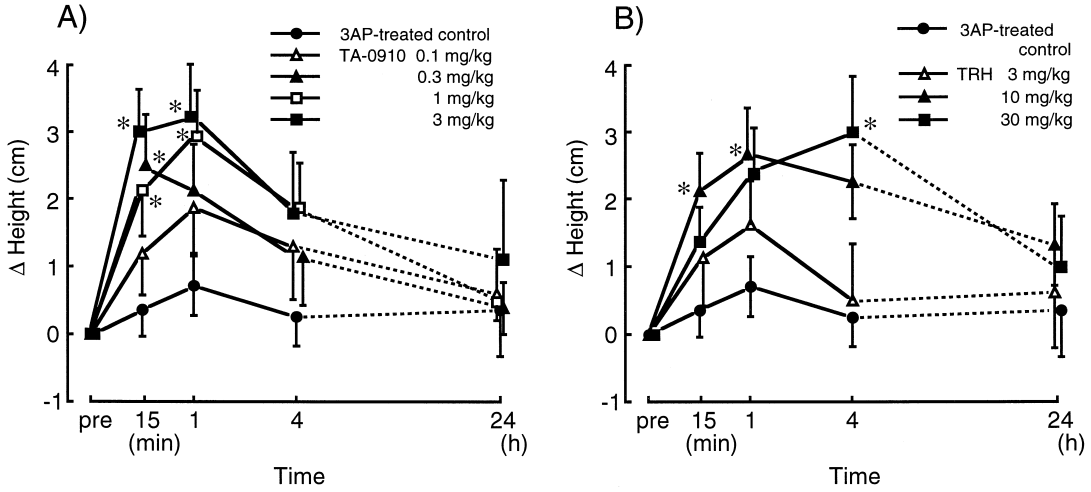

Fig. 2. Changes in Δheight of the maximal height of vertical jump (MHVJ) after intraperitoneal administration of TA-0910 (A) and TRH (B) in 3-acetylpyridine (3-AP)-treated ataxic rats ($n = 15–18$). The mean pre-drug values of MHVJ in each group were 28.3–29.5 cm. Saline was administered to the control. Δheight of the MHVJ of the control group plotted in (B) is the same as in (A). $^{*}P < 0.05$ compared with the control group (Fisher's PLSD).

destruction of the inferior olive nucleus. The maximum height of vertical jump decreased 24 h after 3-acetylpyridine treatment and reached the lowest level 5–7 days after treatment (Watanabe et al., 1997). This effect on the maximal height of vertical jump lasted for 84 days after 3-acetylpyridine injection. Therefore, the actions of TA-0910 (0.1–3 mg/kg, i.p.) and TRH (3–30 mg/kg, i.p.) were evaluated on the 5th day after 3-acetylpyridine treatment, when the behavioral effect of 3-acetylpyridine was stable. Maximal height of vertical jump was measured immediately before (pre-drug level), 15 min, 1, 4 and 24 h after drug administration.

In some experiments, rats were pretreated i.p. with MK-801 (0.1 mg/kg), bicuculline methobromide (0.3 mg/kg), muscimol (0.3 mg/kg), prazosin (1 mg/kg), cyproheptadine hydrochloride (3 mg/kg) or baclofen (1 mg/kg) 15 min before administration of TA-0910 (1 mg/kg) or TRH (10 mg/kg). In these experiments, the maximal height of vertical jump was measured immediately before treatment (pre-drug level) with a transmitter antagonist or agonist and 1 h after the administration of TA-0910 or TRH.

2.5. Statistical analysis

All data were expressed as delta change (cm) against each pre-drug maximal height of vertical jump (Δheight of maximal height of vertical jump, mean ± S.E.) and analyzed by analysis of variance (ANOVA), followed by Fisher's protected least significant difference (PLSD).

3. Results

The maximal height of the vertical jump of the 3-acetylpyridine-untreated animals (naive group) was 41.6–42.4 cm and was constant throughout the experimental period. In contrast, the maximal height of the vertical jump of the 3-acetylpyridine-treated animals on the 5th day was 26.4–31.0 cm (data not shown).

3.1. Effects of TA-0910 and TRH on the Δheight of maximal height of vertical jump in 3-acetylpyridine-treated rats

TA-0910 (0.3, 1 and 3 mg/kg) produced a dose-dependent increase in Δheight of maximal height of vertical jump (Fig. 2A). The maximum effect of TA-0910 (1 and 3 mg/kg) was observed 1 h after drug administration. Thereafter, the effect of TA-0910 declined and disappeared by 24 h after the administration.

TRH (10 and 30 mg/kg) also produced an increase in Δheight of maximal height of vertical jump (Fig. 2B). The maximum effect of TRH was observed at 1 h with a dose of 10 mg/kg and 4 h after administration of a dose of 30 mg/kg. The animals treated with the highest dose of TRH appeared to show a slight tremor-like behavior. These effects disappeared by 24 h after administration.

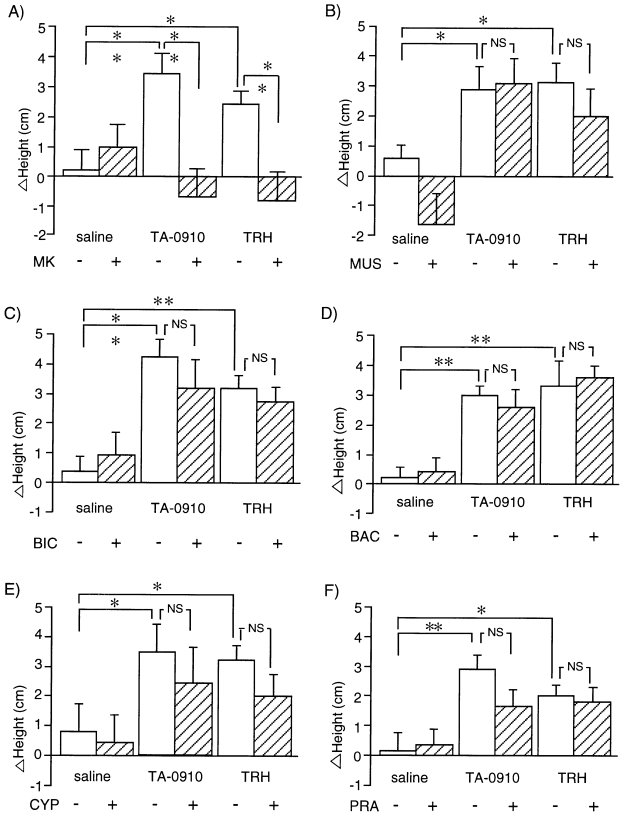

Fig. 3. Effects of pre-drug treatment (i.p.) with MK-801 (0.1 mg/kg, A), muscimol (0.3 mg/kg, B), bicuculline (0.3 mg/kg, C), baclofen (1 mg/kg, D), cyproheptadine (3 mg/kg, E) and prazosin (1 mg/kg, F) on the increase in Δheight of the maximal height of vertical jumps (MHVJ) elicited by TA-0910 (1 mg/kg) and TRH (10 mg/kg) in 3-acetylpyridine-treated rats (n = 9–13). The mean pre-drug values of MHVJ in each group were 26.4–31.0 cm. 15 min after administration of antagonist and agonists, TA-0910 or TRH was i.p. administered. MHVJ was measured 1 h after TRH drug administration. $^{**}P < 0.01$, $^{*}P < 0.05$ (Fisher's PLSD). MK: MK-801, MUS: muscimol, BIC: bicuculline, BAC: bacrofen, CYP: cyproheptadine, PRA: prazosin, NS: no significance.

3.2. Effects of pretreatment with a transmitter antagonist or agonist on the increase in Δheight of maximal height of vertical jump by TA-0910 and TRH

The increase in Δheight of maximal height of vertical jump produced by TA-0910 (1 mg/kg) and TRH (10 mg/kg) was antagonized by pretreatment with MK-801 (Fig. 3A). However, muscimol, bicuculline, baclofen, cyproheptadine and prazosin did not antagonize the effects of TA-0910 and TRH on the Δheight of maximal height of vertical jump (Fig. 3B–F).

4. Discussion

TA-0910 is a TRH receptor agonist which passes the blood–brain barrier and is more resistant to enzymatic

degradation than TRH (Chishima, 1994). 3-Acetylpyridine is a neurotoxin and causes motor dysfunction in rats (Denk et al., 1968; Desclin and Escubi, 1974; Llinas et al., 1975). Although TA-0910 (3 mg/kg, i.p.) and TRH (30 mg/kg, i.p.) did not influence the maximal height of vertical jump in naive rats in our preliminary experiment (data not shown), these drugs clearly ameliorated the ataxia in 3-acetylpyridine-treated rats. Amelioration by both TRH and TA-0910 of the motor dysfunction indicates that stimulation of TRH receptors in the CNS counteracts the 3-acetylpyridine-induced neuronal damage. However, there is no satisfactory explanation for the observation that the effect of TRH lasted as long as that of TA-0910, despite the rapid degradation of TRH in the body. While TA-0910 was more potent than TRH in terms of antagonism of ataxia, it was less potent in terms of TSH release (Moriyasu et al., 1996). The dissociation between the potency of peripheral hormone-releasing effect and the motor effects indicates that the effect on motor deficits is not mediated by released TSH following peripheral administration of TRH receptor agonists.

MK-801, an NMDA receptor antagonist, suppressed the effects of both TA-0910 and TRH in 3-acetylpyridine treated rats. GABA receptor agonists and antagonists, serotonin receptor antagonist and adrenoceptor antagonist were without effect. Dopamine receptor antagonist was not used, because the minimum effective dose to increase the locomotor activity of TA-0910 is more than 10 times greater than that for the anti-ataxic effect of TA-0910 (Yamamura et al., 1990); MK-801 is the only inhibitor available at the moment. Glutamate neurotransmission is, therefore, likely to mediate the action of these TRH agonists. There is however no evidence for the direct binding of TRH to NMDA receptors. TA-0910 displaces the specific binding of [^{3}H]methyl TRH but not that of ligands for NMDA, $GABA_A$, $GABA_B$, adrenaline, acetylcholine, dopamine and serotonin receptors in rat brain homogenates (Asai, personal communication). Therefore the activation of NMDA receptors is not due to direct stimulation by TA-0910 and TRH but is likely to result indirectly by stimulation of TRH receptors in the CNS.

Alternatively, TRH receptor agonists might locally enhance glutamate neurotransmission. It has been shown that TRH potentiates NMDA-receptor-mediated EPSPs in neocortical neurons and hippocampal CA1 neurons (Kasparov et al., 1994; Stocca and Nistri, 1995). It would be expected that MK-801 suppresses the jumping response in the absence of TRH receptor agonists, provided that TRH receptor agonists exert the effect solely by causing local acceleration of glutamate neurotransmission. MK-801 alone, however, did not suppress nor enhance the jumping response in 3-acetylpyridine-treated rats. Thus, it is unlikely that TRH receptor agonists ameliorate the motor deficits only through the local potentiation of glutamate transmission.

Patients with olivo-ponto-cerebellar atrophy have been reported to suffer degeneration of neurons in the olivocerebellar tract (Koeppen and Barron, 1984). Similar selective degeneration is induced by 3-acetylpyridine in neurons of the inferior olive nucleus in rats (Deutch et al., 1989). The result of the present study favors the use of TRH receptor agonists for the treatment of motor dysfunction due to degeneration of cerebellar motor system.

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