YM-14673, a thyrotropin-releasing hormone analogue, injected into the nucleus accumbens and the striatum produces repetitive jaw movements in rats

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Abstract

Bilateral injections of the thyrotropin-releasing hormone (TRH) analogue, Nα-[(S)-4-oxo-2-azetidinyl]-carbonyl-L-histidyl-L-prolinamide dihydrate (YM-14673, 0.1 μg and 1 μg/0.2 μl), into the nucleus accumbens, the dorsal and ventrolateral striatum produced repetitive jaw movements in a dose-dependent manner. The effects were greatest in the nucleus accumbens and smallest in the ventrolateral striatum. Pattern of the movements differed from that produced by injections of a mixture of SKF 38393 (5 μg) and quinpirole (10 μg); frequent tongue protrusions were evident in rats treated with the mixture but those were not seen in YM-14673-treated rats. TRH (1 μg, 10 μg and 30 μg/0.2 μl) did not evoke jaw movements from any of the sites. The non-selective dopamine receptor antagonist, cis-(Z)-flupentixol (10 μg), significantly reduced the response to administration of YM-14673 (1 μg) into the nucleus accumbens or dorsal striatum, while the 5-hydroxytryptamine (5-HT)2a receptor antagonist, 2-(2-dimethylaminoethylthio)-3-phenylquinoline hydrochloride (ICI 169,369, 0.2 μg), did not affect the response to YM-14673 (1 μg). Given intrathecally (0.5 μg/5 μl), both YM-14673 and TRH produced wet-dog shakes. Although the mechanisms giving rise to the display of jaw movements after intrastriatal injections of YM-14673 remain unknown, stimulation of the dopamine D1/D2 receptors may at least partly contribute to these effects. Anyhow, these mechanisms differ from that underlying the ability of YM-14673 and TRH to elicit wet-dog shakes, a mechanism that is known to involve serotonergic processes.

Keywords: Repetitive jaw movement; YM-14673; TRH (thyrotropin-releasing hormone); Nucleus accumbens; Striatum; Dopamine receptor

I. Introduction

Thyrotropin-releasing hormone (TRH) has been shown to elicit a variety of biological activities in animals and man (Prasad, 1987; Yamamoto and Shimizu, 1987, 1988, 1989; Yamamoto et al., 1990; Matsui et al., 1994). Many of these effects appear to share a common dopaminergic mechanism; for example, (a) potentiation of the behavioural effects of L-3,4-dihydroxy phenylalanine (Plotnikoff et al., 1972), (b) induction of rotational behaviour in apomorphine-treated mice (Rips and Boschi, 1985), and (c) activation of tyrosine hydroxylase (Yokoo et al., 1987). Furthermore, it has been found that inhibition of dopamine D1/D2 receptors attenuates TRH effects (Popoli et al., 1991). Indeed, dopaminergic terminal areas such as the nucleus accumbens receive a dense innervation of TRH-containing fibres (Johansson et al., 1983) and contain a high concentration of TRH receptors (Taylor and Burt, 1982). The mechanism underlying TRH augmentation of dopamine-dependent behaviours is still under discussion. Apart from the conflicting reports concerning the ability of TRH to enhance the release of dopamine (Miyamoto et al., 1981; Sharp et al., 1982; Xu et al., 1990; Méndez et al., 1993), it is also able to inhibit the uptake of dopamine (Prasad, 1987, 1991). Although
these properties may underlie the ability of TRH to alter dopamine-dependent effects, it cannot underlie the wet-dog shakes that are elicited by TRH, since 5-HT$_{2A}$ receptors appear to be involved in this TRH effect (Fone et al., 1989a).

Today, there are several TRH analogues that are known to produce TRH effects. Previously, we have shown that the TRH analogue, $N^\alpha-[[(S)-4$-oxo-2-azetidinyl]-carbonyl]-L-histidyl-L-prolinamide dihydrate (YM-14673), elicits dopamine-like behaviours such as sniffing and hyperlocomotion (Yamamoto et al., 1990). The goal of the present study was to investigate whether YM-14673 is indeed able to elicit dopamine-specific behaviour. For that purpose, YM-14673 was injected into the nucleus accumbens, the ventral striatum, and the dorsal striatum in order to study its ability to elicit dopamine-specific repetitive jaw movements. For a long time, it is known that stimulation of dopamine D$_1$ and D$_2$ receptors in these brain regions can generate or modulate such repetitive jaw movements (Koshikawa et al., 1987, 1989, 1990a,b; Bordi et al., 1989; Bordi and Meller, 1989; Kelley et al., 1988, 1989; Delfs and Kelley, 1990; Kikuchi de Beltrán et al., 1992; Prinssen et al., 1992; Koene et al., 1993). To study the specificity of the response of YM-14673, the ability of the dopamine D$_1$/D$_2$ receptor antagonist cis-(Z)-flupentixol (Puech et al., 1981) and the 5-HT$_{2A}$ receptor antagonist ICI 169,369 (Blackburn et al., 1988) to attenuate the response of YM-14673 was analysed. Finally, YM-14673 was given intrathecally in order to investigate whether this TRH analogue can elicit wet-dog shakes, a TRH effect that requires an enhanced serotonin activity at the level of 5-HT$_{2A}$ receptors (Fone et al., 1989a,b).

2. Materials and methods

2.1. Measurement of jaw movements

Surgical procedure

Male Sprague-Dawley rats (260–300 g) were anaesthetized with ketamine HCl (10 mg/kg i.p.), supplemented during surgery with halothane (0.5–4% as appropriate). The surgical and recording procedures were as described previously (Koshikawa et al., 1989, 1990a,b, 1991). A small light-emitting diode was fixed to the head of the animal and the animal was placed in a stereotactic frame so that the head was kept in constant relation to a light-sensitive transducer which detected the vertical and horizontal movements of the diode. Bipolar electrodes were placed into the masseter and digastric muscles to record electromyographic (EMG) activity. The spinal cord was transected at C1 level to confine drug-induced jaw movements to the head region. The jaw movements were recorded on a polygraph for later quantification and were counted automatically with a spike trigger. Stainless steel guide cannulas (0.5 mm o.d., 0.3 mm i.d.) were implanted bilaterally into the nucleus accumbens (anterior = 10.6 mm from interaural line; lateral = 1.7 mm from midline; vertical = 3.2 mm from interaural line), the dorsal striatum (anterior = 9.2 mm; lateral = 3.5 mm; vertical = 6.2 mm) or ventral striatum (anterior = 8.6 mm; lateral = 4 mm; vertical = 3.5 mm), according to the atlas of Paxinos and Watson (1986) (Fig. 1). The nucleus accumbens cannulas were angled 20° from the mid-sagittal plane to avoid the ventricular system. After surgery, the animals were maintained under anaesthesia by continuous infusion of ketamine (10 mg/h i.v.). Lignocaine HCl (2%) gel was applied to all incisions and the rectal temperature was maintained at 37°C with a thermostatically controlled heating pad. Monitored concentrations of expired O$_2$ and CO$_2$ during the experiment were 19–21% and 2.0–2.5%, respectively.

Behavioural observations

The number of jaw openings greater than 1 mm (measured from the diode displacement) was counted in consecutive 5 min periods for 240 min according to our paradigms previously described (Koshikawa et al., 1991; Kikuchi de Beltrán et al., 1992). Jaw movements and EMG activity were also recorded on a polygraph for further analysis of the pattern of jaw movements (see also Koshikawa et al., 1991; Kikuchi de Beltrán et al., 1992).

YM-14673 (0.1 and 1 µg) was bilaterally injected into each brain structure. For sake of comparison, TRH (1, 10 and 30 µg) was tested in a separate set of experiments. The specificity of the responses of YM-14673 was studied by co-administering the dopamine D$_1$/D$_2$ receptor antagonist cis-(Z)-flupentixol (10 µg) or the serotonin 5-HT$_{2A}$ receptor antagonist ICI 169,369 (0.2 µg) with YM-14673 (1 µg), intracerebral...
injections were made in 0.2 μl solution (per side) of a mixture of the respective two drugs. The doses of the antagonists have previously been found to block effectively apomorphine-induced jaw movements (Koshikawa et al., 1990b; Kikuchi de Beltrán et al., 1994). Since YM-14673 produced responses that could be attenuated by cis-(Z)-flupentixol, it was decided to compare the EMG pattern of YM-14673 with that of the combined administration of the dopamine D1 receptor agonist SKF 82958 (5 μg) and the dopamine D2 receptor agonist quinpirole (10 μg), a treatment known to elicit dopamine-specific, repetitive jaw movements (Koshikawa et al., 1991; Kikuchi de Beltrán et al., 1992). All drugs were dissolved in saline and injected bilaterally into the brain sites over 30 s in a volume of 0.2 μl, and the needle was left in situ for 30 s after drug injection.

Histology

At the end of the experiments, the rats were deeply anaesthetized with sodium pentobarbitone, perfused transcardially with 10% formaldehyde solution and the brains were removed. Injection sites were identified from 50 μm coronal sections stained with cresyl violet. The data were analysed only for those animals in which injections were correctly placed. Experimental groups consisted of six rats and animals were used only once.

2.2. Measurement of wet-dog shakes

Wet-dog shakes occurring in the individual male Sprague-Dawley rats were counted for consecutive 5 min periods during 90 min after intrathecal injection of YM-14673 or TRH. For the intrathecal injections, the rats were first anaesthetized with sodium pentobarbitone (50 mg/kg i.p.) to allow a polyethylene catheter (8.5 cm length, 0.5 mm o.d.) to be implanted along the spinal subarachnoid space, so that the caudal tip of the catheter was at the thoraco-lumbar junction of the spinal cord (Yaksh and Rudy, 1976). Animals were allowed 7 days to recover from surgery; rats showing motor dysfunction were eliminated from the study. Subsequently, YM-14673 (0.5 μg) and TRH (0.5 μg) were tested. The dose of TRH has previously been found to elicit wet-dog shakes (Fone et al., 1989a). Intrathecal injection was made over 30 s in a volume of 5 μl followed by 5 μl saline for washing.

2.3. Drugs

The drugs used were YM-14673 (N*-[(S)-4-oxo-2-azetidinyl]-carbonyl]-L-histidyl-L-prolinamide dihydrate, Yamanouchi Pharmaceutical Co.), thyrotropin-releasing hormone (TRH, Peptide Institute), SKF 38393 hydrochloride (1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol hydrochloride, Research Biochemicals International), quinpirole hydrochloride (Research Biochemicals International), cis-(Z)-flupentixol dihydrochloride (Lundbeck) and ICI 169,369 (2-(2-dimethylaminoethylthio)-3-phenylquinoline hydrochloride, ICI Pharmaceuticals).

2.4. Statistical analysis

All values are expressed as means ± S.E.M. Time-course data were statistically analysed with a two-way repeated analysis of variance (ANOVA) followed by a Newman-Keuls multiple comparison test. Total jaw movements data were analysed with a one-way ANOVA followed by a Newman-Keuls test. Differences were considered significant when \( P < 0.05 \).

![Fig. 2. Effects of bilateral injections of saline (0.2 μl (○)) or YM-14673 (0.1 μg (□) or 1 μg (■) in 0.2 μl per side) into the nucleus accumbens (A), the dorsal striatum (B) or the ventrolateral striatum (C). The data are expressed as mean numbers (six rats in each group) of jaw movements occurring in 5-min observation periods. Vertical bars indicate S.E.M.](image-url)
3. Results

3.1. Effects of YM-14673 or TRH injection into the nucleus accumbens on jaw movements

Bilateral injection of YM-14673 (0.1 and 1 μg/0.2 μl per side) dose-dependently elicited repetitive jaw movements. The effects of YM-14673 reached maximum level at 20 min after injection and remained stable until at least 4 h after injection (Fig. 2A). For sake of comparison, YM-14673 was also given into the ventrolateral or dorsal region of the striatum. YM-14673 (0.1 and 1 μg) injected into the dorsal striatum elicited repetitive jaw movements, but the effect was smaller and slower in onset (the effect reached maximum at 60 min after injection, Fig. 2B); the smallest effect with slowest onset was found, when the compound was given into the ventrolateral striatum: the effect reached maximum at 150 min after injection (Fig. 2C). In contrast, TRH (1 μg and 10 μg/0.2 μl) failed to evoke any effect (Fig. 3); even a dose of 30 μg TRH remained ineffective, when injected into the nucleus accumbens (data not shown).

3.2. Effects of cis-(Z)-flupentixol on jaw movements induced by YM-14673 injected into the nucleus accumbens or dorsal striatum

Cis-(Z)-Flupentixol (10 μg) injected into the nucleus accumbens or the dorsal striatum significantly reduced the production of jaw movements induced by YM-14673 (P < 0.01 for the nucleus accumbens; P < 0.05 for the dorsal striatum, Newman-Keuls test) (Fig. 4).

3.3. Effects of ICI 169,369 on jaw movements induced by YM-14673 injected into the nucleus accumbens or dorsal striatum

ICI 169,369 into the nucleus accumbens or the dorsal striatum failed to affect the jaw movements induced by YM-14673 (Fig. 5).

3.4. The pattern of jaw movements including EMG changes induced by YM-14673 and the combination of SKF 82958 and quinpirole

The pattern of jaw movements occurring after administration of YM-14673 (1 μg) into the nucleus accumbens is illustrated in Fig. 6. The upper traces show the jaw movements made in the vertical and horizontal direction, whereas the lower traces show the EMG recordings of the m. digastricus (jaw-opening muscle) and m. masseter (jaw-closing muscle), respectively. It can be seen that YM-14673 produced large amplitude
Fig. 5. Effects of bilateral injections of ICI 169,369 (0.2 μg) into the nucleus accumbens (A) or the dorsal striatum (B) on jaw movements induced by YM-14673 (1 μg, ■). For the co-administration of ICI 169,369 and YM-14673, a mixture of the two drugs (in 0.2 μl per side, □) was injected intracerebrally. The data are expressed as mean numbers (six rats in each group) of jaw movements occurring in 5-min observation periods. Vertical bars indicate S.E.M.

opening and closing movements in the vertical direction that are followed by small amplitude opening and closing movements; the latter were very rapid in comparison with the former. Jaw movements in the horizontal direction (lateral jaw movements) were also present. The jaw movements were associated with a pronounced activity of the digastric muscle and the masseter muscle. The movements were not accompanied by tongue protrusions. This pattern differed from that produced by injections of the dopamine D₁ and D₂ receptor agonists combination (SKF 82958 5 μg + quinpirole 10 μg/0.2 μl) into the nucleus accumbens. This combination produced a continuous series of opening and closing movements of jaw with varying amplitude. Digastric and masseter muscles showed continuous activity throughout the period of maximum drug effect (Fig. 6). Frequent tongue protrusions occurred during the large amplitude jaw openings. This pattern was similar to that described previously (Koshikawa et al., 1991; Kikuchi de Beltrán et al., 1992).

Fig. 6. Overall picture of jaw movements (JM) and of electromyographic (EMG) activity of the right anterior digastric muscle (Dig.) and right masseter muscle (Mass.) induced by bilateral injections of saline (0.2 μl), YM-14673 (1 μg/0.2 μl) or combination of SKF 82958 (5 μg) and quinpirole (10 μg) in 0.2 μl per side into the nucleus accumbens. Vertical (VERT) and horizontal (HOR) movements of the diode attached to the mandibular protuberance are shown along with EMG recordings.

Fig. 7. Wet-dog shakes induced by intrathecal injection of YM-14673 or TRH. (○) YM-14673 (0.5 μg/5 μl), (■) TRH (0.5 μg/5 μl). Each value is the mean of six rats. Vertical bars indicate S.E.M.
3.5. Effects of YM-14673 or TRH on wet-dog shakes induced by intrathecal injection

Intrathecal injection of both YM-14673 (0.5 μg) and TRH (0.5 μg) elicited wet-dog shakes: the effect of YM-14673 was greater and longer lasting than that of TRH (Fig. 7).

4. Discussion

YM-14673 injected into the nucleus accumbens, dorsal striatum or ventrolateral striatum elicited repetitive jaw movements in a dose-related manner. The response to accumbens injections was the greatest, whereas that to injections into the ventrolateral striatum was the smallest. These findings exclude the possibility that YM-14673 diffused from the nucleus accumbens to the dorsal striatum, or vice versa. Thus, it can be concluded that both the nucleus accumbens and the dorsal striatum play a role in jaw movements elicited by YM-14673. Since the response of YM-14673 was attenuated by the dopamine D1/D2 receptor antagonist cis-(Z)-flupentixol, but not by the 5-HT2A antagonist ICI 169,369, it is suggested that YM-14673 directly or indirectly enhanced the dopamine activity at the level of dopamine D1/D2 receptors, but not the serotonin activity at the level of 5-HT2A receptors. Indeed, stimulation of dopamine D1 and D2 receptors in the nucleus accumbens also elicits repetitive jaw movements. However, there are at least three findings indicating that YM-14673 must have additional effects. First, the pattern of jaw movements induced by YM-14673 is not identical to that elicited by stimulation of dopamine D1 and D2 receptors (Fig. 6). Although the differences in number and amplitude of jaw movements may have resulted from differences in the degree in which the dopaminergic postsynaptic activity is altered by uptake inhibition (YM-14673) and direct receptor stimulation (SKF 82958 + quinpirole), the absence (YM-14673) or presence (SKF 82958 + quinpirole) of tongue protrusions cannot be ascribed to such differences. Secondly, YM-14673 was nearly ineffective after injections into the ventrolateral striatum, but reasonably effective after injections into the dorsal striatum (Fig. 3). In contrast, stimulation of dopamine D1 and D2 receptors is most effective after injections into the ventrolateral striatum, but ineffective after injections into the dorsal striatum (Koshikawa et al., 1989). Finally, the effects of YM-14673 could be blocked only partially by a dose of the dopamine D1/D2 receptor antagonist cis-(Z)-flupentixol that is highly effective in blocking the effects of dopamine receptor agonists upon jaw movements (Koshikawa et al., 1990b). These data together strongly suggest that YM-14673 has additional effects, apart from those upon the dopamine transmission. The finding that YM-14673 like TRH elicits wet-dog shakes underscores this conclusion, since TRH-induced wet-dog shakes appear to need an enhanced serotonin activity (Fone et al., 1989a,b). However, the present study excludes the possibility that changes in the serotonin transmission contribute to the effect of YM-14673 upon jaw movements, since the 5-HT2A receptor antagonist remained devoid of any effect.

The finding that TRH itself was unable to elicit jaw movements is difficult to understand. Since TRH is more susceptible to peptidases than YM-14673 (Griffiths et al., 1980; Nakamura et al., 1991), it can be speculated that TRH was too rapidly metabolized in the brain structures under study in order to stimulate sufficiently the receptors involved.

Taking the above-mentioned findings and considerations together, it appears that the TRH analogue YM-14673 can induce its behavioural effects by at least two different mechanisms. First, it can stimulate TRH receptors and, consequently, produces wet-dog shakes involving alterations in the serotonin transmission. Second, it can directly and/or indirectly enhance the dopamine activity at the level of dopamine D1/D2 receptors in certain brain structures. Although the latter mechanism may at least partly contribute to the ability of YM-14673 to elicit jaw movements after injections into the nucleus accumbens, the dorsal striatum and, possibly, the ventrolateral striatum, it is evident that additional mechanisms must play a role in the display of jaw movements.

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