Effect of a TRH Analog (DN-1417) on Tyrosine Hydroxylase Activity in Discrete Areas of Rat Brain

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Summary: Tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis was assayed in 12 regions of rat brain following chronic treatment with DN-1417 (β-butyrolactone-β-carbonyl-histidyl-prolinamide), a synthetic TRH analog. Chronic DN-1417 treatment (20 mg/kg i.p., 7 daily injections) increased the TH activity in the ventral tegmental area, nucleus arcuatus and nucleus locus coeruleus, and decreased the TH activity in the frontal cortices, olfactory tuberculum and nucleus paraventricularis. No significant change in TH activity was observed in the nucleus accumbens, nucleus caudatus putamen, median eminence and substantia nigra. These results suggest that DN-1417 exerts some of its effects through dopaminergic neurons, as well as noradrenergic neurons; and the mesolimbic and mesocortical dopamine system may be intimately involved in the central actions of DN-1417.

Key words: Thyrotropin-releasing hormone analog (DN-1417) — Tyrosine hydroxylase — A9 Dopamine neuron — A10 Dopamine neuron — A12 Dopamine neuron

Introduction

Thyrotropin-releasing hormone (TRH) is a hypophysiotrophic hormone known to evoke the release of thyroid stimulating hormone. TRH is widely distributed in extrahypothalamic regions of the brain (Jackson and Reichlin, 1974; Morley, 1979), and has been shown to exert a number of neuropharmacological actions (Yarbrough, 1979). Administration of TRH causes a variety of central effects (Morley, 1979), including increased motor activity (Havlíček et al. 1976), shortened pentobarbital- or ethanol-induced sleeping time (Breese et al. 1974) and blockade of the depressant action of α-methyl-p-tyrosine (Kulig, 1975). In addition, TRH has been reported to potentiate the behavioral changes produced by L-DOPA (Green and Grahame-Smith, 1974). This effect has been demonstrated both in normal and hypophysectomized animals (Plotnikoff et al. 1972). These findings suggest that TRH serves a physiological role in the brain which is distinct from its actions on the hypothalamus-pituitary-thyroid axis. In biochemical studies, TRH has been found to alter the metabolism of brain catecholamines (Breese et al. 1974; Keller et al. 1974; Agarwall et al. 1976, 1977) and to increase the activity of tyrosine hydroxylase (TH) (Rastogi, 1979), the rate limiting enzyme in catecholamine biosynthesis.

DN-1417 (β-butyrolactone-β-carbonyl-histidyl-prolinamide citrate), a synthesized
TRH analog, also has neuropharmacological actions, but has weaker thyrotropin-releasing effect, approximately one-fortieth of TRH (Miyamoto et al. 1981). This peptide may be useful to analyze the central actions of TRH. The present study examines the influence of chronic treatment with DN-1417 on TH activity in various brain regions of normal rats.

Materials and Methods

Locomotor activity was measured with an Automex meter (Tokai Rika Co.) which was adjusted to a sensitivity of 20 μA to count primarily large movements. Locomotor counts were recorded on a printer. The locomotor activity was measured for 2 hours after the drug injection.

Male wistar rats, weighing 250–300 g, were used. DN-1417 (20 mg/kg) was administered, intraperitoneally, daily for 7 days. Two hours after the dose on the 7th day, the rats were killed by decapitation. The whole brain was rapidly removed and carefully cut into three portions, containing the diencephalon, mesencephalon and pons, with a razor blade on an ice-cold glass plate. The tissue blocks were quickly frozen at −80 ºC. Serial slices of 300 μm thickness were made in a cryostat at −12 ºC. The brain regions were dissected free-hand or punched out with a hollow needle under a stereomicroscope. Schematic drawings of the dissected areas or punched out nuclei are shown in Fig. 1. The dissected or punched out frozen tissues were im-

Fig. 1. Shematic drawings of the dissected or punched out areas. Frontal sections through mesocortical, mesolimbic and nigrostriatal dopaminergic cell bodies and terminals and noradrenergic cell bodies and terminals are shown with the coordinates (in μm) from the atlas of König and Klippel (1974) and Jacobowitz and Palkovits (1974). Abbrevations: FCP, prefrontal cortex polar field; FCM, prefrontal cortex medial field; FCL, prefrontal cortex lateral field; AC, nucleus accumbens; TO, tuberculum olfactorium; CP, nucleus caudatus putamen; PA, nucleus paraventricularis; AR, nucleus arcuatus; ME, median eminence; SN, substantia nigra pars compacta; VTA, ventral tegmental area; LC, locus coeruleus.
TRH ANALOG ON TYROSINE HYDROXYLASE

Immediately placed in the microhomogenizer which was cooled to -45°C to prevent thawing. The tissues were homogenized in 10 μl of ice-cold 50 mM Tris-acetate containing 0.2% Triton X-100 and the homogenate was centrifuged at 10,000×g for 15 min at 4°C. Proteins in the homogenate were quantified by the method of Lowry et al. (1951). The supernatant was used to determine TH activity. TH activity was assayed by a modification of the method of Nagatsu et al. (1971). The incubation mixture contained 140 mM sodium phosphate buffer (pH 6.0), 0.7 mM FeSO₄, 3000 units/μl catalase, 3.6 mM 2-amino-4-hydroxy-6-methyl-tetrahydropteridine (6MPH₄), 0.1 M 2-mercaptoethanol, 0.05% Triton X-100, 110 mM L-[¹⁴C(U)] tyrosine (specific activity 220 mCi/mmmole) purified by passing through alumina columns at pH 8.6. Five μl of the incubation mixture was added to 2 μl of the supernatant in a microtube. After preincubation for 30 min at 0°C, the assay mixture was incubated for 15 min at 30°C. The reaction was terminated by adding 100 μl of a solution composed of 2.5 μM L-DOPA, 0.1 mM L-tyrosine, 35 mM EDTA, 25 mM NaHSO₃, 50 mM KH₂PO₄, and NaOH to give a final pH of 8.9. The diluted samples were kept for 30 min in an ice-water bath. ¹⁴C-DOPA was isolated by a modification of the method of Coyle (1972). The sample was transferred to an alumina column (Pasteur pipette, 0.7×15 cm, packed with 50 mg of alumina), and the column was then washed 4 times with 1.5 ml of water. The ¹⁴C-DOPA was eluted with 1.5 ml of 0.2 N acetic acid and the eluate was counted in 10 ml of Triton phosphor.

Results

The administration of 20 mg/kg, i.p., of DN-1417 markedly increased spontaneous motor activity, as described by Miyamoto et al. (1981). This effect lasted for at least 2 hours after the injection and occurred with each injection throughout the 7-day treatment period (Fig. 2).

The TH activity, 2 hours after the last

![Fig. 2. The effect of chronic treatment with DN-1417 on locomotor activity of rats. The locomotor activity was counted after 7 daily injections with saline (closed circles) or DN-1417 (20 mg/kg, i.p.) (open circles).](image-url)
of the 7 daily injections of DN-1417 (20 mg/kg), is summarized in Table 1. A significant decrease in TH activity was found in the polar (-49 %), medial (-37 %) and lateral (-28 %) fields of the prefrontal cortex, mesocortical dopamine terminals which originate in the A10 dopamine cell group. In the mesolimbic dopamine terminal regions of the A10 dopamine cell group, the activity was significantly decreased in the olfactory tuberculum (-28 %) but unchanged in the nucleus accumbens. There was a significant increase of the activity (49 %) in the ventral tegmental area. A marked increase in TH activity (49 %) was observed in the locus coeruleus which contains A6 noradrenaline cell bodies; however in the terminal region, the nucleus paraventricularis, the enzyme activity was decreased (-35 %). In the tuberoinfundibular dopamine system, the TH activity in the arcuate nucleus (A12 dopamine cell group) was increased (35 %); however the activity in the median emi-

| TABLE 1 |
|———|———|———|
| saline | DN-1417 |
| FCP | 0.66±0.02 (6) | 0.34±0.03 (6)*** |
| FCM | 1.12±0.04 (6) | 0.70±0.04 (6)*** |
| FCL | 0.42±0.04 (11) | 0.30±0.03 (10)** |
| AC | 23.9±0.7 (6) | 26.0±0.8 (6) |
| TO | 29.5±1.8 (6) | 21.2±2.0 (6)** |
| CP | 38.1±2.9 (11) | 46.8±3.0 (9) |
| PA | 4.58±0.14 (6) | 2.66±0.32 (5)*** |
| ME | 28.9±1.8 (9) | 34.8±2.2 (10) |
| AR | 9.99±0.50 (10) | 13.4±1.4 (10)* |
| SN | 34.0±2.7 (6) | 33.3±3.3 (6) |
| VTA | 50.6±1.9 (6) | 75.5±1.4 (5)*** |
| LC | 20.9±2.4 (6) | 31.1±2.8 (8)* |

The results are expressed as the mean±S.E.M. (n moles/mg protein/h) with the number of determination in parentheses. Statistical significance was determined by means of the Student's t-test.

*P<0.05 **P<0.025 ***P<0.005

Discussion

The present study indicates that chronic DN-1417 treatment increased the TH activity in A6 noradrenergic cell bodies and did not change or decreased the TH activity in the terminals, when the activity was assayed at optimal pH and in the presence of saturated cofactor. The increased activity in A6 noradrenergic cell bodies may be due to an increase in TH concentration. Thus, Zigmond et al. (1974; 1979) have shown that the TH activity in the locus coeruleus is increased after administration of reserpine or after exposure to cold stress. This increase in the activity results from an increase in the number of TH molecules (Reis et al. 1974). Furthermore, Black (1975) has demonstrated that the TH activity is increased in the cerebral cortical and cerebellar axon terminals, as well as the locus coeruleus, after reserpine; but the enzyme activity peaked at 3 days in the locus coeruleus, 6 days in the cerebellum, and 11 days in the frontal cortex. The failure to detect the increased activity in the terminal regions, prefrontal cortices and paraventricular nucleus, suggests that the TH molecules induced by DN-1417 in A6 noradrenergic cell bodies do not reach the terminal regions by the 7th day after initiating the treatment. On the other hand, biochemical studies suggest that TRH enhances noradrenaline turnover in the brain. For example, Reigle et al. (1974) reported that the 3H-normetanephrine level was elevated following intracisternal administration of 3H-noradrenaline in rats pretreated chronically with TRH. Keller et al. (1974) ob-
served high levels of 4-hydroxy-3-methoxyphenyl glycol, a metabolite of noradrenaline, in rat brains after acute administration of TRH. Moreover, chronic TRH treatment has been shown to induce a dose-dependent increase of TH activity in rat brain stem without changing the noradrenaline levels (Agarwal, 1977). Chronic administration of DN-1417 may also induce an increase in noradrenaline release which could be followed by TH induction in the locus coeruleus.

The TH activity measured at optimal pH and in the presence of saturated pterin cofactor is known to be relatively insensitive to short term activation (Zivkovic et al. 1974; Lloyd and Kaufman, 1975). Therefore, the increased activity found in the ventral tegmental area suggests that TH molecules may be induced in A10 dopamine cell bodies following chronic DN-1417 treatment. This is of interest since it is the first evidence for the induction of TH molecules in the A10 dopamine system. The measurement of immunoprecipitable TH molecules is necessary to demonstrate increased synthesis of the enzyme. In spite of the increased TH activity in the A10 cell bodies, the activity was not elevated in their terminals, the prefrontal cortices, olfactory tuberculum and nucleus accumbens. The basis for the differential responses of TH in cell bodies and nerve terminals is unclear. It is probable that there is a time lag between the initial increase of TH in the cell bodies and the appearance of increased TH in the nerve terminals; if the TH molecules are transported by axonal transport, as described in the noradrenergic neuron system. In addition, the decreased activity found in the terminal regions may be related to either enzyme degradation or release from the terminals (Black, 1975).

The TH activity in the nigrostriatal dopamine system was not affected by DN-1417, when the activity was assayed at optimal pH and in the presence of saturated cofactor. However, it is possible that DN-1417 enhances the dopamine synthesis in the terminal regions, because the striatal TH is activated in vivo by increasing the affinity of existing molecules for pterin cofactor (see a review by Mandel, 1978). Since the activity measured at suboptimal pH and in the presence of subsaturated cofactor is sensitive to short term activation, further studies are necessary to detect the effect of DN-1417 on the TH activation in the nerve terminal regions.

In behavioral pharmacological studies, a number of investigations suggest that the behavioral changes induced by TRH treatment, are associated with the central dopaminergic system. Agarwal et al. (1976) observed that repeated administration of TRH caused a marked increase in the spontaneous locomotor activity which was both time- and dose-dependent. Miyamoto and Nagawa (1977) reported that intraperitoneal injection of TRH induced both behavioral excitation and increased locomotor activity. We also observed a marked increase in spontaneous motor activity for at least 2 hours after intraperitoneal injection of DN-1417 (20 mg/kg/day).

This phenomenon was reproducible for 7 days. The increase in spontaneous motor activity following the injection of DN-1417, confirms a previous report (Miyamoto et al. 1981). Bilateral injections of TRH (Miyamoto and Nagawa, 1977; Heal and Green, 1979) or TRH analogs, DG-2509 and CG-3703, (Heal et al. 1981) into the nucleus accumbens increase locomotor activity, and the effect of intracerebroventricular injection of TRH was abolished by pretreatment with haloperidol or 6-hydroxydopamine. Biochemical studies have also shown that TRH (Kerwin and Pycock, 1979) or DN-1417 (Narumi et al. 1982) stimulates dopamine release from slices of rat nucleus accumbens. These findings suggest that the locomotor stimulant action of TRH or its analogs is mediated by the
mesolimbic dopamine system. Our results also provide further evidence for the intimate involvement of mesolimbic and mesocortical dopamine systems in the central actions of DN-1417. On the other hand, chronic DN-1417 increased TH activity in the nucleus arcuatus (A12 dopamine cell bodies) but did not change the TH activity in the median eminence which includes their nerve terminals. Although DN-1417 has a weak thyrotropin-releasing effect, the analog should exert indirect effects on pituitary function via the A12 dopamine system.

References


