Down-regulation of thyrotropin-releasing hormone (TRH) receptors in spinal cord after transection as revealed by quantitative autoradiography

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Summary. The autoradiographic localization of thyrotropin-releasing hormone (TRH) receptors was investigated in the rat spinal cord after transection at the level of T8-T9. The discrete distribution of [3H]-MeTRH binding was measured with a computerized image analyzer at the cervical (C6-C7) and lumbar (L2-L3) level, one week and three weeks after injury. The TRH receptor density was expressed in fmol/mg protein. There was no significant change in the density of TRH receptors below the injury site. In the cervical region, TRH receptor concentration in the dorsal gray matter did not differ from normal controls; in contrast we found a time-dependent change in lamina 10 and in the ventral gray, with a significant decrease (25% and 19%, respectively) of TRH receptor binding sites one week after transection and a return to control levels by three weeks. From these data and the known increase of TRH immunoreactivity above a spinal injury, a down-regulation of spinal cord TRH receptors in response to elevated levels of TRH is suggested.

Key words: Thyrotropin-releasing hormone (TRH) – Receptors – Autoradiography – Spinal cord – Experimental transection – Rat

Introduction

Thyrotropin-releasing hormone (TRH) and its receptors are widely distributed in the central nervous system of mammals (Hökfelt et al. 1975a; Manaker et al. 1986). Most of TRH in spinal cord should derive from supraspinal sources, especially medullary raphe region, projecting to the ventral horn. TRH immunoreactivity has been described in the ventral horn at varicose fibers in close association with motoneurons (Hökfelt et al. 1975b) and in the dorsal horn into both cell bodies and fibers (Harkness and Brownfield 1986). TRH function in spinal cord is not fully elucidated, but some reports provide evidence that TRH has a neurotransmitter-like action, and plays an important role in motor system function. It facilitates depolarization of motor neurons and increases the amplitude of monosynaptic or polysynaptic spinal reflexes (Ono and Fukuda 1982; Clarke and Stirk 1983); it provides rapid symptomatic relief of weakness in amyotrophic lateral sclerosis and other motor-neuron related disorders (Engel et al. 1983; Serratrice et al. 1986); TRH and its analogs significantly improve neurologic outcome after experimental spinal injury (see Faden and Jacobs 1985). In addition, the presence of TRH and TRH receptors in the dorsal horn supports a role of the peptide in sensory processing.

Comprehension of the physiological and pharmacological effects of TRH in the spinal cord requires a study on distribution of TRH receptors, as well as of the factors influencing TRH receptor distribution. Recently, the changes in the binding of [3H]-3-methyl-his²-TRH ([3H]-MeTRH) after experimental spinal cord injury were studied biochemically (Faden et al. 1986b). A time-dependent decrease of TRH receptors at the injury site was evident. In the present study we utilized quantitative autoradiography to study the effects of experimental spinal cord transection on TRH receptor binding in discrete spinal cord regions.

Material and methods

Male adult Sprague-Dawley rats (300-400 g) were used in this study. Cordotomy was performed at the thoracic 8-9 level following laminectomy under sodium methohexitol anesthesia (50 mg/kg i.p.)
with additional topical lidocaine. The animals were maintained on a regular laboratory diet. One week or three weeks after spinal transection, the rats were killed by decapitation. The spinal cord was rapidly removed and cervical (C6–C7) and lumbar (L2–L3) segments were frozen in powdered dry ice and stored at −70 °C until used. Unoperated age-matched animals served as controls. After mounting the tissue on cryostat chucks with tragacanth gum, 30 μm thick transverse sections were cut at −20 °C. The sections were thaw-mounted on acid cleaned, chrome alum and gelatin coated microscope slides, and stored with dessicant at −70 °C overnight.

The binding procedure previously described by Manaker et al. (1985b) was used with some modifications. The sections were brought to room temperature and pre-incubated for 10 min in Tris-HCl buffer (50 mM, pH 7.4) containing 5 mM MgCl₂ and 0.2% bovine serum albumin (BSA). The sections were then chilled to 4 °C and allowed to air dry. Incubation was done at 4 °C for two h in a moist chamber in potassium phosphate buffer (20 mM, pH 7.4) containing 5 mM MgCl₂, 0.2% BSA, 1 mM EGTA, 20 μM bacitracin, 0.5 mM iodo-acetamide and 10 nM [³H]-MeTRH. Nonspecific binding was determined as the binding of tritiated MeTRH in immediately adjacent sections in the presence of 10 μM unlabeled MeTRH. After washing with ice-cold incubation buffer (without [³H]-MeTRH) four times for 30 s each, the sections were dipped in ice-cold distilled water and then rapidly dried at 60 °C on a slide drier. The slides were placed on a X-ray film cassette in apposition to tritium-sensitive UTLROFILM (LKB). After 2 months of exposure at 4 °C, the UTLROFILM was developed in Kodak D19, washed and fixed.

Quantitative analysis of autoradiograms was performed according to a previously described method (Rainbow et al. 1984), permitting conversion of optical densities to molar quantities of receptor-bound radioligand. In brief, tritium brain-mash standards were made by mixing rat forebrain tissue with different quantities of [³H]-Leucine. The individual brain pastes were frozen in isopentane cooled in liquid nitrogen and 30 μm thick sections were cut in a cryostat. They were thaw-mounted onto subbed slides and exposed against UTLROFILM at similar conditions as for spinal cord experiments. Additional serial sections were used for radioactivity measurement by mixing them in 1 ml Protosol, storing them two days and then assaying in 10 ml of Aquassure scintillation cocktail, or for protein content determination.

**Fig. 1A, B.** Autoradiographic localization of [³H]-MeTRH binding in normal rat spinal cord. Dark regions represent areas with high receptor densities. A Transverse section at the cervical C6–C7 level. B Transverse section at lumbar L2–L3 level.

**Fig. 2.** Density of TRH receptors in different areas of rat cervical (C6–C7) spinal cord after thoracic transection (mean ± SEM). Significant decrease in TRH receptor binding sites was found at 1 week after injury in lamina 10 (* = p < 0.02) and in ventral gray (** = p < 0.01), with a return to control levels by 3 weeks. (C. = Control animals, n.4; 1 w. = 1 week after cordotomy, n.5; 3 w. = 3 weeks after cordotomy, n.4)