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Diazepam affects the nuclear thyroid hormone receptor density and their expression levels in adult rat brain

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Abstract

Thyroid hormones (THs) are involved in the occurrence of anxiety and affective disorders; however, the effects following an anxiolytic benzodiazepine treatment, such as diazepam administration, on the mechanism of action of thyroid hormones has not yet been investigated. The effect of diazepam on the in vitro nuclear T_3 binding, on the relative expression of the TH receptors (TRs) and on the synaptosomal TH availability were examined in adult rat cerebral hemispheres 24 h after a single intraperitoneal dose (5 mg/kg BW) of this tranquillizer. Although, diazepam did not affect the availability of TH either in blood circulation or in the synaptosomal fraction, it decreased (33%) the nuclear T_3 maximal binding density (B_{max}). No differences were observed in the equilibrium dissociation constant (K_d). The TRα2 variant (non-T_3-binding) mRNA levels were increased by 33%, whereas no changes in the relative expression of the T_3-binding isoforms of TRs (TRα1, TRβ1) were observed. This study shows that a single intraperitoneal injection of diazepam affects within 24 h, the density of the nuclear TRs and their expression pattern. The latest effect occurs in an isoform-specific manner involving specifically the TRα2 mRNA levels in adult rat brain.

Keywords: Diazepam; Adult brain; Nuclei; Triiodothyronine; Thyroid hormone receptors; Synaptosomal T_3

1. Introduction

Thyroid hormones (TH) are important in maintaining cognitive and affective homeostasis (Bauer and Whybrow, 1988) and are implicated in the pathophysiology of psychiatric illnesses (Arem and Cusi, 1997). TH exert their physiological role mainly through binding of triiodothyronine (T_3) to specific nuclear receptors, which are acting as ligand-dependent-transcription factors. T_3 nuclear receptors (TRs) are encoded by two different protooncongenes, *c-erbAα* and *c-erbAβ* (Lazar, 1993). Each gene has several alternative mRNA splicing products including the predominant isoforms of thyroid hormone receptors: TRα1, TRα2, TRβ1 and TRβ2 (Thompson et al., 1987; Koenig et al., 1988; Mitsuhashi et al., 1988; Hodin et al., 1989). TRα1, TRα2 and TRβ1 isoforms are widely expressed in the adult rat brain (Strait et al., 1990), while TRβ2's expression is pituitary specific (Hodin et al., 1989). However, all these isoforms display quantitative differences in their expression levels as well as differential regulation in several T_3-target tissues (Lazar, 1993).

TRα1, TRβ1 and TRβ2 isoforms are known to bind T_3 with high affinity and they also bind to thyroid hormone response elements (TREs) on chromatin, regulating transcriptional processes in several target tissues, including adult rat brain (Yen, 2001). Binding of unliganded TRs on TREs inhibits the T_3-dependent gene expression, due to formation of complexes with nuclear proteins (co-repressors) which private the activation of transcription (Chen and Evans, 1995; Horlein et al., 1995; Seol et al., 1995; Sande and Privalski, 1999). However, the above T_3-depedent gene repression is easily reversed upon binding of T_3 on its specific nuclear receptors (Yen et al., 1993; Li et al., 1999; Wu and Koenig, 2000; Ito and Roeder, 2001).

The TRα2 variant, an alternative splicing product of the *c-erbAα* gene, cannot bind T_3 because it lacks an intact ninth heptad hydrophobic repeat in its ligand-binding domain,

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characterized thus by a unique carboxyl-terminal region in comparison with the T_3-binding TR isoforms (Mitsuhashi et al., 1988; Koenig et al., 1989; Katz et al., 1992). This structural differentiation of TRα2 is responsible for its inability to up-regulate the T_3-dependent gene expression. Moreover, the TRα2 variant has been suggested as inefficient suppressor of target gene expression in comparison to the unliganded T_3-binding TR isoforms, despite its endogenous and resident unliganded conformation (Koenig et al., 1989; Yang et al., 1996; Tagami et al., 1998). However, recent studies have shown that TRα2 variant is capable of DNA binding on TREs, which are optimal either for TR monomeric binding or for heterodimeric TRα1-RXR binding (Katz and Koenig, 1994; Yang et al., 1996). The physiological role of this non-T_3-binding isoform of TRs remains unknown; it has been suggested that TRα2 may inhibit T_3 action due to competition with the T_3-binding TR isoforms for the same TREs on chromatin (Katz and Lazar, 1993).

Despite the accumulated knowledge regarding the mechanism of action of thyroid hormones at the cellular level, the bidirectional correlation of thyroid function and affective disorders is rather complicated since it has been shown that psychiatric disorders may be caused by thyroid diseases (Clower, 1984) and also may be responsible for several thyroid dysfunctions (White and Barraclough, 1988); altered neural activity and mood stabilizing drugs affect several parameters of the TH mechanism of action at the cellular level. Notably, the effect of altered neural activity and/or LiCl treatment on the TH mechanism of action was very prompt, almost instant (within 24 h) (Bolaris et al., 1995, 2005; Constantinou et al., 2005). Furthermore, it has been shown that thyroid hormones are involved in the occurrence of anxiety under thyrotoxic conditions (Kragie, 1993) and that they affect the in vivo and in vitro binding of benzodiazepines on their specific membrane receptor (Nagy and Lajtha, 1983; Medina and de Robertis, 1985). Additionally, it has been presumed that benzodiazepines and TH may interact at the binding sites of benzodiazepine receptor, membrane transport of TH and serum protein binding sites (Kragie, 1993).

Diazepam is one of the most prescribed tranquillizers in current use (also known as valium or stedon) and belongs to the benzodiazepine derivatives which are known to produce their anxiolytic action by activating benzodiazepine receptor coupled with gamma aminobutyric acid (GABA) receptor type A (Costa and Guidotti, 1979; Tallman et al., 1980; Olsen, 1981). However, the effects following such an anxiolytic treatment on the mechanism of action of thyroid hormones have not yet been investigated.

It has been shown that increased neural activity, as it is caused by a single convulsion dose of pentylenetetrazole, affects promptly (within 24 h) the number of T_3 nuclear binding sites and the expression pattern of TRs in adult rat cerebral hemispheres (Bolaris et al., 2005). The object of this study was to investigate whether diazepam, a common anxiolytic drug, affects several cellular and molecular parameters of the TH mechanism of action within the same short period of 24 h after a single intraperitoneal injection of this tranquillizer. In particular, we examined the in vitro nuclear triiodothyronine (T_3) binding, the relative expression of all thyroid hormone receptors (TRs) and the synaptosomal availability of TH in adult rat cerebral hemispheres 24 h after a single intraperitoneal injection of diazepam.

2. Methods and materials

2.1. Animals and treatment

Adult Wistar rats (40 days) of both sexes were bred in our laboratory, housed four per cage, given laboratory chow and water ad libitum and exposed to regular light–dark cycle (light period: 7 am to 7 pm; dark period: 7 pm to 7 am; at 22 ± 1 °C) for at least 1–2 weeks prior to diazepam treatment and until sacrifice. All animals were treated and killing according to the standards of the international statues on animal handling (86/609/EEC); experimental animals received an intraperitoneal injection of diazepam (stedon) [5 mg/kg of body weight (BW)] and control animals were injected with an equal volume of 0.9% NaCl. All animals were sacrificed by decapitation 24 h after the injections. Anesthesia was not used, since it was reported that hypnotic/sedative drugs affect the kinetic characteristics of the in vitro nuclear T_3 binding in adult rat cerebral hemispheres (Bolaris et al., in press). Brain and liver were rapidly removed in a sterile cooled glass plate; the cerebral hemispheres, cerebellum and liver were isolated and weighed.

Plasma T_3 and T_4 levels were determined utilizing specific radioimmunoassay kits (T_3-RIA and T_4-RIA) from Hellenic Center of Natural Research, ''Demokritos''.

2.2. In vitro nuclear T_3-binding assay

The effects of diazepam on the in vitro specific nuclear binding of T_3 were evaluated by performing equilibrium competitive binding assays of cerebral nuclei prepared from control and experimental animals. Briefly, nuclei isolation was performed according to the method of Eberhardt et al. (1978). The final, pure, nuclear preparations were sampled and the DNA content in each one was estimated by the method of Burton (1956) and the protein levels by the method of Bradford (1976). The T_3-binding constants (maximal binding capacity: B_{max}; equilibrium dissociation constant: K_d) in cerebral nuclei from control and diazepam-treated animals were determined by performing equilibrium competitive binding assays as described previously by Bolaris et al. (1995). Finally, the binding constants were estimated by Scatchard analysis (1949) of the binding data.

The in vitro total ^{125}I-T_3-binding in cerebral hemispheres, cerebellum and liver from control and diazepam-treated