Novel translational repressor (NAT-1) expression is modified by thyroid state and age in brain and liver

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Abstract

The technique of reverse transcriptase-polymerase chain reaction differential display was used to identify thyroid hormone (TH) responsive mRNAs in the adult rat cerebral tissue. A partial cDNA (0.76 kb) was cloned and sequenced. Comparison of the sequence to the GenBank data base showed almost 100% homology to mouse translational repressor (NAT-1) mRNA 3'-end. In a northern blot analysis this cDNA hybridized with a mRNA whose expression in hyperthyroid rat cerebral tissue was approximately 6-fold higher than in euthyroid rats. The time course studies showed a rapid induction of this mRNA within 3 h following thyroxine administration. This mRNA is widely expressed in various tissues, and in hepatic tissue it is also TH responsive.

To determine if TH responsiveness of this mRNA persists during aging, 25-month-old aged rats were studied and the results were compared with those of 4-month-old rats. Unlike young mature rats, the TH responsiveness of NAT-1 mRNA in both the cerebral and hepatic tissue of aged rats was blunted. It is concluded that cerebral tissue in aging rats beyond the developmental stages, like the hepatic tissue, is associated with altered TH responsiveness.

Introduction

Thyroid hormones (TH) have profound effects on cognitive and affective disorders (1). The molecular mechanisms of these effects remain largely unknown. Although it is generally believed that the major site of action of TH is at the nuclear receptors (2, 3) and that TH and its receptors are abundant within the cerebral tissue (4-6), there is a paucity of known TH responsive genes in the cerebral tissue of mature animals (7-10).

To identify some of the genes within the cerebral tissue which remain TH responsive in adult animals, we used the technique of differential display of mRNA and identified several candidate cDNAs which recognize TH responsive mRNA species in cerebral tissue of mature rats. In this communication, we report the isolation of a 0.76 kb cDNA clone with 100% homology to mouse translation repressor (NAT-1) mRNA 3'-end (11). Since aging in rats is associated with reduced translational rate of proteins (12, 13) and aged animals have reduced responsiveness to thyroid hormones (14, 15), known modulators of protein translation, we hypothesized that the age-related changes in translational apparatus may be partly the result of changes in the NAT-1 content. To address these questions, the cerebral and hepatic tissue contents of NAT-1 mRNA were measured in rats at various ages during the euthyroid, hypothyroid and hyperthyroid states.

Experimental procedures

Materials

L-Thyroxine, 3,5,3'-tri-iodothyronine (T3), and methimazole were purchased from Sigma Chemical Co. (St Louis, MO, USA). Sodium pentobarbital was purchased from The Butler Company, (Columbus, OH, USA). Sequencing and PCR primers were obtained from Life Technologies (Grand Island, NY, USA) and the Taq polymerase from Perkin Elmer Corp. (Norwalk, CT, USA). All reagents for polyacrylamide and agarose gels were purchased from Research Organics (Cleveland, OH, USA).

Animal groups

Male Fisher 344 rats at 4, 12 and 25 months of age, were obtained from the National Institute of Aging Colony maintained by Harlan Industries (Indianapolis, IN, USA). The rats were housed under the standard conditions of our animal care facility. The rats were maintained on regular rat chow (Teklad, Milwaukee, WI, USA) and water available ad libitum. Hyperthyroidism was induced by daily intraperineal injection of 15 μg T3/100 g body weight for 10 days. Hypothyroidism was induced by 0.025% methimazole in drinking water for 4 weeks. For the time-course experiments, a
group of adult euthyroid rats was injected intravenously with 200 μg l-thyroxine/100 g body weight. The thyroxine was dissolved in physiological saline containing 1% bovine serum albumin. Each rat received approximately 200 μl of this solution. Groups of rats (n = 3) were killed at various time intervals following the injection. The rats were anesthetized with sodium pentobarbital (45 mg/kg) intraperitoneally and killed by exsanguination through the abdominal aorta. Tissues were harvested, rinsed in cold phosphate buffered saline (PBS) and immediately frozen in liquid nitrogen.

The body weight changes and daily food intake of the rats during hyperthyroidism and hypothyroidism have been published recently elsewhere (16).

**Differential display of mRNA and cloning**

Total cellular RNA from euthyroid, hyperthyroid and hypothyroid rat cerebria was analyzed with reverse transcriptase-polymerase chain reaction (RT-PCR) based differential display as described by Liang and Pardee (17) and modified by Liang et al. (18). DNA-ase treated total cellular RNA (250 ng) was reverse transcribed. The primer used for reverse transcription was T\(_1\)\(_3\)CA (5'-TTTTTTTTTTTTCA) and T\(_3\)\(_2\)GC (5'-TTTTTTTTTTTTGC). The subsequent amplification by PCR was performed with the same T\(_1\)\(_3\)CA and T\(_3\)\(_2\)GC as 3' primers and 3 different arbitrary 5' primers: AP I (5'-CTGATCCATG), AP II (5'-CTTGATTGCC) and AP III (5'-CTCGTCTCTA). The amplified cDNAs were separated on a 6% DNA sequencing gel. Approximately 8% of the bands appeared to be differentially increased in hyperthyroidism. However, when these candidate sequences were tested with northern blots, only two were found to be clearly increased in hyperthyroidism. The sequence of one such cDNA with no significant homology to any known genes was reported recently (19). Another cDNA band which was differentially displayed to be very prominent in hyperthyroid animals was also used in northern blots of RNA from three different treatment groups in various thyroidal states. The northern blot analysis confirmed that a mRNA species is over-expressed in hyperthyroid rat cerebria. This particular cDNA clone was used to screen rat brain cDNA library Uni-ZAP XR (Stratagene, LaJolla, CA, USA) as described previously (19). A 0.76 kb clone tested positive in northern blots. This clone was sequenced by the dyeoxy chain-termination method of Sanger et al. (20) with the phage M13 universal and reverse primers and synthetic oligonucleotide primers, in both directions, using a commercially available sequencing kit (USB, Cleveland, OH, USA). Comparison of the sequence to the GenBank data base using the BLAST program showed almost 100% homology to mouse translational repressor (NAT-1) mRNA 3'-end (11). The complete cDNA sequence of NAT-1 has been published previously (11).

**RNA isolation and northern blot analysis**

Total cellular RNA was extracted by the original procedure of Chomczynski and Sacchi (21). RNA (20 μg) was electrophoresed in 1.0% agarose gels in the presence of 2.2 mol/l formaldehyde (22, 23), transferred to the nylon membrane by diffusion blotting and hybridized with \(^{32}\)P-labeled 0.76 kb cDNA probe overnight (24). The loading efficiency was determined by stripping and reprobing this same membrane with 18S ribosomal cDNA. The specificity of the findings was established by reprobing the membranes with glyceraldehyde 3-phosphate dehydrogenase (G3PDH) cDNA. The density of the bands found with northern gels was determined using the densitometer from Molecular Dynamics (Sunnyvale, CA, USA).

**Statistical analysis**

All results are reported as means ± S.E.M. The statistical analysis was carried out by one-way ANOVA. When rats at different ages with various thyroidal states were compared, two-way ANOVA was carried out. A P of less than 0.05 was the limit of statistical significance.

**Results and discussion**

To confirm the results of RT-PCR differential display of mRNA, northern blot analysis was performed. A single 3.8 kb mRNA species hybridized with the probe (Fig. 1). Based on this observation and the near 100% sequence homology between our clone and NAT-1 mRNA 3'-end, it was assumed that our probe was detecting NAT-1 mRNA. It is noteworthy that although the isolated clone is nearly 100% homologous to NAT-1 and the size of the mRNA species is identical to NAT-1, it does not necessarily indicate the identity of the isolated clone with NAT-1 since only 20% of full length NAT-1 cDNA was cloned and sequenced. The expression of this mRNA in T\(_3\)-treated rat brains was approximately 6-fold higher compared with euthyroid controls (550.3 ± 82.9% vs 101.1 ± 18% of the mean euthyroid values, P < 0.01). A 50% decrease in hypothyroid rats (52.8 ± 24.1%) compared with euthyroid rats did not reach statistical significance (Fig. 1). It is possible that if a larger number of animals had been studied or more sensitive assays had been used, this difference would have reached statistical significance.

Time course studies indicated that a significant response to TH occurs as early as 3 h following thyroid hormone injection (Fig. 2). The levels start to decline after 48 h. This is a very rapid response considering that TH equilibration in cerebral tissue requires at least 3 h (25). At the present time, it is not known if TH increases the transcription rate of this gene or stabilizes the mRNA, or both. The rapid response of NAT-1 mRNA to thyroxine in vivo suggests that it is transcriptionally regulated by TH. In the absence of a genomic clone, and
thus promoter sequences, and lack of demonstration of thyroid response element consensus sequences, it cannot be ascertained whether TH has a direct effect on the expression of this mRNA. It is possible that TH responsiveness is an epiphenomenon to another metabolic change. In this regard, the finding that TH increases the expression of a gene whose product reduces translation is counter-intuitive and suggests that the observed response is an indirect effect of TH.

Thyroxine rather than T3 was used in the time course experiments because the transport of thyroxine to the brain is more efficient than that of T3 (26), and up to 80% of T3 in the brain arises locally from thyroxine (25).

Tissue distribution studies showed that this mRNA is widely expressed in various tissues examined, including liver, lung, heart, kidney, intestine and testicles. This is consistent with previously published studies showing that NAT-1 is expressed ubiquitously (11).

The NAT-1 expression in cerebral tissue relative to G3PDH mRNA is less than 0.2%. The length of exposure of filters to obtain a good signal for NAT-1 mRNA in euthyroid rats was at least 4 days while a good G3PDH mRNA signal was detectable within less than 4 h. This suggests a rather low level of expression of NAT-1 relative to G3PDH.

To determine the age-related changes in NAT-1 and its TH responsiveness, 25-month-old rats were compared with 12- and 4-month-old rats. The youngest age group studied was 4 months old because the focus of the investigation was to address the changes seen with senescence rather than with pubertal development. Hyperthyroidism and hypothyroidism were documented by plasma thyroxine measurements and assays of hepatic malic enzyme activity as previously reported elsewhere (16). The body weight changes and daily food intake of the rats during hyperthyroidism and hypothyroidism have been published previously (16).

In the euthyroid state, basal cerebral NAT-1 mRNA relative to G3PDH mRNA was significantly increased in aged rats (25-month-old, 2.10 ± 0.21) and 12-month-old rats (0.95 ± 0.06) compared with 4-month-old rats (0.62 ± 0.12) (P < 0.001). Basal NAT-1 mRNA in hepatic tissue of 25-month-old rats (0.95 ± 0.09) was significantly lower than that in 12-month-old (2.69 ± 0.31) or 4-month-old rats (2.49 ± 0.37) (P < 0.01). All units are arbitrary.

Unlike the cerebral tissue of young rats, in aged rats the thyroidal state did not alter the level of expression of this mRNA (Fig. 3). Thus in T3-treated aged rats, the cerebral content of this mRNA (140 ± 14% of internal control) was not significantly different from the levels found in euthyroid (99.9 ± 7%) and hypothyroid (127 ± 3%) aged rats. In the hepatic tissue of young rats, this mRNA was clearly TH responsive. The concentration of this mRNA in hyperthyroid young rats (350 ± 36% of internal control values) was 3- to 4-fold higher than the concentration in hypothyroid young rats (105 ± 16%, P < 0.01). The euthyroid values (250 ± 37%) were also significantly higher than hypothyroid values (P < 0.01). In contrast, the
hypothyroid aged rat liver values (128 ± 10%) were not significantly different from the values in euthyroid (95 ± 3%) or hyperthyroid (164 ± 33%) aged rat livers. These data clearly show that the expression of NAT-1, a translational repressor, is modulated by thyroid hormone. The TH effects of NAT-1 mRNA were significantly altered in aged rats. This is in agreement with the known age-related alterations in TH responsiveness of cerebral tissue (27) and hepatic tissue (15). It is noteworthy that in cerebral tissue, the NAT-1 mRNA levels clearly increased with age. In contrast, hepatic tissue basal levels of NAT-1 decreased with age. It is not known whether these changes would contribute to the age-related decrease in cerebral protein synthesis (28, 29) or the age-related increase in hepatic protein synthesis (30, 31).

The identification of various TH responsive genes in cerebral tissue of mature adult rats provides new tools in the investigation of the central nervous system effects of TH.

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References


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