Neurotrophin-3 Induced by Tri-Iodothyronine in Cerebellar Granule Cells Promotes Purkinje Cell Differentiation

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Abstract. Thyroid hormones play an important role in brain development, but the mechanism(s) by which tri-iodothyronine (T3) mediates neuronal differentiation is poorly understood. Here we demonstrate that T3 regulates the neurotrophic factor, neurotrophin-3 (NT-3), in developing rat cerebellar granule cells both in cell culture and in vivo. In situ hybridization experiments showed that developing Purkinje cells do not express NT-3 mRNA but do express trkC, the putative neuronal receptor for NT-3. Addition of recombinant NT-3 to cerebellar cultures from embryonic rat brain induces hypertrophy and neurite sprouting of Purkinje cells, and upregulates the mRNA encoding the calcium-binding protein, calbindin-28 kD. The present study demonstrates a novel interaction between cerebellar granule neurons and developing Purkinje cells in which NT-3 induced by T3 in the granule cells promotes Purkinje cell differentiation.

THE development of the rat cerebellum mainly takes place postnatally and it is influenced by a variety of epigenetic signals such as growth factors and hormones. The external granule layer (EGL)[1] gives rise to the cerebellar granule neurons which divide and subsequently migrate inwards to reach the internal granule cell layer (IGL). Granule neurons establish synaptic contacts with the developing Purkinje cells in the molecular layer (ML) (Altman, 1972).

Thyroid hormones are important for normal brain development (Legrand, 1979; Lindholm, 1984; Dussault and Ruel, 1987) and a lack of tri-iodothyronine (T3) is known to adversely influence the highly ordered structure of the developing rat cerebellum (Legrand, 1979). Thus, the differentiation of Purkinje cells which is characterized by an extensive branching of dendrites is retarded in hypothyroidism (Legrand, 1979). Moreover, migration, cell survival, and differentiation of cerebellar granule neurons are also influenced by T3 (Nicholson and Altman, 1972; Legrand, 1979; Heisenberg et al., 1992). Administration of T3 during early postnatal life is known to alleviate cellular changes in brain observed in hypothyroidism (Legrand, 1979; Dussault and Ruel, 1987). In the developing cerebellum, the first three postnatal weeks seem to represent a period of particular sensitivity to thyroid hormones (Legrand, 1979). However, the mechanisms involved and the genes specifically regulated by T3 during cerebellar development are largely unknown (Samuels et al., 1988; Brent et al., 1991).

Neurotrophin-3 (NT-3) is a recently discovered member of the NGF family (Ernfors et al., 1990; Hohn et al., 1990; Jones and Reichardt, 1990; Kaisho et al., 1990; Maisonpierre et al., 1990*a*; Rosenthal et al., 1990) which in addition to NT-3 includes NGF, brain-derived neurotrophic factor (BDNF) (Leibrock et al., 1989), and neurotrophin-4/neurotrophin-5 (Hallböök et al., 1991; Berkemeier et al., 1991; Ip et al., 1992). NT-3 has been shown to support the survival and differentiation of various populations of peripheral, neural crest, and placode-derived, sensory neurons in vitro (Ernfors et al., 1990; Hohn et al., 1990; Maisonpierre et al., 1990*a*). NT-3 is expressed in many different regions of the rat brain during embryonic development including the developing cerebellum (Maisonpierre et al., 1990*b*; Ernfors et al., 1992). The NT-3 expression is developmentally regulated, and in adult animals NT-3 mRNA expression is mainly confined to hippocampus, cortex, and cerebellum (Hohn et al., 1990; Maisonpierre et al., 1990*a*).

The putative tyrosine kinase receptor (TrkC) for NT-3 has been shown to be expressed in the adult mouse brain (Lamballe et al., 1991) and recently also in the embryonic rat cerebellum (Ernfors et al., 1992). NT-3 at high concentrations also binds to trkB (Soppet et al., 1991; Squinto et al., 1991) which is the functional receptor for BDNF (Glass et al., 1991; Klein et al., 1991). However, using in situ hybridization, Ernfors et al. (1992) could not detect trkB in the em-

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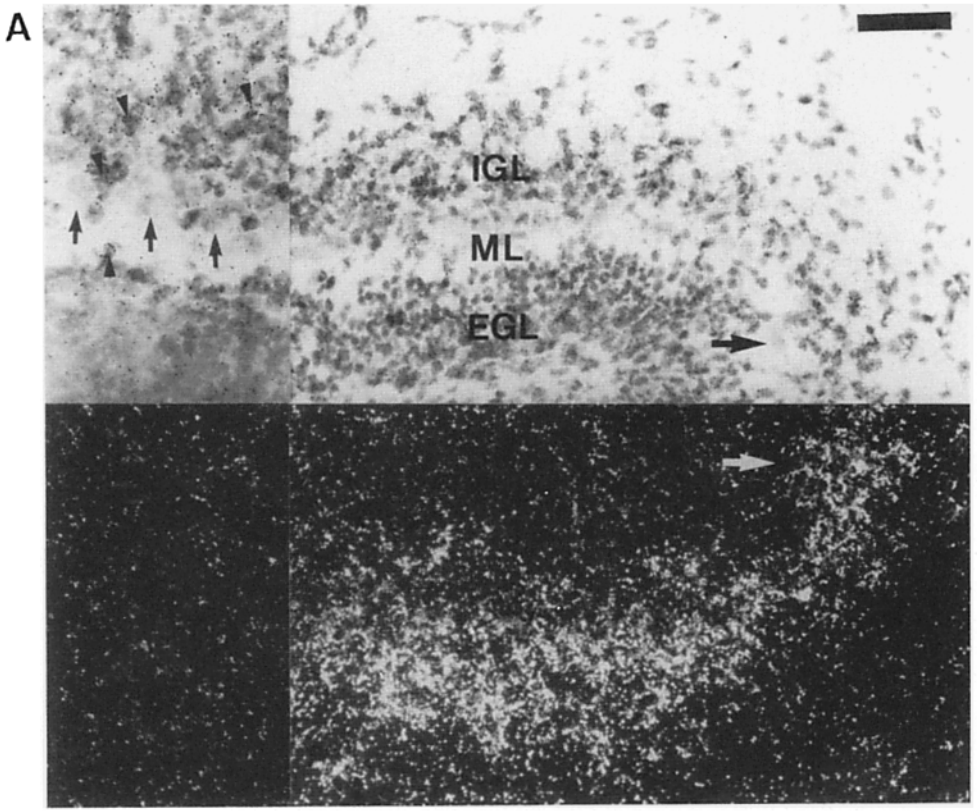
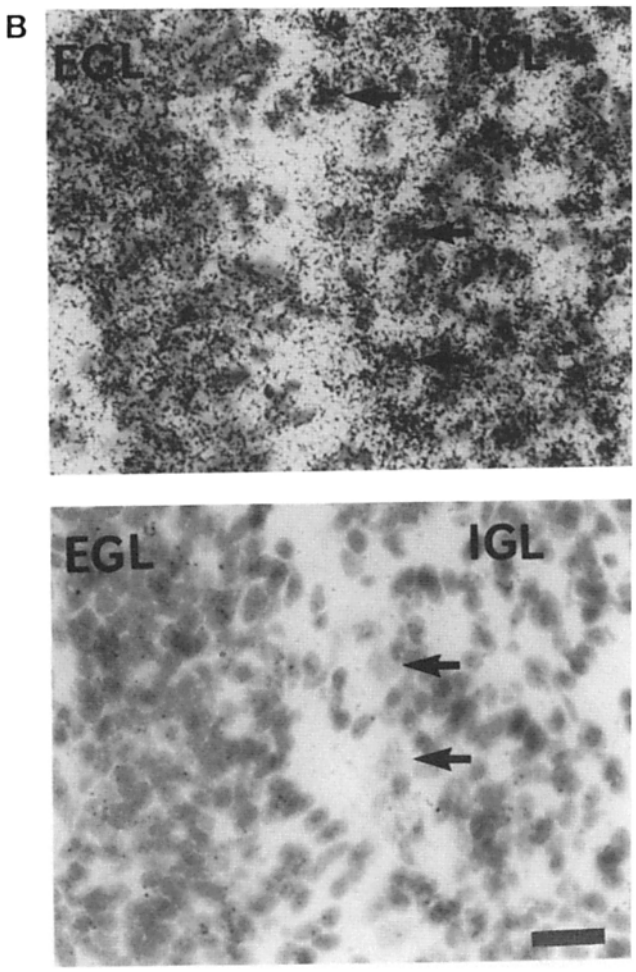
1. *Abbreviations used in this paper*: BDNF, brain-derived neurotrophic factor; EGL, external granule layer; bFGF, basic FGF; IGL, internal granule layer; IL-1, interleukin-1; ML, molecular layer; NT-3, neurotrophin-3; T3, tri-iodothyronine; Trk C, tyrosine kinase receptor C.





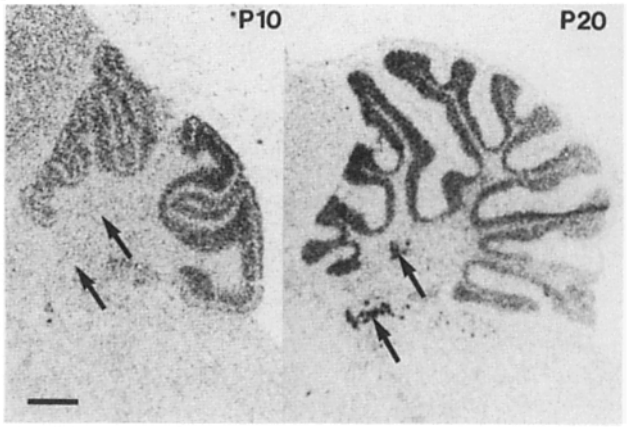


Figure 1. In situ hybridization of NT-3 and trkC in developing rat cerebellum. Rat cerebellum was prepared for in situ hybridization as described in Materials and Methods section. (*A*) NT-3 expression. Lower right panel shows specific NT-3 grains over the internal granule layer (*IGL*) whereas the molecular layer (*ML*) and the external granule layer (*EGL*) showed only background hybridization. Upper right panel, cresyl violet staining of the section. The arrows point to an unlabeled Purkinje cell. Bar, 50 μm. Upper left panel demonstrates unlabeled Purkinje cells (*arrows*) among labeled granule neurons. Lower left panel shows hybridization with the control probe. (*B*) TrkC expression. Upper panel, trkC-specific hybridization in both granule neurons and Purkinje cells. The arrows point to a heavy accumulation of grains over some Purkinje neurons. Lower panel, hybridization with control probe. Bar, 25 μm. (*C*) NT-3 expression in P10 and P20 rats. Arrows indicate the localization at the deep cerebellar nuclei. Bar, 7 μm.

bryonic cerebellum. The levels of BDNF-mRNA present during early cerebellar development are also very low as compared with those of NT-3 (Maisonpierre et al., 1990*b*; Rocamora et al., 1992).

Here we demonstrate that NT-3 mRNA is highly expressed in developing granule neurons but not in Purkinje cells of P5 cerebellum. The levels of NT-3 mRNA in rat cerebellum were decreased in hypothyroid rats and markedly increased by T3 during the first three postnatal weeks. T3 also upregulated NT-3 mRNA in cultured cerebellar granule neurons. Retinoic acid and basic FGF (bFGF) had a similar but smaller effect. As shown by in situ hybridization, Purkinje cells express trkC, the signal-transducing receptor of NT-3, but not NT-3 mRNA. Conversely, NT-3 added to early cerebellar cultures induced sprouting and hypertrophy of the immature Purkinje cells which were identified by calbindin-28 kD staining (Jande et al., 1981). NT-3 also increased the mRNA levels of calbindin-28 kD in these cultures but T3 alone had no effect. The results show that Purkinje cells are a direct target for NT-3 action in the developing rat cerebellum. The fact that T-3 induces NT-3 mRNA levels in cerebellar granule cells without directly affecting the differentiation of Purkinje cells seems to indicate a new concept of neurotrophin action, namely a hormonal regulation of NT-3 which then regulates the differentiation of Purkinje cells.

Materials and Methods

Animal Treatments

T3 was injected subcutaneously, 2 mg per kg, to rats of different ages. After various time periods, the rats were killed by decapitation. Cerebellum and hippocampus were rapidly dissected, and then frozen on dry ice. Total RNA was isolated and analyzed by RNA hybridization. To produce hypothyroid animals, newborn rats were fed a low-iodine diet (LID C 1042, Altromin, Lage, Germany) and were given propylthiouracil (1 g/l) in their drinking water (Stübner et al., 1987). Controls received a normal diet and normal water. The diet was maintained for 10 d, and then one half of the animals were treated for 4 h with T3.

Cell Cultures

Cerebellar granule neurons were prepared from 7-d old (P7) rat cerebella as described previously for hippocampal neurons (Zafra et al., 1990). The cells were cultured for 2 d on poly-DL-lysine–coated dishes (2.5×10^6 cells per 35 mm) in a serum-free medium devoid of T3 (Heisenberg et al., 1992). The cultures received T3 or other growth factors for various time periods before RNA extraction.

To enrich Purkinje cells, cultures were prepared from the cerebellar anlage of E16-E17-old rats. The cells were incubated for 8 d in the absence or presence of 30 ng per ml of recombinant NT-3, and either stained with the anti-calbindin-28 kD antibody or used for RNA analyses.

RNA Analyses

Total cellular RNA was isolated from neuronal cultures or cerebellar tissue by the method of Chomzynski and Sacci (1987). Purified RNA was glyoxylated, electrophoresed on a 1.3% agarose gel, and transferred to a nylon membrane (Lindholm et al., 1992). The filters were hybridized at 65°C in 50% formamide, 3× SSC (1× SSC is equal to 150 mM NaCl, 15 mM Na-citrate), 5 mM Na EDTA, 0.5% SDS, 5× Denhardt's solution, 250 μg per ml salmon sperm DNA, and 50 mM phosphate buffer, pH 7.0. The hybridization was performed overnight in the presence of radiolabeled mouse NT-3 cRNA probe (Hofer et al., 1990) made in vitro in the pGemini system. After washing at increasing stringency, the filters were exposed to x-ray films. They were subsequently rehybridized in the presence of a radiolabeled GAP-43 cRNA probe (Karns et al., 1987). The rat calbindin-28 kD cRNA probe (Hunzicker and Schrickel, 1988) was used to hybridize with RNA obtained from the embryonic cerebellar cultures.

The specific transcripts present on the autoradiograms were quantified using a laser scanner device (LKB, Bromma, Sweden). The amounts of NT-3 mRNA present were expressed either relatively to that of GAP-43 or per mg of cerebellar tissue corrected for RNA recovery.

In Situ Hybridization

12-μm thick frozen sections were acetylated with acetic anhydride (Lindholm et al., 1992) and hybridized overnight at 42°C with a single-stranded ^{35}S-labeled cDNA probe transcribed from either NT-3 or trkC sense or antisense (control probe) cRNA with reverse transcriptase and random priming (Castren et al., 1992). Sections were washed in 0.5× SSC at 60°C, dried, and exposed to NTP-3 autoradiographic, Kodak emulsion. The trkC cDNA was 2,035 bp in length, cloned from rat brain, and is specific for trkC.

Immunocytochemical Staining

P10-old rats were perfused with 4% paraformaldehyde in PBS. After postfixation and washing in increasing concentrations of sucrose, the brains were frozen and 12-μm sections of cerebellum were cut on a freezing microtome, mounted on glass slides (Lindholm et al., 1992), and incubated with monoclonal antibodies against calbindin-28 kD (Jande et al., 1981) (diluted 1:400; Sigma Chem. Co., St. Louis, MO). Sections were then incubated with a biotinylated secondary antibody and the immunoreaction was visualized using the avidin-biotin method and the Vectastain ABC kit (Vector Labs., Burlingame, CA).

Materials

Bovine bFGF was from Amersham, United Kingdom, IL-1β was from Biogen. PDGF-AA and PDGF-BB were kind gifts from Dr. C.-H. Heldin, Uppsala, BMC, Sweden. T3 and retinoic acid were from Sigma Chem. Co.

Results

In Situ Hybridization of NT-3 and trkC in the Developing Rat Cerebellum

To study which cells synthesize NT-3 and its receptor trkC in cerebellum during early postnatal development, in situ hybridization experiments were performed. Granule neurons in the IGL of P5 rat cerebellum contained relatively high levels of NT-3 mRNA, whereas the developing Purkinje cells did not express NT-3 mRNA (Fig. 1 *A*). However, Purkinje cells were heavily labeled with trkC specific grains which were also present over the granule neurons (Fig. 1 *B*). The deep cerebellar nuclei which are the target of Purkinje cell axons did not express NT-3 mRNA in P10 but only in P20 rats (Fig. 1 *C*).

T3 Elevates NT-3 mRNA in Cultured Cerebellar Granule Neurons

Cerebellar granule neurons isolated from postnatal cerebellum have been used extensively to study mechanisms of neuronal differentiation and survival (Burgoyne and Cambray-Deakin, 1988). Granule neurons kept in culture for 2 d in a medium devoid of hormones expressed only low levels of NT-3 mRNA (Fig. 2 *A*). However, the NT-3 mRNA levels increased about fivefold by the addition of physiological concentrations of T3 (Fig. 2 *A*), whereas GAP-43 mRNA remained unchanged (data not shown). The half-maximal effect of T3 on NT-3 mRNA levels was reached at a concentration of ~0.03 ng/ml (Fig. 2 *A*). T3 elevated NT-3 mRNA in granule neurons already after 1 h and the maximum increase was observed after 6 h (Fig. 2 *B*). The effect of T3 on NT-3 mRNA was blocked by the transcription inhibitor actinomycin-D (10 μg/ml) (data not shown). In contrast to

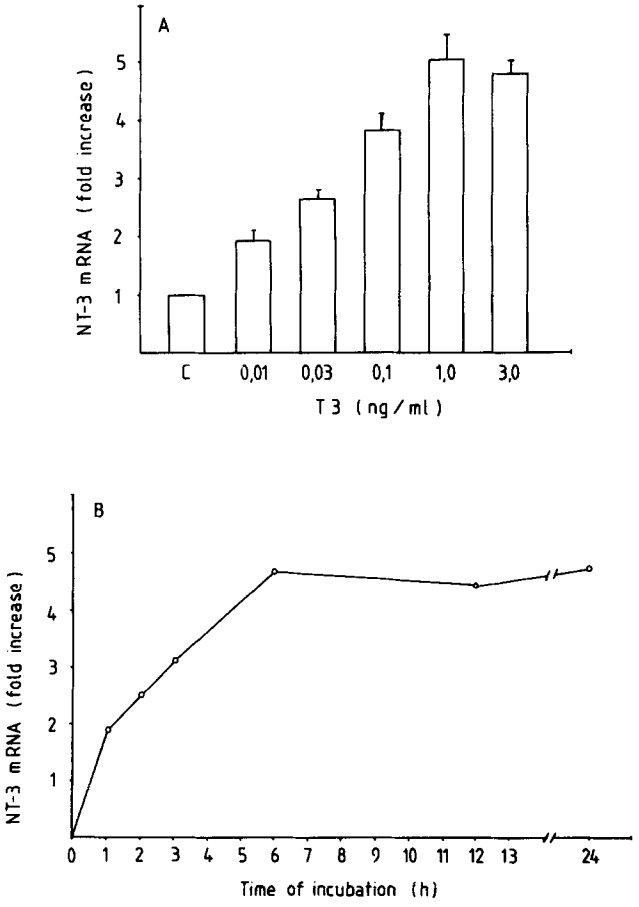


Figure 2. Effect of T3 on NT-3 mRNA levels in cultured cerebellar granule neurons. (*A*) Cerebellar granule neurons were treated for 3 h with different concentrations of T3 and total cellular RNA was then prepared and analyzed by Northern blots. Values represent the mean ± SD of three different experiments. *C*, controls; *T3*, triiodothyronine treated. (*B*) Time-course of induction of NT-3 mRNA by T3. The granule neurons were incubated with 3 ng T3 per ml for various time periods before RNA analyses. Values are the average of two determinations.

T3, retinoic acid only slightly elevated (1.7-fold) NT-3 mRNA in the granule neurons (Fig. 3). Growth factors such as PDGF, AA and BB isoforms, and interleukin-1 (IL-1) did not significantly affect the NT-3 mRNA levels. Likewise, kainic acid which is known to increase NGF and BDNF mRNA levels in rat hippocampal neurons (Zafra et al., 1990) did not influence NT-3 mRNA in the cerebellar granule neurons (Fig. 3). bFGF elevated NT-3 mRNA in these neurons about twofold (Fig. 3).

Hypothyroidism Decreases and T3 Increases NT-3 mRNA Levels in Developing Rat Cerebellum

As in cell culture, T3 also elevated NT-3 mRNA levels in the developing cerebellum of euthyroid rats (Fig. 4 *A*). The increase in cerebellar NT-3 mRNA levels was observed in newborn, P7 and P14, but not in older rats (Fig. 4 *B*). In addition, in P10 hypothyroid rats the NT-3 mRNA levels were ~50% of those in controls, and treatment with T3 elevated NT-3 mRNA more than fourfold (Fig. 4 *C*). These results suggest that the NT-3 expression in developing rat cerebellum is regulated by circulating T3. However, T3 did not elevate NT-3 mRNA in postnatal rat hippocampus (Fig. 4 *A*).

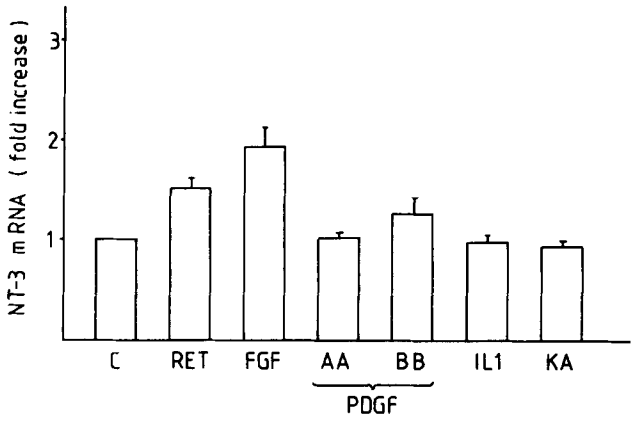


Figure 3. Effect of various growth factors and agents on NT-3 mRNA levels in granule neurons. The granule neurons were incubated for 24 h in the absence or presence of the factors indicated. *RET*, retinoic acid 1 μM; *PDGF-AA* and *PDGF-BB*, PDGF *isoforms AA* and *BB*, 5 ng per ml; *FGF*, basic FGF, 5 ng per ml; *IL-1*, IL-1β, 30 U per ml; *KA*, kainic acid, 25 μm. Values are the mean ± SD of more than three experiments. $p < 0.05$ for *RET* vs *C* and *FGF* vs *C*.

NT-3 Induces Sprouting of Purkinje Cells and Elevates Calbindin-28 kD mRNA

Developing Purkinje cells are heavily affected by hypothyroidism (Legrand, 1979; Legrand and Clos, 1991) and characteristically exhibit a lower degree of dendritic arborization as compared with control rats (Fig. 5). To study whether NT-3 affects Purkinje cell differentiation, cerebellar cultures enriched for these cells from embryonic rats were established. Addition of recombinant NT-3 (Götz et al., 1992) to these cultures led to a vigorous outgrowth of neurites of cultured Purkinje cells, which were identified by staining with an antibody against the calcium-binding protein calbindin-28 kD (Fig. 5, *lower panel*). Likewise, NT-3 also increased Purkinje cell body size without increasing their number (Fig. 5).

To study whether NT-3 acts directly on Purkinje cells, the levels of mRNA encoding calbindin-28 kD, exclusively expressed in these cells in cerebellum (Jande et al., 1981), were measured using a specific cDNA probe. Fig. 6 shows that NT-3 induced (2.5-fold) calbindin-mRNA in these cultures whereas T3 did not. Thus, mRNA data corroborate those obtained by staining of the cells with a specific antibody (Fig. 5) and demonstrate that NT-3 promotes the differentiation of Purkinje cells, whereas the T-3 effect is an indirect one regulating the NT-3 expression in granule cells which then regulates the differentiation of Purkinje cells in an orthograde manner.

Discussion

The results of the present study show that NT-3 is expressed by developing rat cerebellar granule neurons but not by Purkinje cells. The NT-3 mRNA levels in rat P10 cerebellum were decreased in hypothyroidism and conversely T3 induced NT-3 expression in rat cerebellum in vivo during early

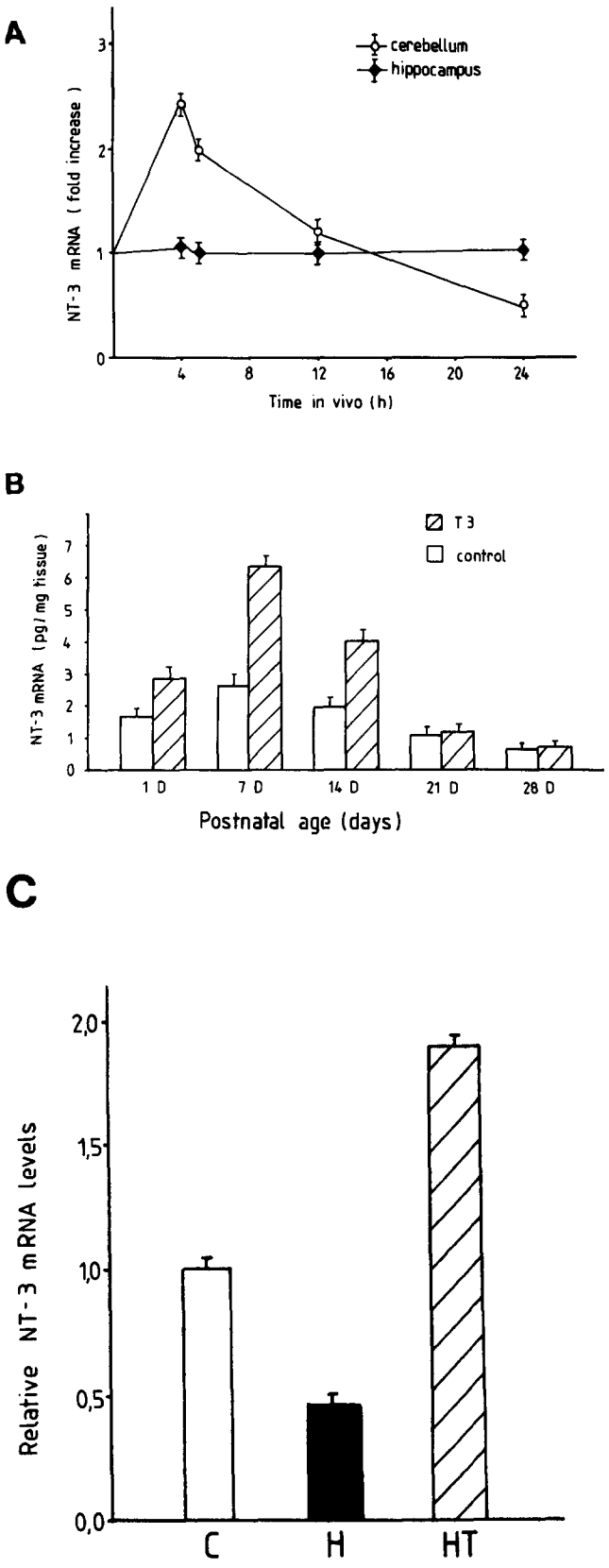


Figure 4. Effect of T3 on NT-3 mRNA levels in vivo. T3 was injected ip. to rats and RNA was analyzed in cerebellum and hippocampus as described in Materials and Methods section. (*A*) Time course of the T3 effect on NT-3 mRNA in cerebellum and hippocampus of euthyroid rats. (*B*) Inducibility of cerebellar NT-3 mRNA by T3 for 4 h during postnatal development. $p < 0.05$ for T3 vs C in P1, P7, and P14 rats. (*C*) Decrease in NT-3 mRNA in cerebellum by hypothyroidism and increase by T3 (4 h). *C*, control; *H*, hypothyroid rats; *HT*, hypothyroid rats treated with T3. $p < 0.01$ for *H* vs *C* and *HT* vs *H*. Values represent the means ± SEM of more than three experiments.

postnatal development. NT-3 induced sprouting and hypertrophy of Purkinje cells in culture and also upregulated mRNA encoding the calbindin-28 kD protein, which, in cerebellum, is expressed only by Purkinje cells. In contrast to NT-3, T3 did not significantly upregulate calbindin-mRNA suggesting that this specific effect of T3 on Purkinje cells is mediated by NT-3. In agreement with the present results, Ernfors et al. (1992) very recently reported that NT-3 is expressed in the EGL of embryonic rat cerebellum and that trkC, but not trk nor trkB, is present in developing Purkinje cells.

Cerebellar granule neurons were isolated from P7 cerebella to study the mechanisms of NT-3 gene regulation in neurons. T3 both rapidly (within 1 h) and in low concentrations (<0.1 ng/ml) increased the mRNA levels for NT-3 in these neurons without affecting the GAP-43 mRNA levels. The fact that actinomycin-D blocked the effect of T3 on NT-3 mRNA in the granule neurons suggests a direct effect of the hormone on NT-3 gene transcription. T3 has previously been shown to enhance transcription of responsive genes, such as growth hormone (Samuels et al., 1988; Brent et al., 1991), through a nuclear receptor, which is encoded by the cellular homologue of the v-erb-A oncogene (Sap et al., 1986; Weinberger et al., 1986). The dissociation constant, Kd, of T3 binding to its high-affinity, nuclear receptor is in the order of 0.2 nM (Sap et al., 1986) which roughly corresponds to 0.1 ng/ml of T3, a concentration which significantly induced NT-3 mRNA (Fig. 2 *A*). Besides T3, the retinoic acid receptor has been shown to stimulate gene expression through a T3 response element (Umesono et al., 1988). However, retinoic acid increased NT-3 mRNA levels in the granule neurons less than twofold showing a high selectivity for T3 in this system. Growth factors such as PDGF (Yeh et al., 1991) and IL-1 (Bandtlow et al., 1990), which both are expressed in rat cerebellum by neurons, did not influence NT-3 mRNA levels in these cells. bFGF which probably is synthesized by the cerebellar glial cells (Hatten et al., 1988) elevated NT-3 mRNA twofold in cultured granule neurons. However, bFGF lacks a signal sequence and it is not clear how it is released by an unconventional pathway (Burgers and Maciag, 1989).

Kainic acid stimulates BDNF and NGF-mRNA levels in hippocampal neurons in vivo (Zafra et al., 1990, 1991; Gall et al., 1991; Dugich-Djordjevic et al., 1992) and in vitro (Zafra et al., 1990, 1991; Lu et al., 1991). However, the NT-3 mRNA levels in the granule neurons were not influenced by kainic acid. In accordance with this finding, preliminary results on hippocampal neurons indicate that neither depolarization by 50 mM KCl nor kainic acid treatments increase NT-3 mRNA in these cells. Likewise, T3 did not affect the very low mRNA levels of NGF or BDNF in the cerebellar granule neurons (Lindholm, D., G. Dechant, C.-P. Heisenberg, and H. Thoenen, unpublished results).

As in cell culture, T3 also elevated NT-3 mRNA in the

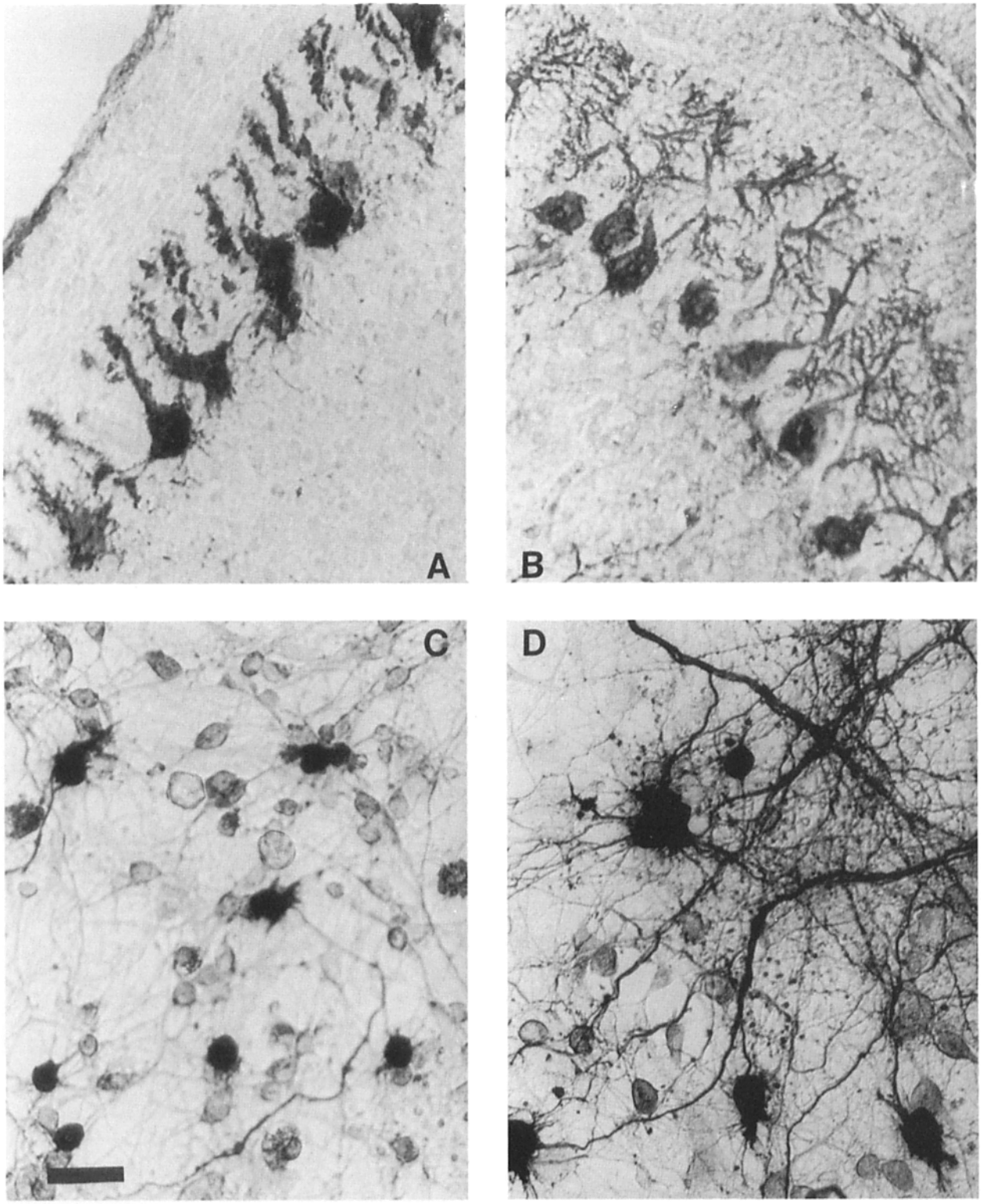


Figure 5. Morphology of Purkinje cells in P10 rats and in culture. Purkinje cells were stained using a monoclonal antibody against calbindin-28 kD protein (see Materials and Methods). Note the increased cell size and vigorous neurite outgrowth in NT-3 treated cultures. There were 58 ± 4 calbindin-28 kD positive cells per field in controls and 60 ± 3 (n = 6) in NT-3–treated cultures. (*A*) Hypothyroid rats; (*B*) Control euthyroid rats; (*C*) Cultured Purkinje cells, controls; and (*D*) cells treated with 30 ng NT-3 per ml. Bar, 25 μm.

cerebellum of euthyroid rats younger than 3 wk. After P10, the cerebellar NT-3 mRNA levels declined and later became unresponsive to the stimulatory effect of T3 (Fig. 4 *B*). The molecular basis of this phenomenon is unknown but the developmental time window for the inducibility of NT-3 mRNA by T3 shown here corresponds to the known critical period of thyroid hormone sensitivity of cerebellar development (Legrand, 1979). Moreover, T3 did not elevate NT-3 mRNA levels in postnatal rat hippocampus (Fig. 4 *A*). Differences in the expression and possibly also in the activa-

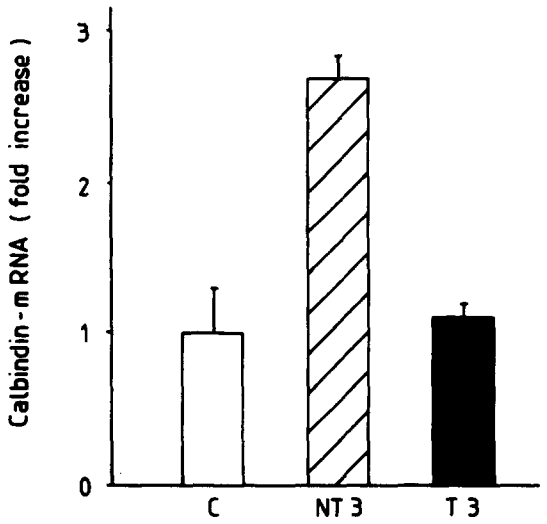


Figure 6. NT-3 but not T3 induces calbindin-28 kD mRNA in cerebellar cultures. Neurons from E17 cerebellum were incubated for 3 d in the absence or presence of either NT-3 (10 ng/ml) or T3 (5 ng/ml). RNA was analyzed as described in Materials and Methods. Values represent the means ± SD of three experiments. $P < 0.01$ for *NT-3* vs *C*.

tion of T3 receptors in different neurons could account for the differential regulation of NT-3 mRNA by T3 in rat brain.

At least two major T3 nuclear receptors, α and β, have been identified in human and rat, and both are present in rat brain (Thompson et al., 1987; Bradley et al., 1989; Mellström et al., 1991). In the developing cerebellum, predominantly α-1 receptors are present and they are confined to the cerebellar granule neurons (Bradley et al., 1989; Mellström et al., 1991). However, the β-subtype of T3 receptors has also been detected in Purkinje cells using immunohistochemical procedures (Strait et al., 1991), but these receptors could not be detected using in situ hybridization techniques (Bradley et al., 1989; Mellström et al., 1992). Interestingly, T3 alone did not significantly increase calbindin-mRNA in our cultures suggesting that T3 does not directly influence the level of calbindin-28 kD protein in the Purkinje cells. However, it cannot be excluded that T3 could directly affect some other aspects of Purkinje cell differentiation besides calbindin-28 kD.

In the present study, Purkinje cells were found to express trkC receptors and respond to recombinant NT-3 with an increase in neurite outgrowth and in cell volume. This effect of NT-3 on Purkinje cells is similar to that produced by NGF in sympathetic and dorsal root ganglia neurons (Levi-Montalcini and Angeletti, 1968; Thoenen and Barde, 1980). NGF has recently been reported to enhance neurite outgrowth by cultured Purkinje cells (Cohen-Cory et al., 1991; Aloe and Vigneti, 1992). This observation is so far puzzling as Purkinje cells only express p75 low-affinity (Pioro and Cuello, 1988), but no trkA receptor, the signal transducing unit for NGF (Ernfors et al., 1992). Since in the experiments by Cohen-Cory et al. (1991) and Aloe and Vigneti (1992) high NGF concentrations have been used, there exists the possibility that the observed effects result from "cross-talk" to other neurotrophic receptors, as has been shown for the survival effects on sensory neurons of the noduse ganglia.

To demonstrate that NT-3 directly acts on Purkinje cells in our culture system, a specific cDNA probe for rat calbindin-28 kD was used (Hunsicker and Schrickel, 1988). Calbindin-28 kD is specifically expressed by Purkinje cells in the cerebellum (Jande et al., 1981) and NT-3 was found to upregulate the mRNA encoding this protein. T3 alone did not significantly affect the levels of calbindin-mRNA.

The dendrites of the Purkinje cells are in synaptic contact with the cerebellar granule neurons via the parallel fibers (Altman, 1972), establishing an important functional connection in the cerebellum. The present results demonstrate that the neurotrophic factor NT-3 plays an important role in Purkinje cell differentiation. The results also raise the interesting possibility that NT-3 might reach the Purkinje cell dendrites in the ML via orthograde transport. It is also interesting to note that the deep cerebellar nuclei which are the target for the Purkinje cell axons do not express NT-3 mRNA in P5 rats during early development but only in P20 rats. The induction of NT-3 mRNA by T3 in cerebellar granule neurons provides a molecular basis for the specific action of T3 in the developing rat cerebellum.

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