cytosine arabinoside (5 μ*M*) and EGF (or other growth factor, where appropriate). Cytosine arabinoside was omitted from subsequent medium changes, which were done every 2 to 3 days. The number of cells bearing neuronal morphology in EGF-treated cultures was approximately 1 to 2% of the total number of cells plated. This is a low estimate of plating efficiency because nonneuronal cell types were included in the cells counted for plating, and both viable and nonviable cells were counted. In addition, of the total number of cells plated, less than 10% were found to be adherent to the plate after 24 hours in culture.
14. Cells were considered to be neurons if they possessed a rounded, phase-bright soma from which extended one or more slender processes. In most experiments, an additional criterion of process length was added. A cell was counted if the longest process extending from the cell body was at least 100 μm (200 μm in some experiments) in length.
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The IL-2 Receptor β Chain (p70): Role in Mediating Signals for LAK, NK, and Proliferative Activities

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Interleukin-2 (IL-2) induces cytolytic activity and proliferation of human blood lymphocytes. Yet, prior to activation, these cells do not express IL-2 receptors recognized by monoclonal antibodies to the Tac antigen. A novel glycoprotein (IL-2Rβ), identified on several lymphocytoid cell lines, has the ability to bind IL-2 alone and to associate with Tac antigen (IL-2Rα) to form high-affinity IL-2 receptors. It is now reported that IL-2Rβ is expressed on both circulating T lymphocytes and large granular lymphocytes in quantities approximately proportional to their responsiveness to IL-2. Studies of the responses of these cells to IL-2 suggest that IL-2Rβ mediates the initial phase of induction of lymphokine activated killer (LAK), natural killer (NK), and proliferative activities. Subsequently, IL-2Rα is induced and functional high-affinity IL-2 receptors are expressed.

THE LYMPHOKINE INTERLEUKIN-2 (IL-2) has several effects on unstimulated human blood lymphocytes. Natural killer (NK) activity can be augmented (*1–4*); lymphokine-activated killer (LAK) activity (*5*) and proliferation (*6–8*) can be induced. Yet, unlike activated lymphocytes, which express both high-affinity (approximate dissociation constant, K_d, 10 p*M*) and low-affinity (approximate K_d, 10 n*M*) IL-2 receptors capable of binding monoclonal antibodies to the Tac antigen, such as anti-Tac (*9–12*), these circulating lymphocytes express little or no IL-2 receptor detectable by anti-Tac (*2, 3, 6, 7, 9*).

Sharon *et al.* detected (*13*) and directly identified (*14*) a 70-kD lymphocyte membrane glycoprotein (p70, IL-2Rβ) which, together with Tac antigen (p55, IL-2Rα), appears to form the high-affinity IL-2 receptor. Other reports have indicated that IL-2Rβ is capable of binding IL-2 and mediating its internalization in the absence of IL-2Rα (*15–17*). We now report evidence that IL-2Rβ mediates the activation of resting lymphocytes by IL-2.

Using Percoll density gradient centrifugation (*18*), we isolated a subpopulation of peripheral blood mononuclear cells (PBMC), large granular lymphocytes (LGLs), which are highly responsive to IL-2 (*3, 4, 7, 8*). As previously reported (*18*), LGLs, but not the denser small T cells, expressed NK activity and this activity is rapidly boosted by exposure to IL-2. After incubation in 1 n*M* IL-2, LGLs typically developed 3 to 5 times more LAK activity and 5 to 20 times more rapid proliferation (thymidine incorporation) than did small T cells.

After further enrichment for IL-2-responsive cells by sorting for the NK cell marker Leu-19 (NKH-1) (*19*), LGLs were examined for the expression of IL-2Rα and IL-2Rβ. Iodine-125–labeled IL-2 was bound and cross-linked to the cells; cell lysates were then analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). IL-2Rβ (p70), when cross-linked to IL-2 (15.5 kD), migrates at 85 to 92 kD, whereas cross-linked IL-2Rα (p55) migrates at 68 to 72 kD (*13*). In each of several experiments, Leu-19$^+$, CD5$^-$ LGLs were highly enriched in IL-2Rβ expression when compared to PBMC or various T cell populations (Fig. 1A). In contrast with our findings in activated T cells (*13*), unstimulated LGLs and T cells (including T cell populations highly purified by flow cytometric sorting) from each of several donors expressed IL-2Rβ in large excess of IL-2Rα (Fig. 1A and 1B). Nevertheless, some IL-2Rα expression was often detected on circulating lymphocytes. When the small subpopulations of LGLs and small T cells expressing IL-2Rα (<5% of total cells) were removed by panning out cells that bound the monoclonal antibody anti-Tac, the remaining cells expressed IL-2Rβ but no detectable IL-2Rα (Fig. 1B). In repeat experiments, these cells, which lack IL-2Rα, retained all of the IL-2–induced LAK and NK activity and >60% of the proliferative activity of the parent populations. These data suggested that IL-2Rβ might mediate the effects of IL-2 on these cells.

We therefore sought to correlate the concentrations of IL-2 required to bind to IL-2Rβ with those needed to activate LGLs and small T cells. In experiments testing the effects of IL-2 concentration—cross-linking to IL-2Rβ (Fig. 2A), induction of cellular aggregation (Fig. 2B), and induction of cytotoxic and proliferative activities (Fig. 2C, open symbols) in unstimulated LGLs—all effects were first observed at 30 to 100 p*M* IL-2, increased through 1 to 10 n*M*, and then reached a plateau. Half-maximal activation was generally observed in the 100 p*M* to 1 n*M* range (approximately 10 to 100 U/ml). Interleukin-2 binding and activation of small T cells was of lower magnitude but occurred at the same concentrations. In contrast, IL-2–induced proliferation of T lymphoblasts, a response mediated by the high-affinity IL-2 receptor, occurred at lower concentrations of IL-2 (Fig. 2C, closed circles).

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Having obtained data implicating IL-2Rβ in the induction of cytotoxic and proliferative responses in LGLs, we further evaluated the role of IL-2Rα in these responses. Anti-Tac directly blocks the binding of IL-2 to IL-2Rα and blocks the IL-2–dependent proliferation of T lymphoblasts (*10*). Prior addition of 67 n*M* anti-Tac had no significant effect on the cross-linking of 1 n*M* IL-2 to IL-2Rβ on LGLs (Fig. 3A); furthermore, 67 n*M* anti-Tac blocked cross-linking to IL-2Rα but not IL-2Rβ on cells expressing both. In functional studies (Fig. 3B), 67 n*M* anti-Tac had no effect on the augmentation of NK activity but blocked all of the proliferation and a significant portion of the LAK activity induced in LGLs by 30 p*M* IL-2. Thus, although unstimulated LGLs express little or no detectable IL-2Rα (Tac antigen), anti-Tac in ≥100-fold molar excess of IL-2 consistently interfered with the 2- to 6-day induction of LAK activity and proliferation but not the 4-hour augmentation of NK activity.

These observations implied that IL-2 might induce on resting lymphocytes the expression of IL-2Rα, which could then associate with IL-2Rβ to form high-affinity receptors capable of conveying further activating signals from IL-2. To test this hypothesis, we incubated cells in medium containing IL-2, then washed them and cross-linked them to ^{125}I-labeled IL-2. Interleukin-2 (1 n*M*) augmented expression of both IL-2Rα and IL-2Rβ on both LGLs and small T cells (Fig. 4).

We next investigated the functional significance of this induced IL-2Rα. In contrast with its effects in 2- to 6-day cultures, anti-Tac (200 n*M*) had no effect on the smaller amount of LAK activity generated in 22-hour cultures of PBMC with 30 p*M* IL-2. In several experiments, we changed culture conditions during the 2- to 6-day period of the generation of LAK and proliferative activities by IL-2 in PBMC, LGLs, and small T cells. Anti-Tac had no significant effect unless present after the first 16 hours; and substitution of high (≥100 p*M*) for low (30 to 50 p*M*) concentrations of IL-2 had their greatest effects early in stimulation (Table 1). These findings indicate that high-affinity receptors containing IL-2Rα function in the later but not the early phase of the generation of LAK activity and proliferation.

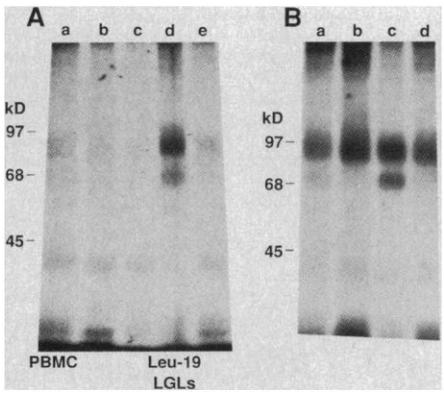

Fig. 1. Affinity labeling of cells with ^{125}I-labeled IL-2. (**A**) Disuccidimidyl suberate (DSS)–mediated cross-linking of 1.5 × 10^6 (lane a) PBMC, (lane b) monocyte-depleted PBMC, (lane c) monocyte- and B cell–depleted PBMC, (lane d) Leu-19$^+$, CD5$^-$ LGLs, and (lane e) small T cells. (**B**) DSS-mediated cross-linking of (lanes a and b) 3 × 10^6 LGLs and (lanes c and d) 1 × 10^7 small T cells, (lanes a and c) before and (lanes b and d) after depletion of IL-2R–bearing cells by panning. PBMC were isolated from volunteer human donor blood using Ficoll-Hypaque (LSM, Litton Bionetics) density gradients. Monocytes were depleted by adherence to plastic and B cells by adherence to nylon wool (Fenwal Laboratories). Nonadherent cells were fractionated on discontinuous Percoll gradients (*18*). The high-density lymphocytes (small T cells) were >95% CD3$^+$ and were >99% small lymphocytes by morphology. The low buoyant density lymphocytes predominantly displayed typical LGL morphology, and, by phenotypic analysis, were <10% monocytes and B cells, 20 to 60% NK cells (Leu-19$^+$), and 40 to 80% T cells (CD5$^+$). Leu-19$^+$, CD5$^-$ LGLs were obtained by sorting on an EPICS 752 flow cytometer (Coulter) with antibodies to the NKH-1 antigen (Leu-19-PE, Becton Dickinson) and a pan T cell antigen, CD5 (Leu-1 FITC, Becton Dickinson). Panning was performed as previously reported (*23*) with anti-Tac. Iodine-125–labeled IL-2 (New England Nuclear) (1 n*M*) was bound to the cells and cross-linked with DSS (*9*). Cross-linked cells were extracted with NP40, and extracts were analyzed by SDS-PAGE under reducing conditions (*9*).

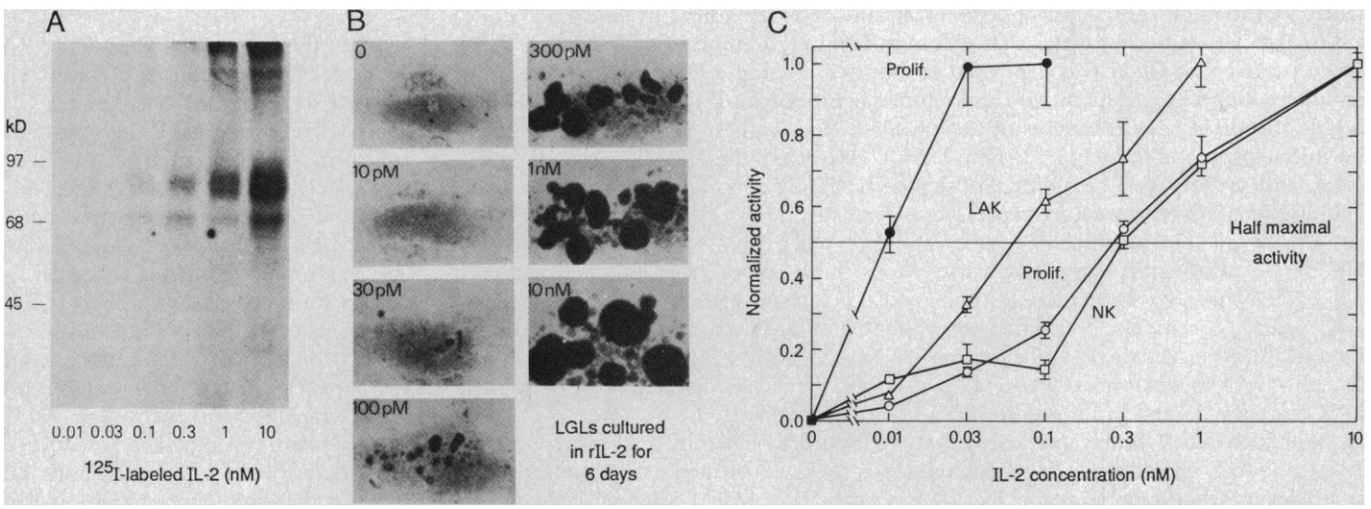

Fig. 2. Effects of the concentration of IL-2 on IL-2 interactions with LGLs. (**A**) Iodine-125–labeled IL-2 was bound and cross-linked to 3 × 10^6 LGLs per lane as in Fig. 1 except that the IL-2 concentration varied. (**B**) LGLs were cultured in 24-well plates at 10^6 cells per milliliter in medium supplemented with recombinant IL-2 at the concentrations indicated. (**C**) LGLs were tested for NK activity (□) and, after culturing as in (B) for 6 days, for LAK activity (△) and proliferation (○). NK activity was measured in a 4-hour assay in 96-well microtiter plates with 5000 ^{51}Cr-labeled K562 target cells per well, effector-to-target ratios of 30:1, 10:1, 3:1, and 1:1, and four replicates per condition. Recombinant IL-2 was added to effector cells immediately after addition of target cells and was present throughout the assay. LAK activity and proliferation were measured in each of four replicate activation cultures per IL-2 concentration. Measurement of LAK activity was similar to that of NK activity, except that NK-resistant Daudi cells were used. Proliferation was measured by 6-hour [*methyl*-^{3}H]thymidine (New England Nuclear) incorporation by 2 × 10^5 cells per well in 96-well microtiter plates. T lymphoblasts were stimulated with phytohemagglutinin for 3 days, grown in IL-2 for 4 days, starved of IL-2 for 1 day, and cultured in varying concentrations of IL-2 for 1 day. Thymidine incorporation was measured as described above (●); ^{3}H counts per minute in the absence of exogenous IL-2 were subtracted as background. NK and LAK activities were calculated in lytic units, the reciprocal of the number of cells required to achieve 30% specific lysis of 5000 target cells. For each activity, the maximal activity level among the various IL-2 concentrations was set to a value of 1.0, the lowest level set to 0, and other levels were proportionally normalized on a scale of 0 to 1.

Our data implicate IL-2Rβ and not IL-2Rα in the initial binding of IL-2 and transmission of activation signals to resting T cells and LGLs:

1) Circulating T cells and LGLs expressed IL-2Rβ consistently but expressed relatively small, and sometimes undetectable, amounts of IL-2Rα. Furthermore, when IL-2Rα–bearing cells were effectively depleted, IL-2 responsiveness persisted and IL-2Rβ remained the principal IL-2–binding protein.

2) Anti-Tac did not impair the ability of IL-2 to induce short-term LAK activity or, as previously reported (*2–4*), to boost NK activity, even when used in 2000- to 6000-fold molar excess of IL-2 (*20*).

3) Leu-19$^+$, CD5$^-$ LGLs, a population of cells highly enriched for IL-2–inducible LAK, NK, and proliferative activities, expressed several times more IL-2Rβ on their surface than did other lymphocyte populations studied.

4) The concentrations of IL-2 at which the response curves are steepest, namely, 0.1 to 1.0 n*M*, are consistent with concentrations needed to bind to IL-2Rβ, as indicated by our cross-linking and by the reported intermediate affinity of IL-2Rβ on the YT cell line (K_d, 0.8 to 1.7 n*M*) (*16*). In contrast, high-affinity IL-2 receptors are nearly saturated at 100 p*M*, leaving little possibility for additional effects of higher concentrations; and low-affinity receptors (IL-2Rα) are very minimally occupied at 100 p*M*.

It has been reported that IL-2 can induce Tac antigen on unstimulated lymphocytes (*7*) and that anti-Tac can inhibit the induction of proliferation (*7*) and LAK activity (*21*) in these cells. We have extended these observations by identifying an early IL-2Rα–independent phase of activation and an IL-2 receptor (IL-2Rβ) on unstimulated lymphocytes that may mediate the effects of IL-2 during that phase. These early effects include the augmentation of NK activity, the induction of early LAK activity, and the induction of the expression of IL-2Rα and high-affinity IL-2 receptors. Subsequently, these receptors facilitate further induction of LAK and proliferative activity.

Unlike IL-2Rβ, IL-2Rα is present in significant amounts only on activated cells and by itself is not known to mediate any biological activity or internalization of ligand (*17, 22*). Therefore, it is conceivable that the principal and perhaps sole physiological role of IL-2Rα is to augment the binding of IL-2 to biologically active IL-2Rβ. What, if any, physiological role is there for the functional IL-2Rβ on resting lymphocytes? Because Leu-19$^+$, CD5$^-$ LGLs are not antigen-specific in their cyto-

Table 1. Four replicate cultures of PBMC per condition tested were incubated for 16 hours at 10^6 cells per milliliter in medium supplemented, where indicated, first with anti-Tac, then 30 minutes later with IL-2. After 16 hours, cells were washed four times, reincubated for 42 hours under the conditions shown, and tested for proliferation by 6-hour [*methyl*-^{3}H]thymidine incorporation and for LAK activity as described in Fig. 1. Here, LAK activity is expressed as percent-specific lysis of targets at an effector-to-target ratio of 30:1. UPC-10 control monoclonal antibody had no detectable effects. Values of proliferation and LAK activity are means ± SEM.

Concentration				Proliferation (count/min)	LAK activity (percent lysis)
0 to 16 hours		20 to 62 hours			
IL-2	Anti-Tac	IL-2	Anti-Tac		
30 p*M*		30 p*M*		6,140 ± 643	31 ± 2
30 p*M*	67 n*M*	30 p*M*		6,970 ± 411	35 ± 1
30 p*M*		30 p*M*	67 n*M*	958 ± 191	6 ± 1
1 n*M*		1 n*M*		18,948 ± 869	69 ± 1
1 n*M*		30 p*M*		11,592 ± 1,097	56 ± 3
1 n*M*		0 p*M*		3,160 ± 907	17 ± 4
30 p*M*		1 n*M*		5,997 ± 377	52 ± 5
30 p*M*		30 p*M*		6,140 ± 643	31 ± 2

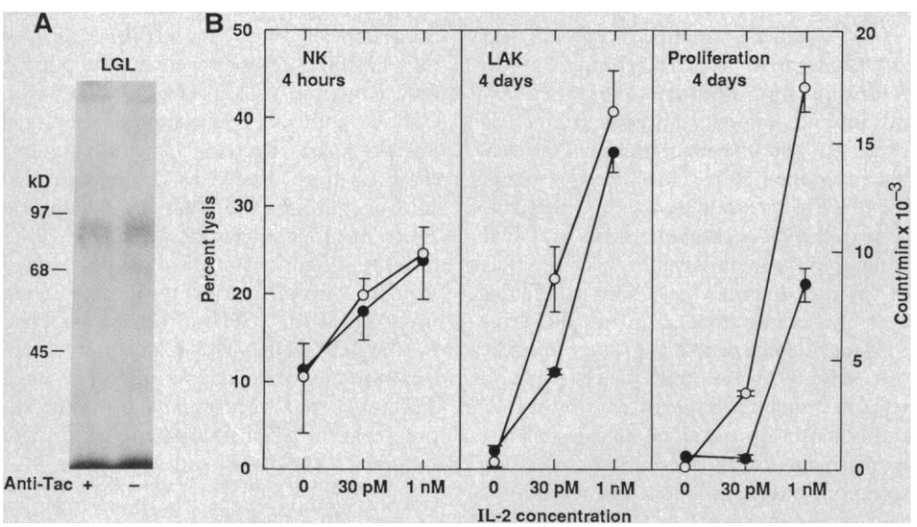

Fig. 3. Effects of anti-Tac on IL-2 interactions with LGLs. (**A**) LGLs were prepared and cross-linked to IL-2 as described in Fig. 1, except that, where indicated, 67 n*M* anti-Tac was added 5 minutes before the addition of ^{125}I-labeled IL-2 and was present throughout the binding phase. (**B**) After 30 minutes of incubation with 67 n*M* anti-Tac (●) or control (○), LGLs were stimulated with IL-2, at the concentrations indicated, in the continuing presence of antibody. NK activity was measured for the first 4 hours, and LAK and proliferative activities were measured as described in Fig. 2 after 4 days. Shown is the mean ± SEM of four replicates. UPC-10 (Litton Bionetics), a monoclonal antibody of the same isotype as anti-Tac but against an irrelevant antigen, was used as a control.

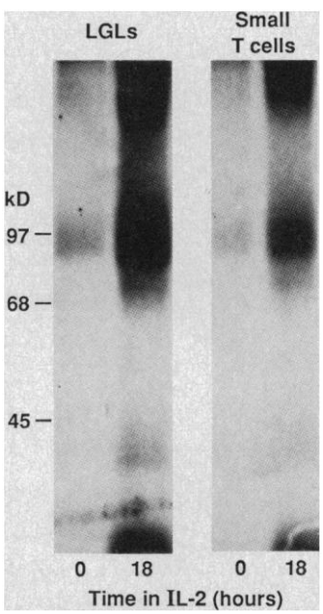

Fig. 4. Induction of IL-2Rα and IL-2Rβ on LGLs and small T cells by IL-2. LGLs and small T cells were cross-linked to ^{125}I-labeled IL-2 as described in Fig. 1 (2×10^6 cells per lane) either with or without prior incubation for 18 hours at 10^6 cells per milliliter in medium containing 1 n*M* IL-2. Before being cross-linked, incubated cells were extensively washed and incubated in the absence of IL-2 over a 4-hour period to allow shedding or internalization of all surface-bound IL-2 (*13*); 3×10^6 cells were then cross-linked to ^{125}I-labeled IL-2 as described in Fig. 1.

toxicity or activation for lymphokine production, we hypothesize that these cells might be activated at sites of antigen-specific immune responses through the interaction of IL-2 with IL-2Rβ. The role of IL-2Rβ on resting T cells is less clear. It is possible that it may serve principally or solely to provide a substrate for formation of high-affinity IL-2 receptors when IL-2Rα is expressed. Thus, the relatively large amount of IL-2Rβ present on LGLs may allow IL-2–induced recruitment of bystander LGLs at the sites of immune responses, while the smaller amount on resting T cells may allow less nonspecific recruitment but rapid expression of high-affinity IL-2 receptors and response after antigen-specific induction of IL-2Rα.

Note added in proof: Since this manuscript was submitted, two other groups have also reported the expression of IL-2Rβ on LGLs (*24*).

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20. The 40-fold greater affinity of the high-affinity IL-2 receptor for IL-2 than for anti-Tac [see (*12*)] necessitates use of considerable excess anti-Tac to prevent binding of IL-2. Our conditions ensured <2% high-affinity receptor occupancy by IL-2. The ratio of anti-Tac to IL-2 used by others is often difficult to ascertain but generally far less than used in this study. Concentrations of anti-Tac sufficient to saturate receptors and to block proliferation of activated T cells (a frequently used control) may not block other IL-2R–dependent responses if there are differences in the requirements for receptor occupancy or the IL-2 concentrations used.
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Thyroid Hormone Regulates TRH Biosynthesis in the Paraventricular Nucleus of the Rat Hypothalamus

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Thyroid hormone is important in the regulation of synthesis and secretion of thyroid-stimulating hormone (TSH) in the anterior pituitary, but its role in the control of hypothalamic thyrotropin-releasing hormone (TRH) is controversial. To determine whether thyroid hormone regulates the function of TRH in the hypothalamic tuberoinfundibular system, a study was made of the effect of hypothyroidism on thyrotropin-releasing hormone messenger RNA (proTRH mRNA) and TRH prohormone in the rat paraventricular nucleus. Extracts of rat hypothalamic paraventricular nucleus were examined by quantitative Northern blot analysis, and coronal sections of rat brain were examined by in situ hybridization histochemistry and immunocytochemistry. A nearly twofold increase in proTRH mRNA was observed in hypothyroid animals; this increase could be obliterated by levothyroxine treatment, suggesting an inverse relation between circulating thyroid hormone and proTRH mRNA. In situ hybridization showed that this response occurred exclusively in medial parvocellular neurons of the paraventricular nucleus. A simultaneous increase in proTRH mRNA and immunoreactive TRH prohormone in this region suggests that hypothyroidism induces both transcription and translation of the TRH prohormone in the paraventricular nucleus.

ALTHOUGH IT IS CLEARLY ESTABLISHED that thyroid hormone exerts negative feedback control on thyroid-stimulating hormone (TSH) secretion by anterior pituitary thyrotropes (*1*), its effect on hypothalamic thyrotropin-releasing hormone (TRH), the principal regulator of TSH secretion, has been a subject of controversy (*2*). Measurements of TRH content in the hypothalamus in altered states of thyroid function have been conflicting, possibly because there are many TRH neurons that are not part of the tuberoinfundibular system (*3, 4*) (and hence might be regulated differently by thyroid hormone), and because content alone may be separately affected by rates of synthesis, transport, secretion, and degradation. The recent isolation of a complementary DNA (cDNA) that encodes the TRH prohormone (proTRH) (*5*) and the development of antisera that interact specifically with proTRH (*6*) have provided the tools to determine whether thyroid hormone regulates the function of the TRH tuberoinfundibular system.

Hypothyroidism was induced in male Sprague-Dawley rats by injecting them intraperitoneally with propylthiouracil (1.0 mg per 100 g of body weight) and putting 0.02% methimazole in their drinking water. Control animals received either an initial intraperitoneal injection of normal saline alone or were treated as the hypothyroid animals but given a daily intraperitoneal injection of levothyroxine (3 μg per 100 g of body weight). Animals were decapitated after 21 days of treatment and their blood was assayed for TSH by reagents supplied by the NIADDK. The brains were snap-frozen in hexanes containing Dry Ice, and a 1.5-mm coronal section containing the entire paraventricular nucleus was cut with

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