Isolation of IL-2 Receptor-β cDNA Clones from AtT-20 Pituitary Cells: Constitutive Expression and Role in Signal Transduction

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Interleukin-2 (IL-2) has been shown to stimulate ACTH secretion by anterior pituitary cells and has been implicated in pathophysiological processes of the pituitary and brain in several major neuropsychiatric disorders. The present study tested the hypothesis that IL-2 receptor-β (IL-2Rβ), a constitutively expressed and essential subunit for IL-2 signaling in lymphocytes, is expressed by AtT-20 pituitary cells and involved in transducing intracellular signals induced by IL-2. We isolated and sequenced three overlapping IL-2Rβ cDNA clones from AtT-20 pituitary cells representing key regions of the gene protein coding sequence. These cDNA clones included conserved sequences shared by growth hormone and prolactin as well as intracytoplasmic Src and JAK family homology domains of nonreceptor protein tyrosine kinases essential for IL-2 signaling in lymphocytes. Their nucleotide sequences were 100% homologous with those expressed by lymphocytes (together they comprised 70% of the full length coding sequence). The IL-2Rβ gene is constitutively expressed by AtT-20 pituitary cells, and its transcription was upregulated after CRF stimulation. Species-specific Il-2 induced intracellular signals in AtT-20 cells known to be mediated by Il-2Rβ, including a transient increase in c-myc nuclear proto-oncogene transcription and the dose-dependent induction of DNA replication as measured by [³H]thymidine incorporation. The IL-2–induced DNA replication signal was not delivered by heat inactivated IL-2 and was partially blocked by a murine anti-IL-2Rβ monoclonal antibody. These studies suggest that IL-2Rβ may be a critical target involved in mediating the neuroimmunological actions of this prototypical cytokine in endocrine cells.
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Much attention has focused on the potential role of interleukin-2 (IL-2) in the pathogenesis of several major neuropsychiatric disorders and disorders of the HPA axis (e.g., Maes et al. 1991, 1995; Quirion et al. 1991; Licinio et al. 1993; Rapaport et al. 1993; Araujo and Lapchak 1994; Sauer et al. 1994; McAllister et al. 1995; Nimgaonkar et al. 1995). This prototypical immunoregulatory cytokine has a variety of actions in the central nervous and endocrine systems (for reviews, see Merrill 1990; Nistico and De Sarro 1991; Nistico 1993). IL-2 has been shown to modulate the physiology of the pituitary gland in both animals and humans. Alterations in ACTH and cortisol secretion, for example, are manifested by cancer patients receiving IL-2 immunotherapy (Lotze et al. 1985; Denicoff et al. 1989). IL-2 has also been shown to stimulate ACTH secretion from anterior pituitary corticotrophs in vivo and in vitro (Farrar 1984; Smith et al. 1989; Naito et al. 1989; Karanth and McCann 1991; Harbuz et al. 1992; Hanisch et al. 1994), and recent studies of invertebrates suggest the possibility

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that corticotrophin-releasing factor (CRF) and IL-2 share a common evolutionary origin (Ottaviani et al. 1994). Knowledge of the mechanisms by which IL-2 transduces intracellular signals in endocrine cells may have important implications for understanding pathophysiological processes of the HPA axis. Little is known, however, about the molecular characteristics of IL-2 receptors expressed by endocrine cells.

Lymphocyte IL-2 receptors (IL-2R) are comprised of a multisubunit receptor complex, where three distinct subunits, α, β, and γ combine via differential expression to form different functional IL-2R subtypes (for review, see Hatakeyama et al. 1989b; Minami et al. 1993; Taniguchi and Minami 1993). The constitutively expressed IL2Rβ and IL2Rγ subunits form a so called "intermediate" affinity receptor ($K_D \cong 1$ nmol/L). The IL2Rβ and IL2Rγ subunits are critical for transducing IL-2 signaling processes in lymphocytes. Activated lymphocytes often co-express the accessory IL-2Rα subunit, which together with IL2Rβ and IL2Rγ form a "high" affinity receptor ($K_D \cong 10$ pmol/L). By itself, IL-2Rα can form a "low" affinity receptor ($K_D \cong 10$ nmol/L), which is not capable of transducing IL-2 signals in lymphocytes. Whereas IL2Rβ and IL2Rγ are members of the superfamily of cytokine receptors, IL-2Rα is not (Bazan 1990; Taniguchi and Minami 1993). Recent molecular cloning studies by our laboratory indicate that IL2Rβ and IL-2Rα genes are expressed by normal murine brain cells (Petitto and Huang 1994, 1995) and IL2Rβ and IL-2Rα mRNA has been identified in the adult human brain tissue and fetal brain tissue, respectively (Otero and Merrill 1995). Forebrain IL-2 receptors appear to be the site of action of those neuroendocrine effects of IL-2 which are initiated at the level of the hypothalamus (Bindoni et al. 1988; Harbuz et al. 1992; Cambronero et al. 1992; Bartholomew and Hoffman 1993; Karanth et al. 1993; Lapchak and Araujo 1993; Hillhouse 1994; Raber and Bloom 1994; Raber et al. 1995).

Landmark studies demonstrated that IL-2 could induce ACTH secretion and proopiomelanocortin (POMC) gene expression from anterior pituitary cells (Farrar 1984; Brown et al. 1987; Smith et al. 1989). Detection of the α-subunit of the IL-2 receptor, a nonconstitutively expressed accessory subunit of the IL-2 receptor heterotrimer complex, has been identified in AtT-20 pituitary cells (Smith et al. 1989; Arzt et al. 1992). Since the interleukin-2 receptor α accessory subunit (IL-2Rα) lacks an appreciable intracellular domain and is not involved in the intracellular signaling events in lymphocytes, it was unknown whether IL-2 signals in pituitary cells are transduced by the IL-2Rβ subunit of the superfamily of cytokine receptors or by a unique subunit expressed by cells of endocrine origin (Merrill 1990; Arzt et al. 1992; Petitto and Huang 1995).

In the present study we therefore sought to test the hypotheses that IL-2Rβ or an IL-2Rβ-like gene is consti-

tutively expressed by AtT-20 pituitary cells and involved in IL-2–induced signal transduction processes in this endocrine cell line. In lymphocytes, two such processes induced by IL-2, which are known to be mediated by IL2Rβ, are c-myc nuclear proto-oncogene activation and growth signals (Hatakeyama et al. 1989a,b; Shibuya et al. 1992; Minami et al. 1993; Taniguchi and Minami 1993; Nelson et al. 1994; Taniguchi 1995). To test these hypotheses, the present study sought to determine specifically: (1) if AtT-20 pituitary cells express IL-2Rβ mRNA; (2) whether key segments of the coding sequence of the IL-2Rβ expressed by AtT-20 cells, in particular regions of the intracellular domain where signaling sequences of the receptor reside, were derived from the same or a gene related to the lymphocyte IL-2Rβ; (3) if IL-2Rβ was constitutively expressed by these pituitary corticotrophs, and upregulated after CRF stimulation; (4) whether species-specific recombinant IL-2 could activate intracellular pathways known to be associated with IL-2Rβ in lymphocytes—increased transcription of c-myc nuclear proto-oncogene and the delivery of an intracellular DNA replication signal to AtT-20 cells and; (5) if the DNA replication signal induced by IL-2 could be blocked by a specific monoclonal antibody to the murine lymphocyte IL-2Rβ.

MATERIALS AND METHODS

Reverse Transcription and Polymerase Chain Reaction (PCR)

The mRNA used for PCR amplification and cloning experiments was isolated from the mouse anterior pituitary cell line, AtT-20 (American Type Culture Collection), using a commercial kit (Microfast Track, Invitrogen) as described previously (Petitto and Huang 1994; Petitto et al. 1994). The cDNA used for PCR amplification was synthesized using a 0.5 μg of poly(A)+ mRNA primed with a mixture of 0.12 μg oligo(dT) and 0.4 μg of random primers. Reverse transcription was conducted with reverse transcriptase (cDNA Cycle, Invitrogen) for 1 h at 42°C. After denaturation of RNA-cDNA hybrids at 95°C for 3 min and cooling on ice, additional reverse transcriptase (5 units) was added to the reaction mixture and reincubated for 1 h at 42°C. The reaction mixture was heated again to 95°C for 3 min and cooled on ice.

PCR primers for IL-2Rβ were designed with the computer assisted program PCRPLAN (Intelligenetics) using the published sequence of the mouse lymphocyte IL-2Rβ (Kono et al. 1990). To determine if an IL-2Rβ (or a sequence homologous to the lymphocyte IL-2Rβ) is expressed by AtT-20 pituitary cells, we designed primers to amplify the WSXWS sequence characteristic of the lymphocyte IL-2Rβ and other members of the cytokine receptor superfamily (Bazan 1990; Taniguchi and Minami 1993). The upstream and downstream primers

were 5′-GTGGACCTCCTTGACATA-3′ and 5′-GTTTCG-TTGAGCTTTGA-CCCTCA-3′, respectively. Subsequent to the isolation of this cDNA clone, primers were then targeted to cytoplasmic domain sequences known to be essential for the signaling functions of IL-2Rβ in lymphocytes. The two pairs of primers used for those experiments were: 5′-GTGAGGGTCAAAGCTCAACG-3′ and 5′-GATCTCCAAGGCATTGGG-3′, upstream and downstream primers, respectively, and; 5′-CCAGCTGCTTCA-CCAACCA-3′ and 5′-GTAAGCCCATCAGGTCACG-3′, upstream and downstream primers, respectively.

The upstream and downstream primers for murine c-myc were 5′-ATGTTGCGGTCGCTACGTC-3′ and 5′-CTGTCCAACTTGGCCCTCTTG-3′, respectively. The upstream and downstream primers for murine β-actin were 5′-GTGGGCCGCTCTAGGCACCAA-3′ and 5′-CTC-TTTGATGTCACGCACGATTTC-3′, respectively. All primers were synthesized at DNA Synthesis Core of the University of Florida Interdisciplinary Biotechnology Center, except for the murine β-actin primers, which were purchased commercially (Clonetech).

PCR reactions were performed using a 50 μl total reaction volume containing 0.4 μmol/L each of forward and reverse primers, 20–40 ng cDNA, 0.2 mmol/L of each dNTP, and 2.5 units Taq DNA polymerase (GIBCO BRL), in 50 mmol/L KCl/0.01% gelatin/10 mmol/L Tris-HCl buffer with 2 mmol/L MgCl₂, and amplified using a thermal cycler with a heated evaporation cover (Ericomp). The cycling parameters were: denaturing 94°C (45 s); annealing 56°C (30 s, for IL-2Rβ and β-actin, and 25 s for c-myc); extension 72°C (2 min) with a final extension step of 7 min. Thirty-five cycles were used for the PCR cloning experiments. In other experiments, the identity of c-myc and β-actin bands were confirmed by size and by select restriction enzyme cuts.

PCR Cloning of IL-2Rβ from Mouse AtT-20 Pituitary Cells

The PCR amplified IL-2Rβ cDNA was cloned from the library of cDNAs obtained from mouse AtT-20 pituitary cells. Direct cloning of PCR products was performed by the T-A overhang method as described previously (Petitto et al. 1994; Petitto and Huang 1994, 1995). In brief, the PCR product was agarose gel purified. Agarose containing the band was removed by centrifugation in a spin-x column (Costar), the cDNA was purified further by phenol extraction and ethanol precipitation, washed with 75% ethanol, dried, and resuspended in TE buffer. The vector (pGEM-T, Promega) and PCR product of interest were ligated at 12°C overnight. The vector was then transformed into competent *Escherichia coli*, spread on LB plates containing ampicillin and X-Gal, and white colonies were selected after overnight incubation at 37°C. Plasmid isolation and restriction analysis were performed using standard methods.

DNA Sequencing

The IL-2Rβ cDNA clones isolated from AtT-20 pituitary cells were sequenced by the chain termination method (Sequenase 2.0, United States Biochemical) using ³⁵S-labeled nucleotides (Amersham). Three independent clones from each of the three different cDNAs amplified from AtT-20 pituitary cells were sequenced. Electrophoresis was performed using a 6% polyacrylamide sequencing gel.

Ribonuclease Protection Assay

The ribonuclease protection assay was performed as described previously (Petitto et al. 1994; Petitto and Huang 1994, 1995). Total RNA was isolated from homogenized tissue from AtT-20 pituitary cells by the guanidine isothiocyanate method. The uniform loading of equal quantities of RNA to each protection assay reaction was ensured by adjusting the amounts of RNA in the samples via OD₂₆₀ nm absorbance using a spectrophotometer, and by subsequent comparison of the 28S and 18S rRNA bands obtained from an aliquot of the sample resolved on an ethidium bromide–stained gel (Ausubel et al. 1995).

A ³²P-labeled antisense riboprobe was synthesized using the T₇ promotor of the PCR ™II vector (Invitrogen) containing a cloned brain IL-2Rβ cDNA (Petitto and Huang 1994) according to standard methods. Briefly, 30 μg of total RNA was co-precipitated with the riboprobe for 30 min at −80°C. The mRNA/probe precipitate was dried, resuspended in 20 μl hybridization buffer (40 mmol/L Pipes/1 mmol/L EDTA/0.4 mol/L NaCl/80% formamide) and incubated at 80°C for 10 min. After brief centrifugation, the mRNA/probe was resuspended and incubated for 16 h at 47°C. This was followed by incubation in RNase A/RNase T₁ digestion buffer at 32°C for 45 min, and incubated for 15 min at 37°C after the addition of SDS (0.6%) and proteinase K (0.03%). After phenol/choloform extraction, carrier RNA was added (5 μg), and ethanol precipitation was performed at −80°C for 30 min. The pellet was washed and dried in ethanol, dissolved in 80% formamide dye, heated to 80°C for 7 min, and cooled on ice for 1 min. Samples were subsequently loaded on a 6% polyacrylamide gel. Autoradiograms were scanned using computer assisted densitometry (MCID System, Imaging Research, Inc.) and were quantified relative to the 28 S rRNA band.

Cell Culture and Murine IL-2 Stimulation of Murine AtT-20 Pituitary Cells

AtT-20 pituitary cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/

ml) at 37°C in a humidified CO_2 incubator (90% air/ 10% CO_2). In some experiments, cells were stimulated with murine recombinant IL-2 (mIL-2) (Biosource International) to determine the effects of this cytokine on: (1) c-myc proto-oncogene induction and (2) cell proliferation. Murine IL-2–induced proliferation of AtT-20 pituitary cells was performed using a [³H]thymidine incorporation assay. Briefly, 10^5 cells were initially subcultured in 6-well plates (Costar #3506) containing only culture media. After 24 h of incubation, 100 µl mIL-2 dissolved in media or 100 µl media only (as control) was added to each culture well to yield a final volume of 1 ml. In experiments where an IL-2Rβ monoclonal antibody was used to antagonize the functional effects of mIL-2, either the monoclonal antibody or media was added 2 h before the addition of mIL-2. The cells were incubated in the presence or absence of mIL-2 for 48 h, pulsed with [³H]thymidine (4 µCi/ml; Amersham) for an additional 24 h, and harvested onto glass-fiber filter paper using a cell harvester (Skatron). These experimental conditions, in particular the culture and pulsing times, were adopted based on the doubling time of this subline of AtT-20 cell (~5.5 days) and on preliminary experiments. Results are expressed as the incorporation index (the ratio of [³H]thymidine incorporation in the presence of mIL-2/[³H]thymidine incorporation in the absence of mIL-2). Across all experiments, basal [³H]thymidine incorporation (in the absence of IL-2) ranged from 66–424 cpm/µl.

One-way analysis of variance (ANOVA) was used to assess differences in IL-2–induced proliferation of AtT-20 pituitary cells across conditions, with preplanned comparisons used to test for differences between select experimental conditions.

amplified DNA resulted from complementary mRNA, and was not the result of contamination from genomic DNA. These include the use of PCR primers spanning one or more introns, and the inability to amplify by PCR a detectable DNA band using purified mRNA that was treated with RNAse prior to reverse transcription (Kono et al. 1990; Shibuya et al. 1990). Subsequently, each of the three different PCR-generated cDNAs amplified from AtT-20 pituitary cells was subcloned, and three independent clones were sequenced for each of the three different cDNAs isolated. Sequence analysis showed that the nucleotide sequences of all three cDNAs, the one cDNA targeting the WSXWS motif (clone A) and the two intracytoplasmic cDNAs (clones B and C) from AtT-20 cells, were 100% homologous with the corresponding segments of the murine lymphocyte IL-2Rβ. These three cDNA clones isolated from AtT-20 pituitary cells overlapped and corresponded to bps 410–750 (clone A), bps 730–1238 (clone B), and bps 1182–1527 (clone C).

Constitutive and CRF-Upregulated Expression of IL-2Rβ mRNA in AtT-20 Pituitary Cells

Ribonuclease protection analysis of AtT-20 pituitary cells was used to confirm the constitutive expression of IL-2Rβ mRNA by AtT-20 pituitary cells and to determine whether the transcription of IL-2Rβ by AtT-20 pituitary corticotrophs could be modified by treatment with 10 nmol/L CRF. Figure 3 shows the results of a representative experiment (one of two independent experiments yielding similar results). As seen in Figure 3,

RESULTS

Nucleotide Sequence of IL-2Rβ cDNA Clones Isolated from AtT-20 Pituitary Cells

Figure 1 shows the results of the initial PCR experiments to determine if AtT-20 pituitary cells express the IL-2Rβ sequence containing the WSXWS motif found among members of the cytokine receptor superfamily. The expected size 341 bp cDNA for IL-2Rβ was amplified from splenic lymphocytes (Kono et al. 1990). A similar size (though less pronounced) cDNA was amplified from AtT-20 pituitary cells. Subsequently, as seen in Figure 2, two additional cDNAs were amplified using primers targeting areas of the intracytoplasmic region encoding intracellular domains of the receptor known to be involved in signal transduction processes by IL-2Rβ in lymphocytes. These cDNAs amplified were the expected sizes of 508 and 345 base pairs, respectively, from both lymphocytes and AtT-20 pituitary cells. Experimental controls were used to ensure that the PCR-
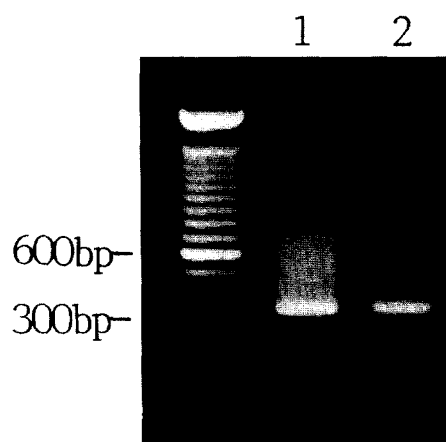

Figure 1. PCR amplification targeting regions of the transmembrane domain and the extracellular domain of IL-2Rβ from AtT-20 pituitary cells, including the WSXWS motif characteristic of the cytokine receptor superfamily. This cDNA was subcloned and sequenced (clone A). The far left lane is the 100 bp ladder. *Lane 1*: 341 bp IL-2Rβ cDNA from splenic lymphocytes. *Lane 2*: 341 bp IL-2Rβ cDNA from AtT-20 pituitary cells (clone A).
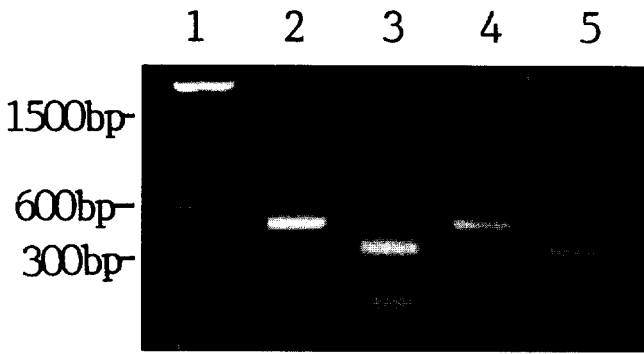
Figure 2. PCR amplification targeting the intracytoplasmic sequences of IL-2Rβ from AtT-20 pituitary cells. These cDNAs were subcloned and sequenced (clone A and clone B). *Lane 1*: 100 bp DNA ladder. *Lane 2*: 5' 508 bp cDNA segment of the intracytoplasmic domain of IL-2Rβ from splenic lymphocytes. *Lane 3*: 3' 345 bp cDNA segment of the intracytoplasmic domain of IL-2Rβ from splenic lymphocytes. *Lane 4*: 5' 508 bp cDNA segment of the intracytoplasmic domain of IL-2Rβ from AtT-20 pituitary cells (clone B). *Lane 6*: 3' 345 bp cDNA segment of the intracytoplasmic domain of IL-2Rβ from AtT-20 pituitary cells (clone C).

ribonuclease protection analysis showed that unstimulated AtT-20 pituitary cells constitutively express IL-2Rβ, though at considerably lower levels than murine splenic lymphocytes stimulated by ConA. Compared to unstimulated AtT-20 pituitary cells, treatment of AtT-20 cells with 10 nM CRF for 24 h produced a modest increase in IL-2Rβ mRNA. Scanning densitometry of autoradiograms showed that the level of IL-2Rβ mRNA expressed by CRF stimulated AtT-20 cells was approximately two times higher than unstimulated AtT-20 cells.

Species-Specific IL-2 Induces Intracellular Signals in AtT-20 Pituitary Cells Ascribed to IL-2Rβ

Since IL-2Rβ is essential to aspects of IL-2–induced intracellular signal transduction including the transcrip-
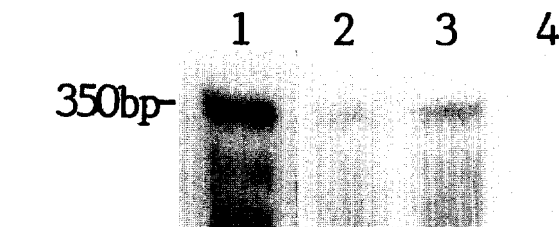
tion of nuclear proto-oncogenes, we sought to determine whether mIL-2 could induce c-myc mRNA expression by AtT-20 murine pituitary cells. As depicted in Figure 4, mIL-2–induced transient expression of c-myc message with the highest level expressed after 60 min of mIL-2 stimulation.

Another function mediated by IL-2Rβ in subsets of lymphocytes is the transduction of a growth signal induced by IL-2. Figure 5 depicts the results of experiments examining the ability of mIL-2 to deliver a DNA replication signal (measured by ^{3}H-thymidine incorporation) in AtT-20 pituitary cells. As seen in Figure 5, mIL-2 produced a dose-dependent increase in [^{3}H]thymidine incorporation [$F(3,35) = 4.8, p < .01$] with the 10 nmol/L concentration being significantly different than baseline control levels (media only) ($p < .01$). Heat-inactivated mIL-2 had no effect on AtT-20 cell proliferation (data not shown).

Figure 6 shows the results of subsequent experiments to determine if this mIL-2–induced DNA replication in AtT-20 cells could be blocked or antagonized by a monoclonal antibody to the murine lymphocyte IL-2Rβ. In preliminary experiments, AtT-20 cells were cultured at various concentrations of the anti-mIL2Rβ monoclonal antibody, and the integrity of the cells was
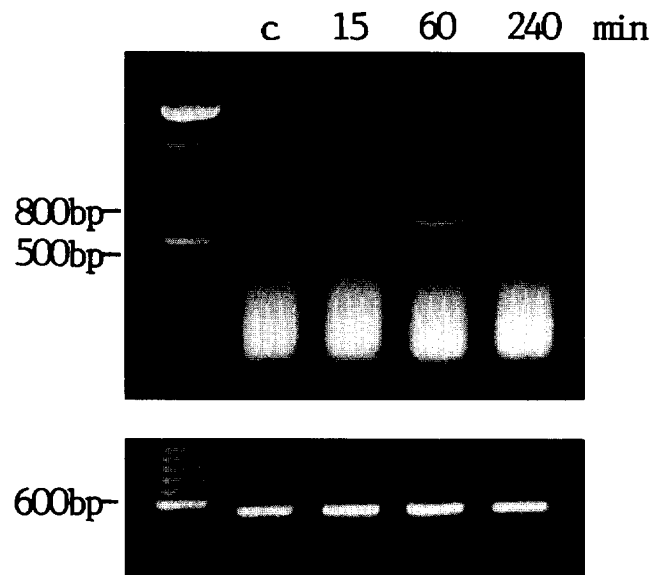

Figure 3. Autoradiogram of ribonuclease protection analysis of IL-2Rβ mRNA from unstimulated and CRF stimulated AtT-20 pituitary cells. *Lane 1*: IL-2Rβ cDNA from ConA stimulated murine (Balb/c) splenic lymphocytes. *Lane 2*: IL-2Rβ cDNA from AtT-20 pituitary cells cultured in media only (unstimulated) for 24 h. *Lane 3*: IL-2Rβ cDNA from AtT-20 pituitary cells treated with 10 nmol/L CRF for 24 h. *Lane 4*: tRNA control.

Figure 4. Induction of c-myc nuclear proto-oncogene gene expression in AtT-20 pituitary cells by mIL-2. AtT-20 pituitary cells were cultured in the presence of 1 nmol/L mIL-2 for various times. The upper panel represents PCR analysis of c-myc gene expression (776 bp) and the lower panel for β-actin (540 bp). The far left lane is the 100 bp ladder. The lane labeled c represents the control (AtT-20 cells cultured in media only for 60 min) for AtT-20 cells stimulated with mIL-2 for 60 min, the time point where the highest level of c-myc gene expression was detected.
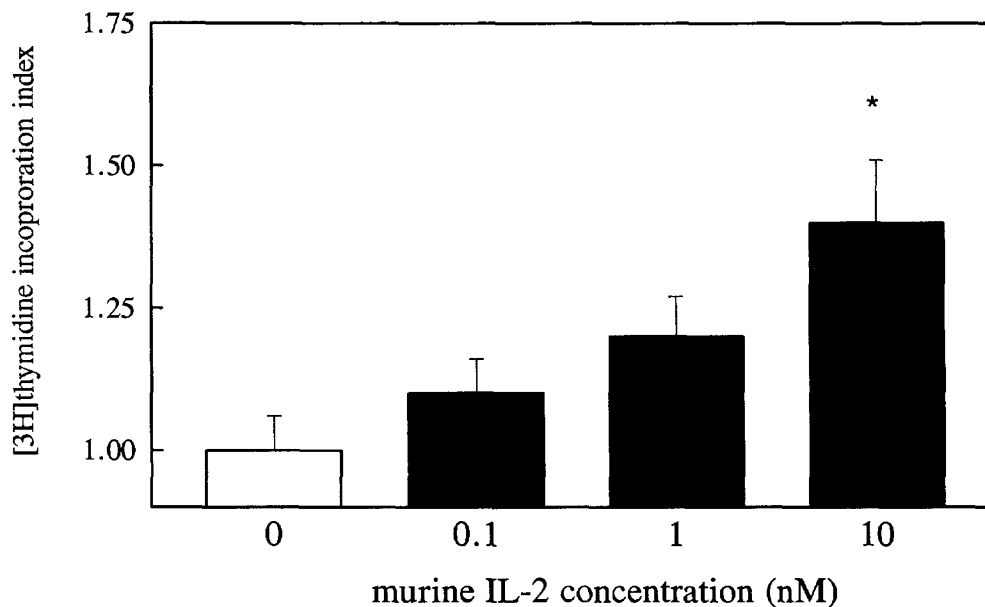
Figure 5. IL-2–induced DNA replication in murine AtT-20 pituitary cells. AtT-20 pituitary cells were cultured in the presence of various concentrations of murine recombinant IL-2 (mIL-2) and [^{3}H]thymidine incorporation was measured. Data are ex-pressed as the [^{3}H]thymidine incorporation index (the ratio of [^{3}H] incorporation in the presence of mIL-2/[^{3}H]thymidine in-corporation in the absence of mIL-2). Each bar represents the mean $\pm$ SEM of nine cultures. Analysis of variance (ANOVA) demonstrated a significant main effect of mIL-2 concentration. $*p < .01$ compared to control (conc. 0).

subsequently examined by trypan blue exclusion (Petitto et al. 1989). Based on these results and the immunological literature, a concentration of 0.05 μg/ml culture of the anti-IL2Rβ monoclonal antibody was selected for these experiments (higher concentrations of the antibody cultured alone with AtT-20 cells were found to significantly inhibit basal [^{3}H]thymidine incorporation). As can be seen (Figure 6), the anti-mIL2Rβ monoclonal partially antagonized (by about one-half) the mIL-2–induced increase in [^{3}H]thymidine incorporation. This antagonism, however, was not statistically significant. In wells where AtT-20 pituitary cells were cultured in

the presence of the anti-mIL2Rβ alone (not shown), there was marked variability on [^{3}H]thymidine incorporation that overlapped significantly with each of the three conditions tested in Figure 6 (n = 10 cultures, mean $\pm$ SEM, 1.67 $\pm$ 0.34, overall appearing to have a growth-enhancing effect).

DISCUSSION

This study demonstrated the IL-2Rβ gene is expressed by AtT-20 pituitary cells. Together, the three overlap-
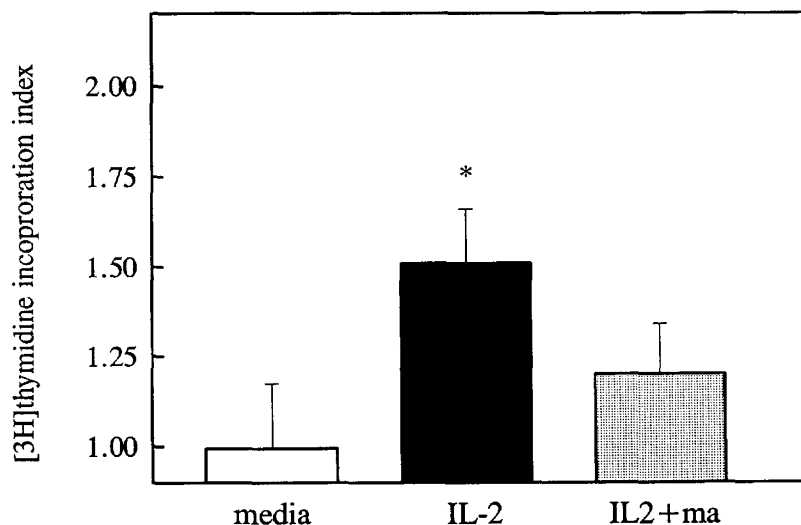

Figure 6. Antagonism of IL-2–induced DNA replication in murine AtT-20 pituitary cells by an anti-mIL2Rβ monoclonal antibody. AtT-20 cells cultured in the presence of 10 nmol/L mIL-2 were pretreated with anti-mIL2Rβ monoclonal antibody. Each bar represents the mean $\pm$ SEM of 9–10 cultures. *Level of [^{3}H]thymidine incorporation significantly different ($p < .05$) from the level of [^{3}H]thymidine incorporation by AtT-20 cells cultured in media only.

ping cDNAs that were isolated and sequenced from AtT-20 pituitary cells coincided with bp 410–1527 (approximately 70% of the full-length coding sequence) of the mouse lymphocyte IL-2Rβ. The nucleotide sequence of these three IL-2Rβ cDNA clones from AtT-20 cells were identical to the lymphocyte IL-2Rβ nucleotide sequence. Clone A (bps 410–750) comprised the most proximal segment of the transmembrane domain and most of the extracellular domain of the receptor, including the WS motif (WSXWS sequence) characteristic of the cytokine receptor superfamily (Bazan 1990; Taniguchi and Minami 1993). Interestingly, this highly conserved region is also shared by two classic endocrine receptors, growth hormone and prolactin (Bazan 1990), and this same gene sequence is also expressed in the mouse forebrain including limbic areas (e.g., hippocampus) known to modulate the activity of the HPA axis (Petitto and Huang 1994). Clone B (bps 730–1238) and clone C (bps 1182–1527) contain nucleotide sequences of the intracytoplasmic domain of IL-2Rβ including the Src and JAK family homology domains of nonreceptor protein tyrosine kinases, which are known to be essential for some of the complex signaling processes activated by IL-2 in lymphocytes. Many cytokine receptors on immune cells, including the IL-2 receptors, are typically multisubunit receptors comprised of subunits that may combine via differential expression to form different functional subtypes (e.g., high versus intermediate IL-2 receptors). Some cytokine receptors share common subunits with one another (e.g., IL-2Rγ is a common γ subunit for lymphocyte IL-4 and IL-7 receptors) (Kondo et al. 1993; Noguchi et al. 1993). Although certain classic neurotransmitter receptors exist as receptor "isoforms" derived from the same gene by alternative RNA splicing (e.g., the D_2 dopamine receptor has long and short isoforms), this is not the case for the lymphocyte IL-2 receptor heterotrimer complex (Minami et al. 1993). Thus, based on our finding that the sequences of the IL-2Rβ clones isolated from AtT-20 pituitary cells comprised more than two-thirds of the coding sequence and were identical to the corresponding lymphocyte IL-2Rβ sequences, it is highly likely that the remainder of the gene coding sequence expressed by AtT-20 pituitary cells is the same as the lymphocyte. Nonetheless, the possibility that the remaining areas of IL-2Rβ expressed by AtT-20 pituitary cells are different from the lymphocytes sequences cannot be ruled out. This is the first demonstration, to our knowledge, that intracytoplasmic sequences of the receptor known to be critical for IL-2 signaling have been cloned and sequenced from a nonimmune tissue. In the immune system, subpopulations of lymphocytes (e.g., natural killer cells, subsets of $C8^+$ T cells) constitutively express IL-2Rβ (Sigel et al. 1987; Takeshita et al. 1992). Our data also show that AtT-20 pituitary cells constitutively expressed IL-2Rβ and that upon stimulation of these corticotrophs

with CRF, IL-2Rβ gene transcription is increased about two-fold.

Although IL-2 has been shown to induce ACTH secretion from pituitary cells including AtT-20 cells, the direct effects of species-specific IL-2 on intracellular signaling in AtT-20 pituitary cells had not been established. Thus, since IL-2Rβ is known to be essential for transducing intracellular signals by IL-2 in lymphocytes, we determined if this pituitary cell line, which constitutively expresses IL-2Rβ, was capable of IL-2 mediated signaling events. These experiments revealed that mIL-2 treatment of AtT-20 cells produced a transient increase in the transcription of the gene for c-myc nuclear proto-oncogene. In lymphocytes, both the intracytoplasmic serine-rich and acidic region of IL-2Rβ (AtT-20 clones B and C) are critical for IL-2–mediated induction of some nuclear proto-oncogene transcriptional regulators (e.g., c-myc, c-fos, c-jun) and the proliferative response (Roussel et al. 1991; Shibuya et al. 1992; Satoh et al. 1992; Taniguchi 1995). A number of important questions remain to be determined regarding whether the complex profile of IL-2Rβ–mediated signaling events in pituitary cells parallels or differs from signaling processes in lymphocytes. Since the time course of this transient increase in c-myc coincidences with the time course of IL-2–induced ACTH release found in rodent pituitary cells in vitro and in vivo observed in some previous studies (Smith et al. 1989; Naito et al. 1989), it will be of interest to determine if this transcription factor is involved in the control of IL-2–induced ACTH secretion by AtT-20 pituitary cells.

The present study demonstrated further that mIL-2–induced a dose-dependent DNA replication signal to AtT-20 pituitary cells. Though not a focus of this investigation, parametric studies optimizing the various culture conditions (e.g., pulse time, cell density, media constituents) could increase further the magnitude of the DNA replication signal by IL-2 (e.g., at 10 nmol/L) that we have observed. In the present study, the increased DNA replication signal to AtT-20 pituitary cells was induced by 10 nmol/L IL-2 (but was not delivered by the same concentration of the heat-inactivated mIL-2) and could be partially antagonized by the addition of an anti-IL2Rβ monoclonal antibody. This anti-IL2Rβ monoclonal antibody (clone TM-β1) is, to our knowledge, the only one available for the mouse. It has been found to exhibit varied and bidirectional effects on growth responses in different IL-2Rβ-bearing immune cell lines (Hatakeyama et al. 1989a). Pretreatment of these pituitary cell cultures with the anti-IL2Rβ monoclonal antibody resulted in approximately a 50% reduction of the magnitude of the IL-2 effect. Unfortunately, as noted earlier, since higher concentrations of the antibody alone appeared to have direct toxic effect on AtT-20 cells in culture, it precluded our ability to reliably attain complete and unequivocal blockade of the IL-2

effect. Future experiments will require a different anti-IL2Rβ monoclonal antibody as well as benefit from additional controls (e.g., an isotype, subclass, and light-chain matched antibody to an antigen not expressed by AtT-20 cells) and an endocrine cell line with growth characteristics more amenable to such manipulations (e.g., cells with a shorter doubling time, cells that grow in monolayers rather than clusters).

In summary, the present experiments confirmed the hypothesis that IL-2Rβ is constitutively expressed by AtT-20 pituitary cells and involved in mediating intracellular signal transduction processes induced by species-specific IL-2 in this endocrine cell line. These functions comprise two signaling parameters that have been previously ascribed to IL-2Rβ in lymphocytes. It will be important to determine in future investigations whether endocrine cells also express an IL2Rγ (or IL2Rγ like) subunit, which may also be constitutively expressed together with IL-2Rβ and involved in the complex array intracellular signaling events induced by IL-2 (Takeshita et al. 1992; Minami et al. 1993; Taniguchi and Minami 1993; Taniguchi 1995). Knowledge of the mechanisms by which IL-2 mediates intracellular signaling processes in pituitary and brain cells could provide better understanding of the molecular basis of altered HPA function in certain neuroendocrinological and major neuropsychiatric disorders.

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