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The Expression of Thyrotropin Receptor in the Brain*

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ABSTRACT

The regulation of the thyroid gland by TSH is mediated by a heterotrimeric G protein-coupled receptor. Nonthyroid effects of TSH have been reported, and expression of its receptor has been described in adipocytes and lymphocytes. We have previously reported the existence of specific and saturable binding sites of TSH and specific TSH effects in primary cultured rat brain astroglial cells. We now report expression of the TSH receptor gene in these cells; the coding sequence of the corresponding complementary DNA is identical to that previously established in thyroid. Using specific antisense RNA probe, expression of this gene was detected in some isolated or clustered glial fibrillary acidic protein-positive primary cultured cells by *in situ* hybridization. With this technique, we further detected TSH receptor messenger RNA (mRNA) expression in rat brain cryoslices in both neuronal cells and astrocytes. Its presence predominated in neuron-rich areas (pyriform and postcingulate cortex, hippocampus, and hypothalamic nuclei) and was mostly colocalized with neuron-specific enolase. In astrocytes, this mRNA was detected in the ependymal cell layer and the subependymal zone, and several isolated cells were also found in the brain parenchyma. We also detected TSH receptor mRNA and protein in primary cultured human astrocytes. The protein was detected as well in both rat and human brain cryoslices. Together, these findings clearly demonstrate the expression of the TSH receptor gene in the brain in both neuronal cells and astrocytes. (*Endocrinology* **142:** 812–822, 2001)

TSH WAS identified a long time ago as the most important factor regulating the production of thyroid hormones by the thyroid gland (1). The effects of TSH follow its binding to a transmembrane receptor. Its complementary DNA (cDNA) has been cloned from thyroid cells in several species (2–6). According to its sequence, the TSH receptor belongs to the superfamily of the heterotrimeric G protein-coupled receptors. The best known signaling pathway of TSH in thyroid, identified long before its receptor, is one that involves an increase in cAMP production (7). Since then, however, other TSH signaling pathways have been described in thyroid (8–12).

Besides its expression in thyroid tissue, the presence of a TSH receptor in nonthyroid tissues has also been described. Birnbaumer and Rodbell reported the stimulation of cAMP production by TSH in adipocytes (13), in which TSH binding was characterized soon afterward (14, 15). Similarly, Pekonen and Weintraub reported the existence of TSH-binding sites at the surface of lymphocytes (16). More recently, expression of TSH receptor messenger RNA (mRNA) has been reported in both cell types (17, 18). These results strongly suggest that TSH may play another role besides regulating the thyroid gland (19). In fact, the presence of TSH in cerebrospinal fluid (20) and brain (21) has been described, raising the question of a hypothetical local function of TSH in the central nervous system.

A few years ago we described the existence of specific [particularly compared with LH/CG (22)] and saturable binding of radiolabeled TSH at the surface of astroglial cells (23) in primary cultures from newborn rat cerebrum. However, TSH failed, surprisingly, to enhance cAMP production in these cells, contrary to what was reported in thyroid. Instead, TSH stimulated the release of arachidonic acid (23). Moreover, we found that activity of the type II 5′-iodothyronine-deiodinase, known to be regulated by other factors in these cells (24), was potently stimulated by TSH in a sustained manner (23). In astroglial cells TSH also induced a stimulation of mitogen-activated protein kinase isozymes, Erk1 and Erk2 (25). Together, these results suggested that a functional TSH receptor was effectively expressed in these cells. Thus, although TSH exerts several effects in astroglial cells, uncoupling of TSH receptor from adenylyl cyclase may suggest the existence of a TSH receptor variant.

In this report we first determined the sequence of the corresponding cDNA in astrocytes. By *in situ* hybridization, using a specific riboprobe, we then showed that, as in thyroid, the TSH receptor gene was expressed in cultured astroglial cells and in brain cryoslices. The protein was detected with anti-TSH

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receptor antibodies in primary cultured astrocytes in humans and in brain cryoslices in both rats and humans.

Materials and Methods

Materials

DMEM, FCS, horse serum, trypsin-EDTA, PBS solution with 1:5000 EDTA (Versene), *Taq* polymerase, and reverse transcriptase (Superscript) were obtained from Life Technologies, Inc. (Gaithersburg, MD). Nucleic acid detection, digoxygenin (DIG) riboprobe, and PCR product purification kits were obtained from Roche (Indianapolis, IN). Rabbit anticow glial fibrillary acidic protein was obtained from DAKO Corp. (Carpenteria, CA), and horseradish peroxidase conjugate antiserum was purchased from Caltag Laboratories, Inc. (South San Francisco, CA). Antineurospecific enolase rabbit polyclonal antibody was a gift from Noel Lamande and Angelica Keller, and antineurofilament rabbit polyclonal antibody was obtained from Sigma (St. Louis, MO). Antidigoxigenin sheep polyclonal Fab fragments coupled to either alkaline phosphatase or fluorescein were obtained from Roche. Bluescript plasmid and DH5α cells (*Escherichia coli*) were purchased from Stratagene (La Jolla, CA). The laser confocal microscope was purchased from Carl Zeiss {[LSM410, (New York, NY)]; lens, ×40; 1.2 C-apochromat; excitation wavelengths: λ_{FITC} = 488 nm and $\lambda_{rhodamine}$ = 543 nm} and was driven by Philippe Leclerc (INSERM, Le Kremlin-Bicêtre, France).

Sequencing of the TSH receptor cDNA

Total RNA was isolated, as previously described (26), from rat primary cultured astrocytes, and cDNA was then synthesized as previously described (27). The entire length of the astrocytic TSH receptor cDNA was sequenced by PCR with eight pairs of oligonucleotides (Fig. 1) designed from the sequence of the TSH receptor cDNA in rat thyroid cells (6): 1) 5′-ACAGCGCGCAACGATGAAGT-3′ and 5′-GCATCAGGGTCTATGTAAGT-3′; 2) 5′-GCGACTGGAGCCACATTCTT-3′ and 5′-GGAAGGAAGAGCAGTAACGC-3′; 3) 5′-CAAAGATGCATTTGGAGGAG-3′ and 5′-CCGGATACTACTCTCATTAC-3′; 4) 5′-TACCCAAGTCACTGCTGTGC-3′ and 5′-AAAGACATTGCCCAGGAGAG-3′; 5) 5′-CAGGAAGAGACTCTACAAGC-3′ and 5′-AGAAGGAAGCAGCAAACCCA-3′; 6) 5′-TGCAACACGGCTGGTTTCTT-3′ and 5′-TTCACATAGCAGGAGCAGAC-3′;7)5′-GTACATCGCCCTTGTTCTCC-3′ and 5′-TGTTGGGACAGACTCTCTGG-3′; and 8) 5′-TTCATCCTACT CAGCAAGTT-3′ and 5′-AATACTCCCTAGAGATGAAC-3′. Both strands of the eight amplimers were then submitted to sequencing (PE Applied Biosystems, Foster City, CA) (28).

Preparation of cultured cells

Astroglial cells were isolated from cerebrum of 2-day-old Sprague Dawley rats, as previously described (29, 30). After seeding in sterile chamber slides, cells were cultured in DMEM supplemented with 10% FCS until day 6. FCS was then replaced by horse serum, and the cells were further cultured until day 10. At the end of the culture period, the cells were rinsed twice with PBS solution, pH 7.4, then placed in a 4% paraformaldehyde solution and kept at 4 C until use. Human astrocytes were cultured as previously described (31). For immunochemistry studies, human astrocytes were rinsed once with ice-cold PBS, incubated with ice-cold acetone for 10 min, then dried and kept at −20 C until use.

Plasmid construction and cloning for rat TSH receptor antisense RNA synthesis

Specific PCR primers were designed from the sequence of the rat TSH receptor cDNA (6), and one restriction site, for *Eco*RI or *Hin*dIII (*underlined*), respectively, was added to the 5′-end of each oligonucleotide: 5′-GTCAGAATTCGCTCTCCTGGGCAACGTCTT-3′ and 5′-GACTAAGCTTGGCGAAGGTGATGGCATA-3′. The PCR product (see Fig. 1), of about 350 bp, was gel extracted, digested with both *Eco*RI and *Hin*dIII restriction enzymes, and inserted by ligation into a Bluescript plasmid, which was then transfected into competent DH5α cells. Positive bacterial cell clones were selected and grown for plasmid extraction and purification (32). Then both strands were sequenced using primers corresponding to T3 and T7 promoters.

In situ hybridization

Rat brains were fixed overnight in 4% paraformaldehyde in PBS and then incubated in 30% sucrose. Brain sections of 15 μm were freeze-cut. TSH receptor riboprobes, sense and antisense, were synthesized with T3 or T7 RNA polymerase (see above). The riboprobes were DIG-labeled (DIG-deoxy-UTP), as described in the DIG-riboprobe kit protocol (Roche). The hybridization of TSH receptor mRNA in brain sections was performed overnight at 50 C with 10 μl of probes (10–20 ng/μl) in a solution containing 50% formamide, 5 × SSC (saline sodium citrate solution), 0.02% SDS, 5% blocking reagent, and 0.1% *N*-lauryl sarcosine. Washing was performed for 30 min at 50 C in 2 × SSC, for 20 min at 60 C in 2 × SSC plus 50% formamide, and for 30 min in 2 × SSC, then for 30 min in 1 × SSC at room temperature. Sections were rapidly rinsed in 100 mM Tris-HCl, pH 7.5, plus 150 mM NaCl and then incubated in blocking reagent. A conjugated antibody (anti-DIG alkaline phosphatase conjugate) was used to detect the probes, and a subsequent alkaline phosphatase-catalyzed color reaction was performed with X-phosphate and nitro blue tetrazolium salt producing a precipitate. The signal obtained with colloidal gold-coupled antibody (Roche) was enhanced by incubating the preparation for a few minutes with BL silver-enhancing blotting kit (British BioCell International, UK) and observed with polarized light. Five *in situ* hybridization experiments were performed independently, with four to five rats in each.

Glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), and neurofilament detection by immunofluorescence

Double staining was performed by *in situ* hybridization with TSH receptor RNA probes, and immunocytochemistry was performed with anti-GFAP, anti-NSE, or antineurofilament antibody. Immunodetection of the RNA probes was realized with an FITC-conjugated antibody (anti-DIG fluorescein isothiocyanate conjugate). After RNA detection, sections were incubated overnight with anti-GFAP (DAKO Corp.), anti-NSE, or antineurofilament antiserum diluted 1:50 in a solution of 0.1%

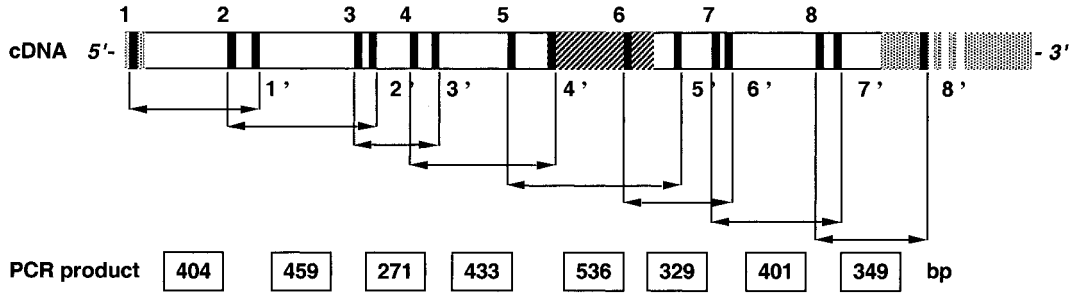

FIG. 1. Sequencing of the astrocyte TSH receptor cDNA. In astrocytes, this cDNA was sequenced with PCR (see *Materials and Methods*) from nucleotide 13 up to nucleotide 2470. In the thyroid, full-length cDNA is 5270 nucleotides long, translation start codon is nucleotides 60–62, and stop codon is nucleotides 2346–2348 (6). ▩, 5′- and part of 3′-untranslated regions; □, translated region; ■, PCR primers; ▨, region corresponding to the RNA probe used for *in situ* hybridization experiments (starting nucleotide 1336). The size of each PCR product is *framed*.

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Triton X-100 in PBS and then with a 1:30 solution of $F(ab')_2$ goat anti-rabbit horseradish peroxidase-conjugate serum (Caltag Laboratories, Inc.) for 2 h. After washing twice with PBS, sections were placed on slides and mounted in Mowiol (mix of glycerol and polyvinyl alcohol) for observation. When possible, a colocalization pattern was determined that was the result of a computer-assisted pixel by pixel analysis. For each pixel, the intensity of the input signal in each wavelength channel was considered significant only when it was above a given threshold. For the significant pixels, these values were reported in a two-axis graph, each axis corresponding to one wavelength channel. A "colocalization cloud" was then obtained, that was submitted to further analysis to determine which pixels had the most significant input signal in both wavelength channels, simultaneously.

Results

Astrocyte TSH receptor cDNA sequencing

We first attempted to establish the sequence of the TSH receptor cDNA in astrocytes using specific primers designed from the sequence of the TSH receptor cDNA (GenBank access no. M34842) that was previously published (6). This was achieved with PCR experiments after a step of RT. We detected a transcript in total RNA extract from primary cultured rat brain astroglial cells (not shown). Therefore, we next performed additional RT-PCR experiments, using newly designed primers (see *Materials and Methods* and Fig. 1) to establish the sequence of the detected cDNA. These PCR products had the same apparent sizes as those observed in thyroid (not shown) and were subsequently sequenced. Comparison of our data to those in GenBank showed that the sequence of the TSH receptor cDNA in astrocytes was identical to that described in the thyroid (6).

TSH receptor gene expression in primary cultured astrocytes

We then performed *in situ* hybridization experiments (Fig. 2) using RNA probes (see *Materials and Methods* and Fig. 1) corresponding to part of the last exon in the human TSH receptor gene (33) (as yet, the structure of the rat TSH receptor gene has not been determined). In primary cultured astroglial cells, the results of experiments performed with sense riboprobe were negative (not shown). With antisense riboprobe, this mRNA was detectable in only some cultured astrocytes, but then at a very high level (Fig. 2, A–C). Many positive cells were associated together in islets (Fig. 2B), but isolated-labeled cells were also found (Fig. 2, A and C). Whereas all of the TSH receptor mRNA-positive cells were also GFAP labeled (Fig. 2, D and E), GFAP-positive cells did not all express the TSH receptor gene (Fig. 2, F and G). In addition, none of the GFAP-negative cells was detected as TSH receptor positive.

TSH receptor gene expression in the brain

To determine whether the TSH receptor mRNA was also detectable in the brain *in vivo*, we further performed *in situ* hybridization experiments in brain cryoslices. The results of experiments performed with sense riboprobe were negative (Fig. 3, B and D). In sharp contrast, staining was observed predominantly in discrete areas of young rats brains in experiments performed with antisense probe (Fig. 3, A and C).

On day 7 several regions of the brain were clearly stained (the hippocampus, piriform, and postcingulate cortex) as well as cell clusters at the base of the brain, which may

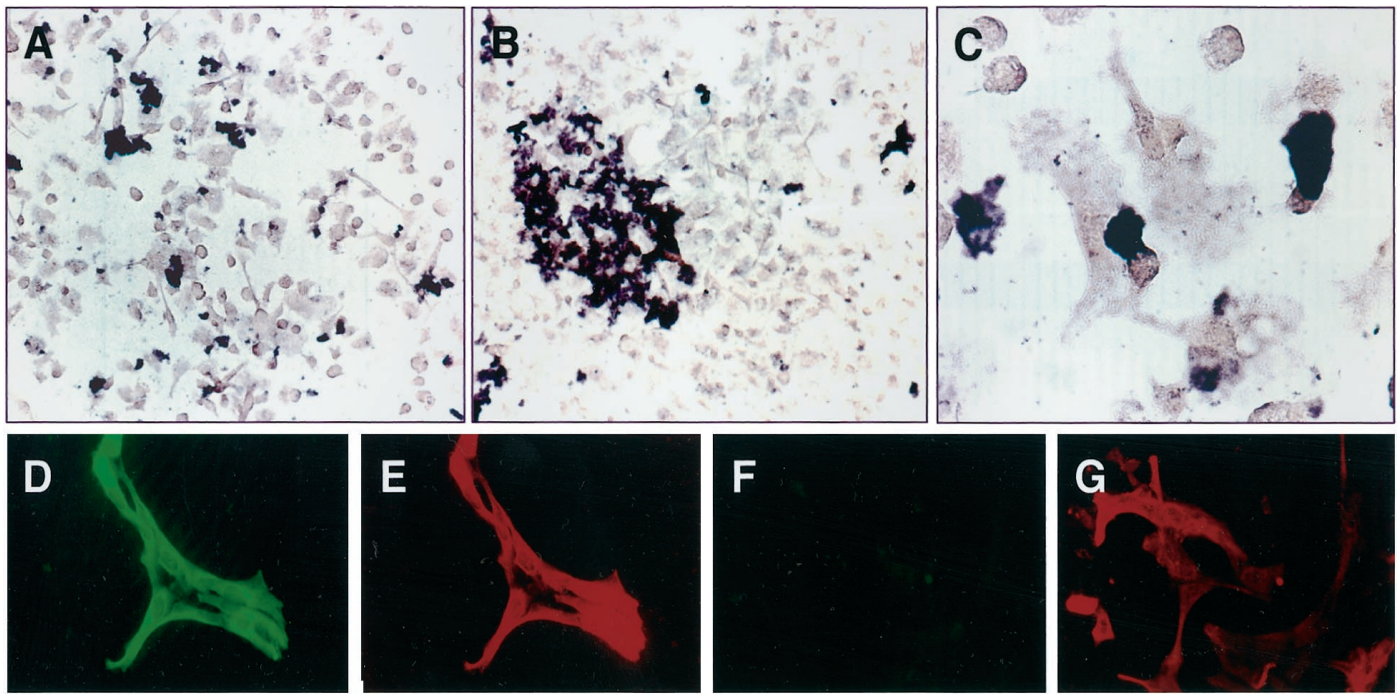

FIG. 2. Expression of the TSH receptor gene in rat primary cultured astrocytes. Astroglial cells were incubated with TSH receptor antisense riboprobe. Hybridization was then detected with phosphatase-coupled antibody and phosphatase-catalyzed color reaction (A–C) or FITC-coupled antibody (D and F). The cells were also incubated with anti-GFAP antibody (E and G), then with rhodamine-coupled antibody. Magnifications: A and B, ×180; D–G, ×360; C, ×600.

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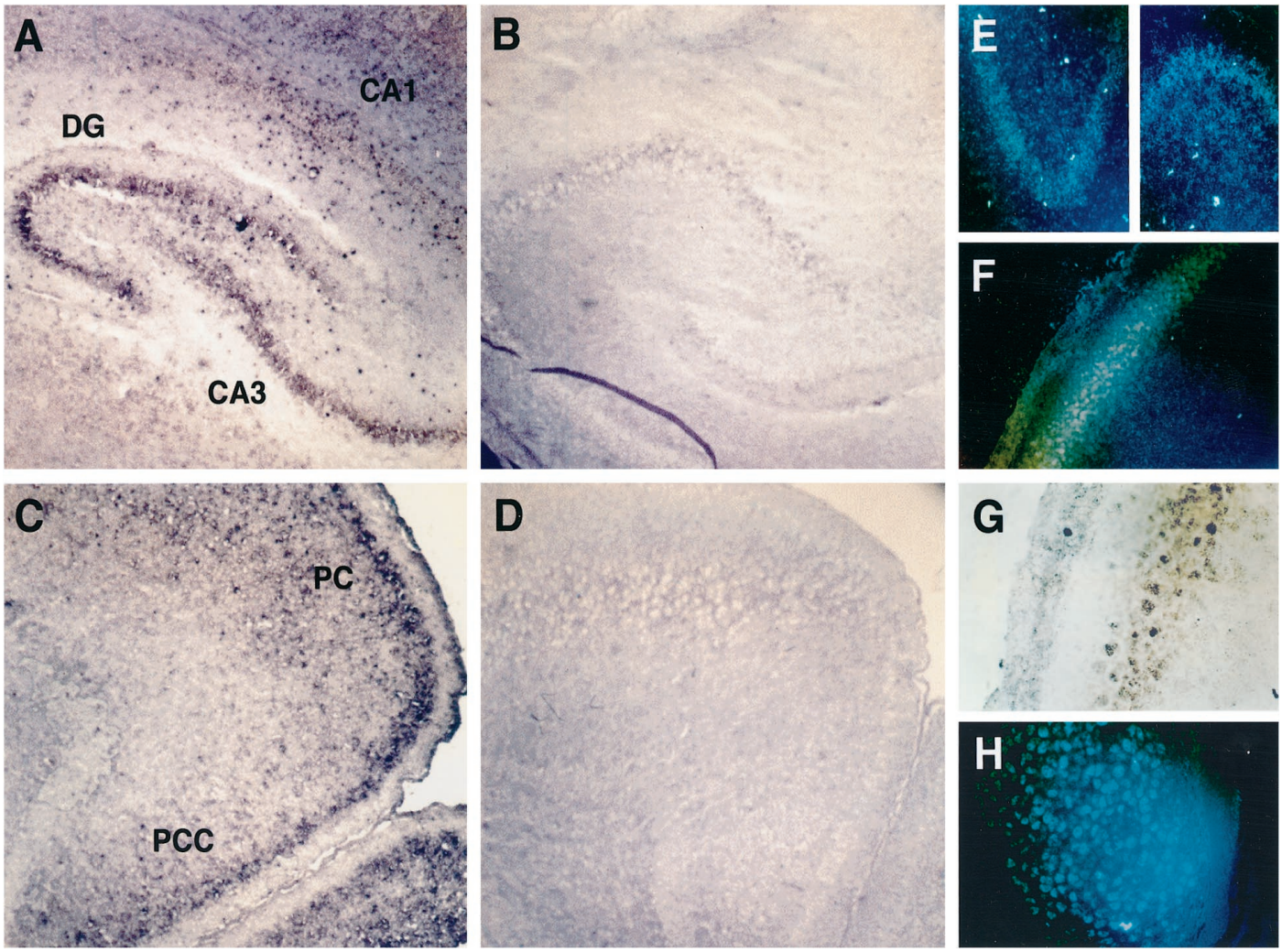

FIG. 3. *In situ* hybridization of TSH receptor antisense RNA in rat brain. Brain cryoslices of 7-day-old rat were fixed, then incubated with TSH receptor riboprobe (see *Materials and Methods*; B and D, sense riboprobe; A, C, and E–H, antisense riboprobe), and hybridization was detected as described in Fig. 2 (A–D) or with colloidal gold-coupled antibody (E–H). A–D, DG, Dentate gyrus; CA, Ammon's horn subfield; PCC, postcingulate cortex; PC, piriform cortex. E, Hippocampus: *left panel*, dentate gyrus; *right panel*, Ammon's horn. F and G, Piriform cortex in polarized (F) or nonpolarized light (G). H, Hypothalamus nucleus. Magnification: A–F, ×90; G and H, ×180.

correspond to hypothalamic nuclei. Clear staining was also visualized in the area corresponding to or surrounding the ependymal cells layer. The choroid plexus and cerebellum (at the level of the Purkinje cell layer) were labeled as well as the meninges, albeit to a lesser extent (not shown). In the brain of 1-day-old rat, staining was weak in the hippocampus, piriform, and postcingulate cortex, but increased up to day 7, when it peaked, and then decreased progressively.

In the hippocampus, both the dentate gyrus and Ammon's horn were labeled (Fig. 3, A and E). However, staining was clearly most prominent in the granule cell layer of dentate gyrus. In Ammon's horn, staining was located at the level of the pyramidal cell layer and spatially decreased from the subfield CA4 to the subfield CA1. In piriform and postcingulate cortex, cortical layers 2–3 were the most stained in a pattern of adjoining spots, whereas in the fiber layer above no staining was detected (Fig. 3, F and G). In the deeper layers of the cortex (piriform and postcingulate cortex) and in the white matter below staining was observed; overall it was much less than in layers 2–3, but contained a few intense spots (Fig. 3C) not corresponding to vessels. In the hypothalamus, only some nuclei were labeled (Fig. 3H). In the brains of adult rats, these regions were not stained.

Combined TSH receptor mRNA and GFAP labeling in brain

As some astrocytes in primary culture expressed the TSH receptor gene, we studied whether this was also the case in brain. Using laser confocal microscopy, TSH receptor mRNA was detected in the surrounding region of the hippocampus (Figs. 3A and 4A). Whereas many GFAP-positive cells were detected in this area (Fig. 4B), only a few of them expressed the TSH receptor gene (Fig. 4, C and D). Hence, most of the GFAP-positive cells were negative for the expression of TSH receptor mRNA (Fig. 4, B–D). The same result was observed in the areas beneath the piriform and postcingulate cortex (not shown).

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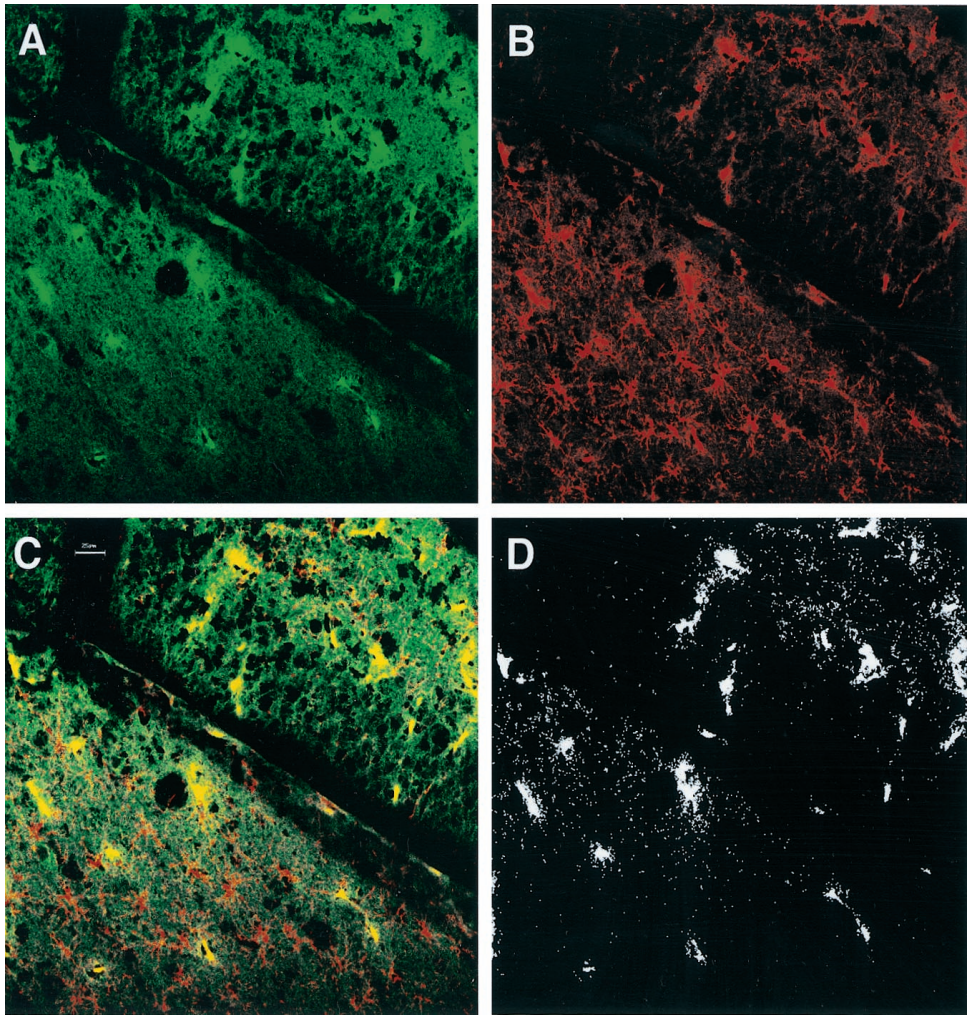

FIG. 4. Combined detection of TSH receptor mRNA and GFAP in rat brain. Rat brain cryoslices (hippocampus area) were incubated with both TSH receptor antisense riboprobe and anti-GFAP antibody. Hybridization/binding were revealed as described in Fig. 2. Then laser confocal microscopy analysis was performed in both fluorescein isothiocyanate (A) and rhodamine (B) wavelength channels. C, Superposition of A and B; D, colocalization pattern (see *Materials and Methods*). *Scale bar*, 25 μm (C).

Combined TSH receptor mRNA and NSE labeling in brain

In the brain, the staining distribution suggested that the TSH receptor gene is expressed mainly within or in the neighborhood of neuron bodies. We therefore performed iterative labeling *in situ* experiments to determine whether *in vivo* the TSH receptor mRNA was colocalized with either NSE, a marker of neuronal cell bodies, or neurofilament, a marker of axons. Using laser confocal microscopy (Fig. 5), staining was detected in NSE-expressing cells in both piriform cortex (Fig. 5, A–D) and hippocampus (Fig. 5, E–L), suggesting that TSH receptor mRNA was indeed located within neuronal cells.

In the dentate gyrus many, but not all, cells expressing the TSH receptor gene were NSE positive (Fig. 5, E–H). This contrasted with the Ammon's horn's area where only a few cells were doubly labeled, but most were singly labeled (Fig. 5, I–L). Moreover, TSH receptor mRNA was not colocalized with axon-specific neurofilament (Fig. 5, M–O), suggesting that it was confined to the neuronal cell bodies (Fig. 5, P–R). We also noticed labeling consistent with the expression of the TSH receptor gene in surrounding blood vessels (Fig. 5, A–D), but it was not colocalized with other labeling.

Combined labeling in the ependymal cell and subventricular area

Interestingly, TSH receptor mRNA was detected in the ependymal cells layer, where coexpression with GFAP was sometimes detected, albeit at very low level (Fig. 6, A–D). It is, however, beneath the ependymal cell layer, in the subventricular zone, that dual labeling was mainly detected (Fig. 6, A–D). Moreover, in the cells of this area that expressed NSE (Fig. 6, E–G) or axon-specific neurofilament (not shown), TSH receptor mRNA was not detected. In the deeper structures of the brain, except for isolated cells, GFAP-positive cells generally did not express the TSH receptor gene (Fig. 6, A–D).

Expression of TSH receptor protein in human primary cultured astrocytes

In this work we also sought TSH receptor expression in primary cultured human astrocytes (31). After RT, PCR experiments allowed us to detect two overlapping PCR products corresponding to the full length of the human TSH receptor cDNA (3–5) (not shown). This suggested that the TSH receptor gene is also expressed in human astrocytes.

We further tested whether the TSH receptor itself could be

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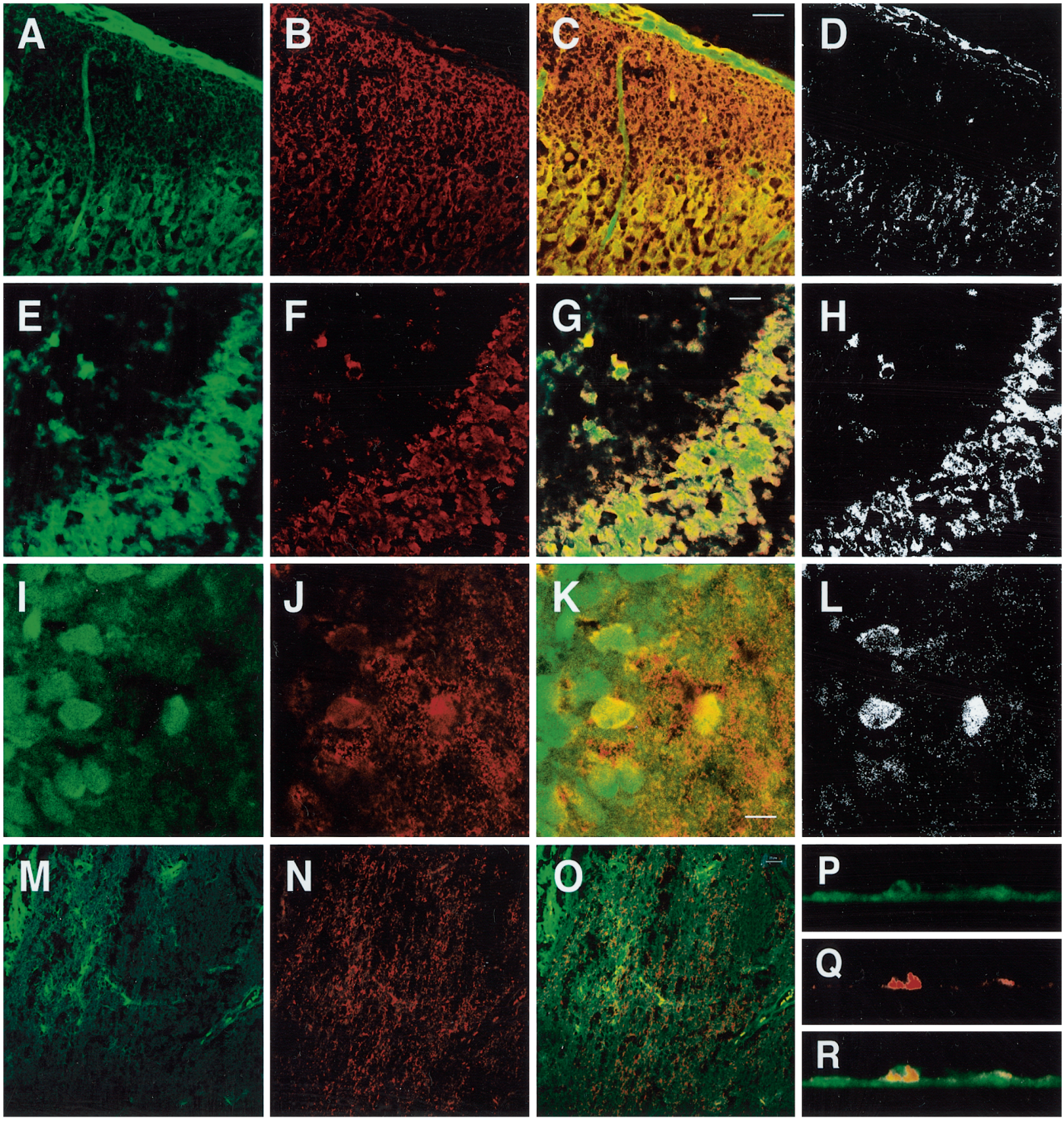

FIG. 5. Combined detection of TSH receptor mRNA and NSE in rat brain. Rat brain cryoslices were incubated with both TSH receptor antisense riboprobe (A, E, I, M, and P) and anti-NSE (B, F, J, and Q) or antineurofilament (N) antibody, then analysis was performed as described in Fig. 4. A–D, Piriform cortex; E–H, dentate gyrus; I–L, Ammon's horn; M–O, fasciculus of nerve fibers; P–R, Ammon's horn cells (transversal section analysis). Superpositions: C = A + B; G = E + F; K = I + J; O = M + N; R = P + Q. D, H, and L, Analysis of the colocalization patterns. *Scale bars*, 25 μm (C, G, and O) or 10 μm (K).

detected in these cells. Figure 7A shows that several astrocytes were labeled using antihuman TSH receptor antibody (provided by Edwin Milgrom), whereas no labeling was detected with nonspecific antibody (Fig. 7B). This labeling was observed with three different antibodies, each directed against a distinct part of the extramembrane subunit of the human TSH receptor (34). This staining was abolished when the anti-TSH receptor antibody was first incubated with the

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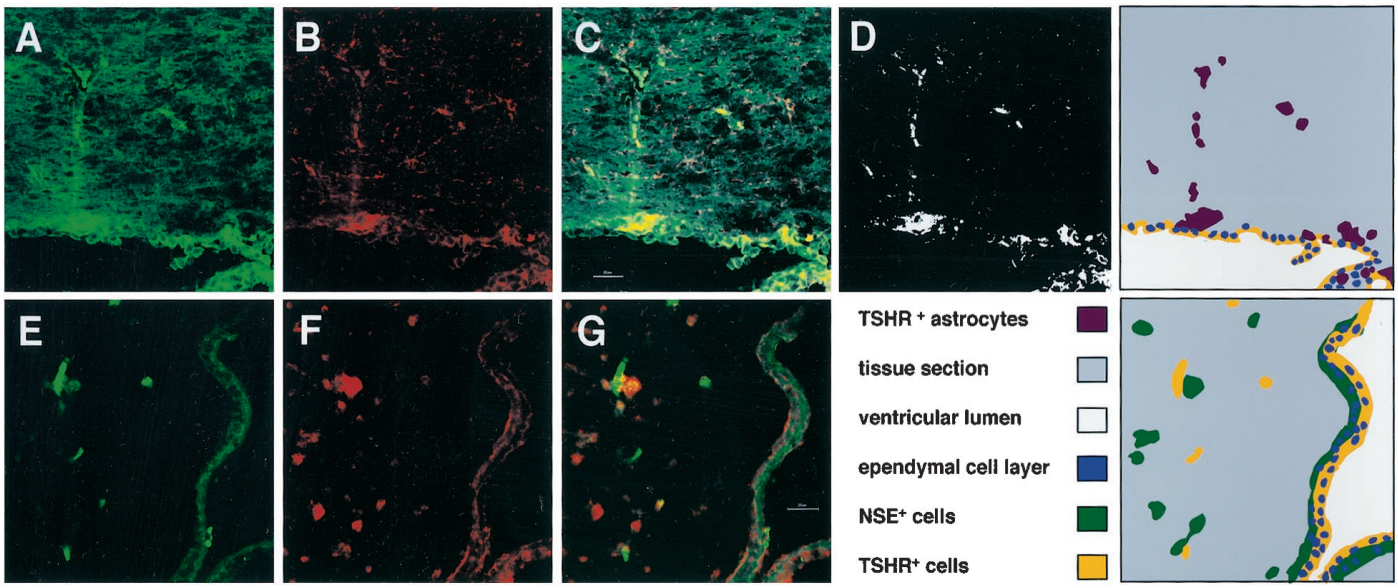

FIG. 6. Detection of TSH receptor mRNA in ependymal area. Brain cryoslices of the paraventricular area were incubated with TSH receptor antisense riboprobe (A and E) and anti-GFAP (B) or anti-NSE (F) antibody, then analyzed as described in Fig. 4. Superpositions: C = A + B, G = E + F. D, Analysis of the colocalization pattern. *Scale bars*, 25 μm (C and D). The diagrams in the *right panels* show the localization of various cell types in the corresponding tissue sections.

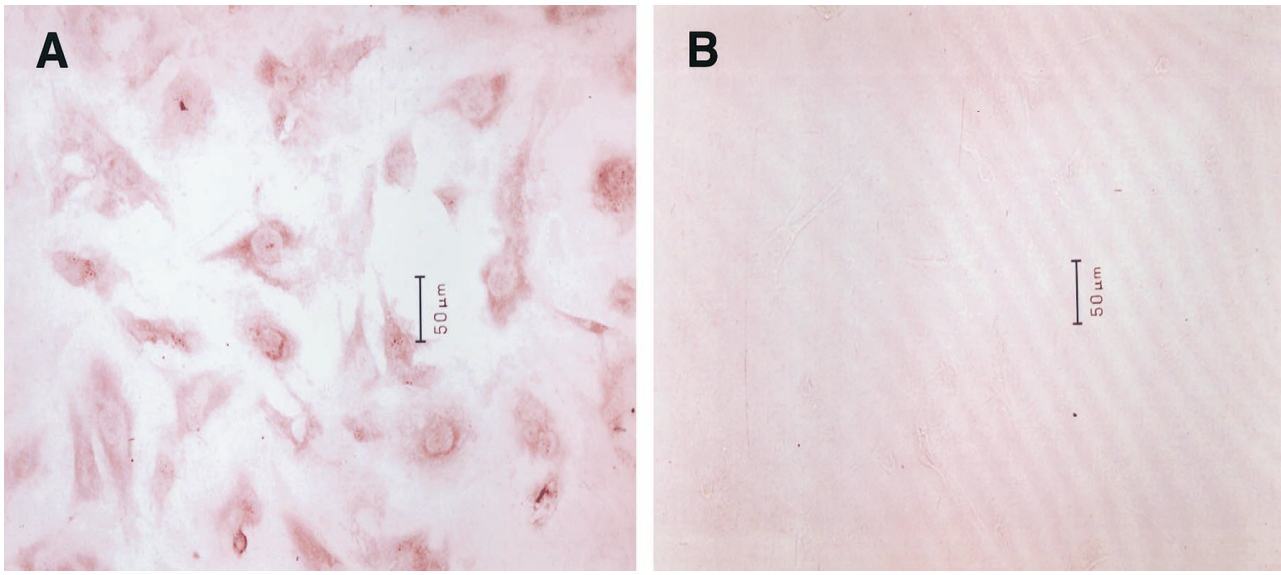

FIG. 7. Expression of the TSH receptor in human primary cultured astrocytes. Cultured astrocytes were fixed with ice-cold acetone, then incubated with either nonspecific (preimmune mouse IgG1 immunoglobulin; B) or anti-TSH receptor antibody (TSHR34; A). Binding was detected with peroxidase-coupled antibody and peroxidase-catalyzed color reaction (A and B). Preincubation of TSHR34 antibody with purified extracellular domain of the TSH receptor gave a result similar to that obtained in A.

purified extramembrane subunit of the human TSH receptor. Together these data suggested that the observed labeling was specific, and therefore that TSH receptor was indeed present in primary cultured human astrocytes.

We then looked for such an expression in both rat and human tissues in thyroid and brain. Experiments were performed to demonstrate that anti-TSH receptor antibody cross-reacted between human and rat thyroid (Fig. 8, A–D and G). Then, rat and human brain cryoslices were incubated with anti-TSH receptor antibody. Albeit stronger in humans, which may be explained by a higher affinity of this antibody for the human TSH receptor compared with that in the rat, a positive signal was detected in both cases (Fig. 8, E and F, and H and I). This demonstrates that the TSH receptor transcript detected in the brain is translated *in vivo* as well.

Discussion

The identification of the TSH receptor in nonthyroid tissue and the finding of TSH in brain and cerebrospinal fluid were reported several years ago. We had previously reported the existence of TSH-binding sites in primary cultured rat as-

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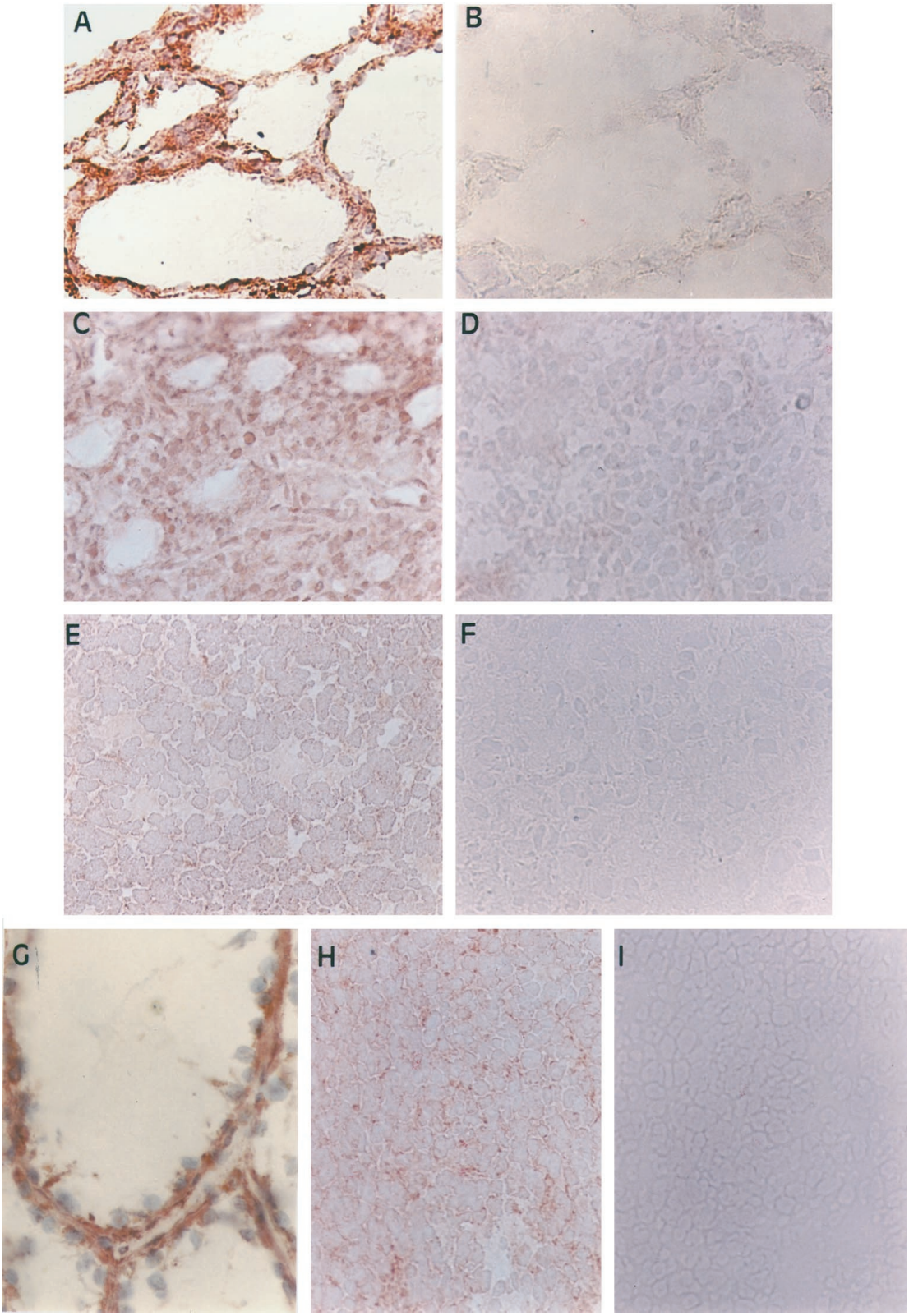

FIG. 8. Detection of the TSH receptor protein in both rat and human brain and thyroid tissue. A and B, Serial sections of adult rat thyroid gland immunostained with 2 μg/ml of the anti-TSH receptor antibody TSHR34 (A) or preimmune mouse IgG1 Ig (B; negative control). C and D, Consecutive sections of 8-day-old rat thyroid gland immunostained with TSHR34 antibody (C) or IgG1 Ig (D; negative control). E and F, Consecutive sections of 8-day-old rat cerebral cortex immunolabeled with TSHR34 antibody (E) or control IgG1 Ig (F; magnification, ×400). G, Adult human thyroid gland immunostained with antibody TSHR34 (magnification, ×1000). H and I, Consecutive sections of human embryo cerebral cortex immunolabeled with TSHR34 antibody (H) or control Ig (I; magnification, ×400).

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troglial cells (23), suggesting the expression of TSH receptor in these cells.

The possibility that TSH receptor was expressed in cultured astrocytes has been previously considered by others (35). Although adenylyl cyclase was functional in these cells and effectively coupled to other G protein-coupled receptors, the lack of stimulation of cAMP production by TSH led some authorities to believe that no functional TSH receptor was expressed in astrocytes (35). Nevertheless, our previous findings in astroglial cells were discordant with the interpretation that an absence of stimulation of cAMP production necessarily meant an absence of TSH receptor. Indeed, in these cells, we identified several TSH effects that appeared to be independent of stimulation of the cAMP pathway and yet still specific for this hormone (23, 25). Thus, all of these TSH effects were specifically blocked by monoclonal anti-TSH antibody, monoclonal anti-idiotypic anti-TSH antibody (36, 37), polyclonal anti-TSH receptor peptide antibody (38), and human Igs containing a high level of anti-TSH receptor-blocking activity (39, 40).

It is unlikely that the product of another TSH receptor gene could explain the uncoupling of adenylyl cyclase from the TSH receptor observed in astroglial cells (23). Indeed, according to the similar dimeric structure of the pituitary glycoprotein hormones (TSH, LH, and FSH) (41), the high sequence homology of the cDNAs (42), and the very close spatial structure (43) of their receptors, for evolutionary reasons (41) these striking similarities make unlikely the existence of another gene coding for a high affinity TSH receptor that would be completely different from that already identified. Lower affinity binding sites were also found in cultured astroglial cells (23), as also described in thyroid (44, 45). The concentration of TSH required to produce specific effects in astroglial cells (23, 25) tends, however, to indicate that high affinity binding sites mediate the effects of TSH. The existence of alternative spliced variants of the TSH receptor transcript has been observed in thyroid (46). In fact, we found that the TSH receptor cDNA in primary cultured rat astroglial cells was identical to that of the main type in rat thyroid cells. *A priori*, this result excluded that an alternate splicing of the TSH receptor pre-mRNA explains the change in functional coupling in astrocytes. Processing of the TSH receptor depends on the type of cell in which it is expressed (47). Such posttranslational differences might tentatively explain the uncoupling of this receptor from adenylyl cyclase in astrocytes.

Using a riboprobe corresponding to the TSH receptor gene, the expression of which was previously identified in thyroid and now in cultured astrocytes, we observed two main types of localization. One type of localization was transient, in neuronal cells, and mostly found in the dentate gyrus of the hippocampus. This was established by detection of TSH receptor mRNA in NSE-positive cells, with laser confocal microscopy analysis. The other localization was in astrocytes, in accordance with our previous reports. The latter expression was mostly detected in the ependymal and subventricular areas. This restriction of expression is, perhaps, comparable to our present observation of primary cultured rat and human cells that suggests that TSH receptor expression is confined to particular astrocytes.

Albeit as yet unknown, the pattern of this expression may suggest various physiological roles. In a previous report we formulated the hypothesis that TSH receptor expression in the brain may be involved in local thyroid homeostasis when we reported the stimulation of type II iodothyronine deiodinase activity with TSH in astrocytes (23). This is consistent with the pattern of expression of type II iodothyronine deiodinase in the brain, predominating in astrocytes and in tanycytes of the ependymal area (48). In addition, the description by others of TSH receptor gene expression in sheep hypothalamus (49) may be consistent with a neuroendocrine feedback of TSH upon TRH release or expression, for example.

The pattern of expression suggests additional physiological roles. In the dentate gyrus, where TSH receptor gene expression in neuronal cells culminated, neuronal precursors have been observed in rodents (50, 51). Progenitor cells, which can ultimately differentiate into both neuronal and astroglial cells (52), are found in the central system during development. Most precursors migrate toward their final location, and they fully differentiate (53). Whereas astroglial cells are well known to keep a potential for proliferation in adult brain, neurons were thought, until recently (50, 51), to have lost this potential. Moreover, enhancement of the TSH receptor transcript level in the brain coincided with a dramatic rise in thyroid hormone β-receptor expression (54, 55). The thyroid system is implicated in neural development within the neonatal period (56). It was therefore tempting to link the enhancement of expression of TSH receptor to that of thyroid hormone β-receptor, perhaps in relationship with the commitment of neuronal precursors in the brain at birth.

Finally, further reports have appeared recently in the literature, consistent with the persistence of stem cells in the central nervous system of adult mammals (57–59). It is generally accepted that embryonic stem cells are initially located within the inner part of the central nervous system. Later, this part will give rise to the ependymal and subventricular areas. As one might expect, stem cells in the adult were found mostly within the ependymal cells layer (57) and/or astrocytes of the subventricular zone (58). Whether astroglial and neuronal intermediary precursors are obligatory steps in their ultimate differentiation process is still under debate (59). Coincidentally, in our study TSH receptor gene expression in NSE-negative cells culminated in those latter areas. Although not yet proved, our observations are consistent with an expression in brain precursor and/or stem cells. This hypothesis would satisfactorily explain the prominent labeling of neuronal cells in the dentate gyrus, the staining detected in ependymal cells and astrocytes of the subventricular zone, and the existence of isolated GFAP-positive cells that might correspond to migrating cells.

The factors allowing TSH receptor expression in the brain remain unknown. Interestingly, TSH receptor gene expression in the hippocampus and cortex was regulated throughout the neonatal period (our data) and correlated positively with the level of circulating TSH in serum (60). In humans, a peak of TSH is also detectable in serum after birth, although it lasts only a few hours (61). In thyroid, TSH-induced regulation of TSH receptor expression has been reported (6), suggesting that TSH may also regulate TSH receptor expression in the brain. In the thyroid, this regulation was, however,

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cAMP dependent; such a mechanism was excluded in astrocytes (23). Nevertheless, other processes have been implicated in TSH receptor expression in the thyroid, such as TTF-1 transcription factor (1). Albeit its expression in the thyroid gland is well known, TTF-1 is, in fact, also important during brain development (62). Moreover, its activity was reported to be regulated in a cAMP-independent manner in nonthyroid tissue (63). Therefore, this left open the possibility for TSH to regulate this expression through TTF-1 in astrocytes also. The presence of TSH or TSH-like peptide in the brain and cerebrospinal fluid that has been reported in rats and primates (21) and in humans (20) might, therefore, be of physiological relevance.

Taken together, our results demonstrate the expression of the TSH receptor gene in both neurons and astrocytes in the brain. Whether TSH receptor expression in the brain is related to the commitment of precursor or stem cells after birth and with the increased level of TSH observed in the serum or plays a role later, as suggested by the more frequent occurrence of neurodegenerative disorders during hypothyroidism with elevated level of circulating TSH (64) or by the description of enhanced TSH receptor expression in Alzheimer's disease (65), are questions remaining to be addressed.

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