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Cloning and Expression of a Brain-Derived TSH Receptor

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Several hormones not only regulate the activity of endocrine cells and non-endocrine tissues but also serve as neuronal transmitters or modulators of neuronal activity. Accordingly, the expression and physiological significance of hormonal receptors in the central nervous system (CNS) could be demonstrated for a whole set of hormones (e. g. hCG/LH, GH, T3, CRF, TRH). The G-protein coupled TSH receptor is densely expressed in the thyroid gland and mediates the production and secretion of thyroid hormones. Not all TSH effects, especially in neurological and psychiatric disease states, can readily be explained by the action of the hormone on the thyroid gland and/or TRH levels. Therefore, it has been suggested that TSH might exert its effects directly in the CNS, although no direct proof for a TSH receptor in the human brain has been provided yet. Here we describe the cloning of a TSH receptor from an ovine hypothalamic cDNA library that is similar to thyroid derived cDNA clones. The comparison of amino acid sequences indicates that several protein domains important for the function and activity of the receptor are highly conserved. RT-PCR and RNA protection assay demonstrated that the TSH receptor mRNA is widely expressed throughout the ovine brain. The expression of a TSH receptor in the CNS indicates that TSH is not only a hormonal messenger for the thyroid gland but can also act directly in the brain. Further studies should focus on the physiological role of TSH in the CNS and the regulation of TSH receptor expression in the mammalian brain.

The hypophyseal hormone thyrotropin (Thyroid Stimulating Hormone: TSH) plays a crucial role in regulating the function of the thyroid gland via the hypothalamo-pituitary axis modulating both (i) the secretion of the thyroid hormones—triiodothyronine (T3) and thyroxine (T4)—and (ii) the gland trophism (For review see: 1, 2). These effects of TSH are mediated by a specific membrane bound receptor on the thyroid cell, TSH receptor (TSHR) (3), which is a member of the guanine nucleotide binding protein (G-protein) coupled family of receptors with seven transmembrane domains. The signal transduction cascade is coupled to two intracellular second messenger systems. The activation of adenylate cyclase by a G_S-protein (4, 5) is the predominant pathway whereas the inositol phosphate (IP_3)/Ca^{2+} response via a G_q/G_{11}-protein plays a minor role (6-8).

After cloning of the TSHR (9, 10) much efforts have been made to elucidate the structure and function of TSHR at the molecular level (for review see 11) since autoantibodies against the TSHR are thought to be involved in the expression of autoimmune thyroid diseases and Graves' disease (for reviews see 12, 13).

Besides the thyroid gland (3) the TSHR has been localized in several tissues like peripheral lymphocytes (14), brown and white adipose tissue (15), retro-ocular fat tissue (16, 17), fibroblasts in culture (18), and in the cardiac muscle (19). Moreover, in the recent years some indirect evidence lead to the supposition that also a brain specific TSHR might exist. It could be shown that hormonal parameters in neurological and psychiatric disorders show a striking correlation between the occurrence of depression and a blunted TSH secretion response (20), a fact that could not solely be explained by a central deficiency of hypothalamic thyrotropin-releasing hormone (TRH).

Several hormones do not only regulate the biosynthesis and secretion of endocrine target cells and non-endocrine tissues but also act as neuromodulators altering neuronal activity via binding to CNS receptor sites, i. e. hCG/LH receptor (21), GH receptor (22-24), T3 receptor (for review see 25), and CRF receptor (for review see 26). The expression of a TSHR in the brain would give further evidence for a comparable role of TSH in the brain.

In this study we report the cloning of a brain derived ovine TSHR that seems to be identical to gene products from thyroid cDNA libraries. Furthermore, we show the expression pattern of the receptor in several different brain regions by RT-PCR and RNase protection assay.

MATERIALS AND METHODS

Construction of a hypothalamic cDNA library. For mRNA preparation ovine hypothalami were carefully dissected and collected in

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liquid nitrogen. Total RNA from PD and PT was isolated according to the procedure of Chomczynski and Sacchi (27) (TRIzol reagent; Gibco BRL, Life Technologies, Eggenstein, Germany). Poly(A)$^+$ RNA was separated using oligo(dT)$^+$-cellulose columns (Pharmacia Biotech, Uppsala, Sweden). 5μg poly(A)$^+$ RNA was used for cDNA synthesis employing the ZAP Express cDNA Synthesis Kit (Stratagene, Heidelberg, Germany). After size fractionation the cDNA was ligated into ZAP Express vector arms and packaged according to the manufacturer's descriptions yielding approximately 7.5×10^5 independent recombinants each.

Cloning techniques. The cloning procedure was based on successive fractionations of a hypothalamus-specific cDNA library (28) and a PCR based detection of positive clones. In brief, 1.2×10^6 phages of the cDNA library (λZAP Express; Stratagene) were incubated with 1×10^8 *E. coli* XL1 Blue MRF', mixed with melted top-agar and plated on ten 150 mm NZCYM-agar plates (29). After overnight incubation at 37°C the phages were washed from the plates with SM-buffer. Each lysat was tested for the presence of TSH-receptor cDNA by PCR [95°C for 30 s, 66°C for 30 s, 72°C for 30 s; 35 cycles; 72°C for 3 min; 20μl PCR SuperMix (Life Technologies, Eggenstein, Germany) containing Taq-polymerase, buffer, dNTP, and Mg^{2+} in a total volume of 22 μl] using DNA primers complementary to the bovine sequence (accession number: U15570; sense primer: -5′-GCCAGCGAGCTGTCTGTGTA-3′-; nucleotides 1568-1587; antisense primer: -5′-CCGGGTTGTAGTGGGGATTTC-3′-; nucleotides 1904-1884; fragment size: 337 bp) revealing 4 positive lysats. The procedure was repeated by plating ten times 1.5×10^4 phages of one positive lysat resulting in only one positive lysat that was again plated to 1.5×10^4 phages/plate. On the next day the agar disk was cut into 372 agar blocks that were put into SM-buffer, 10 % chloroform. Each 14 agar block lysats were pooled to 31 pools that were again pooled to 5 master pools. Via three successive PCRs all pools were tested for TSH-receptor cDNA. The phages of one positive agar block lysats (~40 single plaques) were plated. 100 plaques were picked with Pasteur pipets, put into SM-buffer, 10 % chloroform, pooled to ten pools à 10 single plaques, and tested by PCR as before. The resulting single plaque was in vivo excised from the λZAP Express vector to a pBK-CMV plasmid according to the manufacturers' instructions and sequenced (Thermo Sequenase flourescent labeled primer cycle sequencing kit with 7-deaza-dGTP, Amersham Buchler) in an automatic sequencer (LI-COR 4000L, MWG-Biotech).

PCR techniques. To test the selectivity of the TSHR expression, we employed the PCR methodology. One μg of total RNA was reverse transcribed (SuperScript II, Life Technologies, Eggenstein, Germany) in a total volume of 20 μl using random primers according to the manufacturers' instructions. The TSHR cDNA was detected by PCR in 0.5 μl of the transcription reaction with 50 pmoles of each primer (see fig. 1 for sequence), and 20 μl of PCR SuperMix (Life Technologies, Eggenstein, Germany) (PCR conditions: 95°C for 30 s, 66°C for 30 s, 72°C for 30 s; 35 cycles; 72°C for 3 min). Five microliters of the reaction products were separated in a 2% agarose-gel and visualized by staining with ethidium bromide.

As a control rat specific β-actin primers were used in PCR experiments (accession number V01217; sense primer: -5′-CACCTTCTACAA(C/T)GAGCTGC -3′-; nucleotides 1594-1613; antisense primer: -5′-TTCATGAGGTAGTC(A/C/G/T)GTCAG -3′-; nucleotides 2366-2347; fragment size 308 bp).

RNase protection assay. Total RNA was extracted from the selected tissues (ovine hypothalamus, cortex, cerebellum, thyroid, blood; collected in liquid nitrogen) according to the procedure of Chomczynski and Sacchi (27) (TRIzol reagent, Life Technologies, Eggenstein, Germany). 15 μg of RNA of each tissue were used for the ribonuclease protection assay (HybSpeed RPA, Ambion, USA) and hybridized with 40 ng of a TSH receptor specific complementary riboprobe (2×10^6 cpm).

The [^{35}S]-UTP labeled riboprobe was derived from an ovine TSH receptor cDNA subclone [nucleotides 1-1887; *Sma*I deletion mutant of the excised TSH receptor clone in pBK-CMV; Stratagene]. The DNA was amplified by PCR using a TSH receptor specific sense primer (-5′-GCCAGCGAGCTGTCTGTGTA-3′-; see fig. 1) and an antisense primer that binds to the vector encoded T7 RNA polymerase promotor (-5′-TAATACGACTCACTATAGGG-3′-) generating a PCR fragment of 387 bp containing 335 bp of TSH receptor sequence (nucleotides 1553-1887; see fig. 1), 32 bp vector DNA, and 20 bp encoding the T7 promotor sequence [PCR conditions: 1 ng template DNA, 50 pmoles primer, 20 μl PCR SuperMix (Life Technologies, Eggenstein, Germany) in a total volume of 22 μl; 95°C for 30 s, 66°C for 30 s, 72°C for 30 s; 35 cycles; 72°C for 3 min]. The PCR fragment was isolated from a 2% agarose gel and extracted by a silica gel matrix (Jetsorb Gel Extraction Kit, Genomed, Bad Oeynhausen, Germany). 0.2 μg of the PCR product were transcribed (RNA Transcription Kit, Stratagene) by T7 RNA polymerase for 2 hours at 37°C in the presence of 50 μCi [^{35}S]-UTP (Amersham Buchler) and 2 mM unlabeled UTP to get full length transcripts of 369 nucleotides. After DNAse I treatment (Life Technologies, Eggenstein, Germany) with 5 units for 30 min at 37°C unincorporated nucleotides were separated by size exclusion chromatography (Bio-Spin 6 Chromatography Column, BioRad). The products of ribonuclease protection were separated on a denaturing gel (5% polyacrylamide/8 M urea/1 × TBE) and exposed to β-max Hyperfilm (Amersham Buchler).

RESULTS

Cloning of the TSHR. Using a novel strategy for cloning by subsequent fractionations and PCR based detection we cloned an ovine TSHR from a hypothalamus cDNA library (fig. 1). DNA sequence analysis and comparisons between the ovine brain derived TSHR and TSHRs cloned from the thyroid glands of several other species like bovine, canine, human, murine, and rat TSHR revealed DNA sequence similarities between 97.4% (bovine) and 86.5% (rat) within the 764 amino acids, respectively (fig. 2). Interestingly the sequence identities were not randomly distributed over the entire proteins, although the ovine and bovine sequences only diverged to a little extent. The signal peptide region and the C-terminal 50 amino acids showed significant exchanges of about 25-40% between the ovine and bovine TSHRs and TSHRs of other species. The residues 303-382 belonged to a long region in the C-terminal portion of the extracellular domain whose deletion or exchange had essentially no effect on receptor function (33, 34). Besides these three regions the homologies were about 90.0-98.2%, respectively.

Localization of ovine TSH receptor by PCR and RNase protection assay. PCRs were performed with reversely transcribed RNA from thyroid gland, blood, and different neuronal tissues including hypothalamus, cerebellum, and cortex. Using specific DNA primers for amplification (see fig. 1 for DNA sequences) TSH receptor cDNAs with the correct size of 337 bp could be detected in every tested tissue (fig. 3). Control experiments with primers coding for β-actin (308 bp; for primer sequences see *PCR techniques* above) resulted in discrete bands of nearly identical intensities indicating that the same amount of intact RNA has been used in RT-PCR experiments.

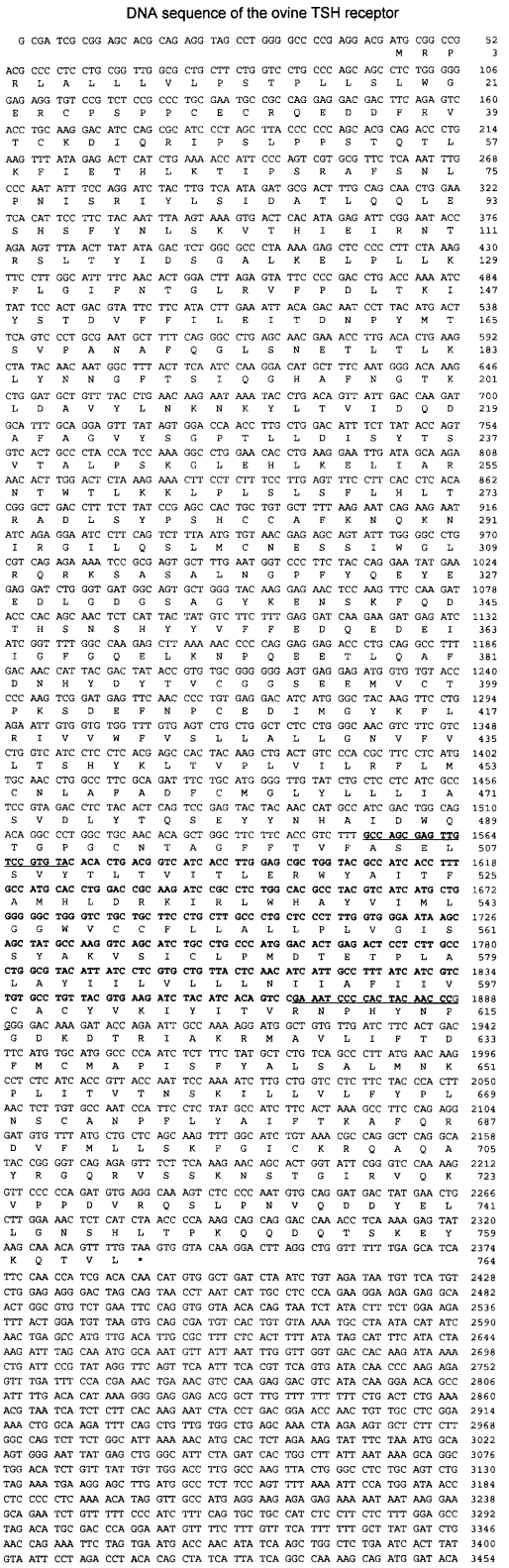

FIG. 1. Nucleotide and deduced amino acid sequence of the ovine brain derived TSH receptor. Sequences that have been used as binding sites for PCR primers are underlined, the DNA fragment used in *in vitro* transcription experiments for constructing the riboprobes is in bold face. The termination codon is indicated by a star.

In RNase protection assays the expression of the TSH receptor could be detected both in peripheral tissues like thyroid gland and blood, and in all three tested neuronal tissues (fig. 4) with almost identical intensities. Compared to the length of the hybridization riboprobe of 369 nucleotides (lane 8) the RNase treated RNA fragments were only composed of TSHR specific nucleotides (335 nucleotides; lanes 1-5) and lacked unhybridized vector sequences. The specificity of RNase treatment was tested by using the [^{35}S]-UTP labeled riboprobe in hybridization experiments without any mRNA showing no detectable band after RNase treatment (lane 6). The same experiment without RNase incubation revealed a main band at 369 nucleotides and a ladder of bands below caused by different secondary structures of the labeled probe mimicking smaller RNA fragments (lane 7).

DISCUSSION

In this study we report the cloning of an ovine TSH specific receptor gene from a hypothalamus cDNA library. Sequence analysis and the comparison of the deduced amino acid sequence with several TSHRs cloned from the thyroid gland revealed only minor sequence differences. With respect to these findings and the detection of the TSHR by RT-PCR and RNase protection assay it has to be stated that:

(i) The transcribed mRNA is an identical isoform known from the thyroid gland. The comparison of the ovine brain derived TSHR with five other receptor proteins reveals species specific average identities of 86.5% to 97.4%. Besides three regions of significant increases of divergencies, i. e. the signal peptide, the C-terminus, and a deletable portion of about 80 residues within the N-terminal extracellular loop the remaining 610 residues are highly conserved ($\geqslant$ 90%). The conserved region contains domains which are important for receptor activity, namely the TSH binding domain within the large N-terminal extracellular loop, the seven transmembrane helices, and the three intracellular loops which are responsible for mediating the TSH signal to the second messenger systems (cAMP, IP_3/Ca^{2+}). The third cytoplasmic loop (between TM5 and TM6) is mainly involved in the coupling of G_q-protein and together with the first cytoplasmic loop (between TM1 and TM2) mediates the IP_2/Ca^{2+} signal (35, 36) whereas the middle portion of the second loop (between TM3 and TM4) is most important for G_s-protein coupling (34).

In contrast to the LH/CG receptor (37) there is no evidence for alternative splicing events of the TSHR in different tissues. Although Tomer *et al.* (38) reported identical mRNAs in normal and abnormal tissues (multinodular goiter; Graves' disease) they detected additional transcripts by liquid hybridization that seem

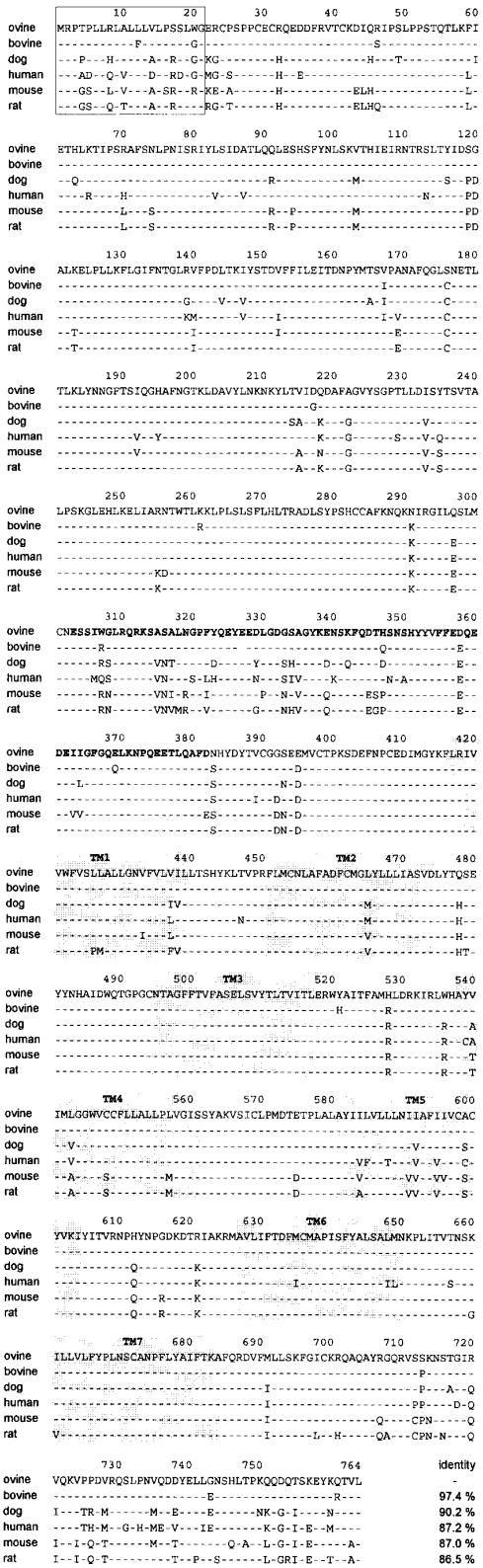

FIG. 2. Comparison of the amino acid sequences of TSHRs from the ovine (see fig. 1), bovine (accession number U15570), canine (9), human (10, 11), murine (31), and rat TSHR (32). Identical residues are compared with the entire ovine sequence (upper line) and are indicated by hyphens. The signal sequence is denoted by an open box, the seven putative transmembrane helices (TM1–7) by shaded boxes. The bold letters show a peptide fragment which could be deleted or exchanged by other residues without any change in TSHR activity.

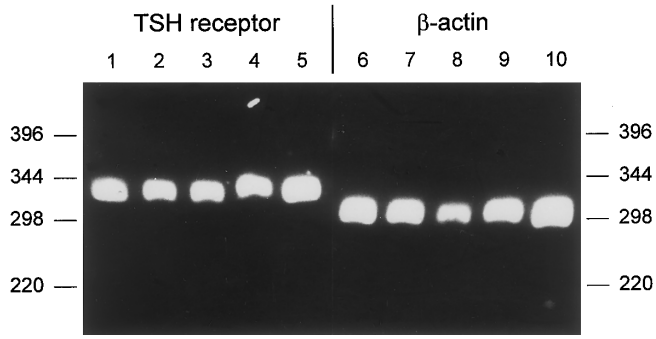

FIG. 3. Comparison of the TSH receptor expression in different ovine tissues by RT-PCR (β-actin was used as a control): lanes 1, 6, thyroid gland; lanes 2, 7, blood; lanes 3, 8, hypothalamus; lanes 4, 9, cerebellum; lanes 5, 10, cortex. 0.5 μl of the reverse transcription experiment was used as template together with 50 pmoles of each primer and 20 μl of PCR SuperMix (Life Technologies, Eggenstein, Germany) in a total volume of 22 μl. Five microliters of the β-actin PCR and eight microliters of the TSHR PCR were separated in a 2% agarose gel and visualized with ethidium bromide. The sizes of the DNA fragments are given in base pairs (1 kb DNA ladder; Life Technologies, Eggenstein, Germany).

to lack physiological importance. Until now only one alternatively spliced form could be detected (39). This human TSHR cDNA encodes a protein of 253 amino acids for the N-terminal half of the extracellular domain containing exons 1-8 and an additional unidentified DNA tract, presumably an intron. The authors postulated that this truncated TSHR might be secreted and functions as a TSH binding protein.

(ii) The TSHR is localized in several brain areas, at least in the hypothalamus, cerebellum, and cortex. As shown by RT-PCR and RNase protection assay the signal intensitites between the thyroid gland and the different brain areas are almost identical. Provided the prominent expression of the TSHR mRNA in the brain is correlated with the mRNA and protein expression in the thyroid gland, a yet unknown role of the TSHR in central nervous structures is likely.

In analogy to other hypophyseal hormones like LH/CG (21) and GH (22-24) which bind to receptors localized in the brain, the TSHR could also play a role in an additional feedback mechanism on neuroendocrine control of the thyroid gland. Such a role could be related to sensing the actual TSH serum concentrations in the hypothalamus thereby controlling TRH secretion through both thyroid hormone and TSH levels.

RT-PCR experiments, however, showed that the localization of the TSHR was not only restricted to the hypothalamus. The signal intensities of the detected

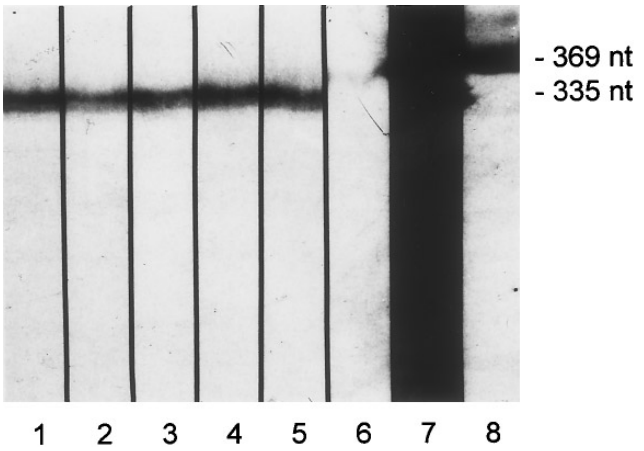

FIG. 4. RNase protection assays of different tissue specific RNAs (lane 1, thyroid gland; lane 2, blood; lane 3, hypothalamus; lane 4, cerebellum; lane 5, cortex). 15 μg of total RNA was hybridized with the [^{35}S]-UTP labeled TSH receptor specific riboprobe and processed according to the manufacturers' instructions (Ambion, USA). Lanes 6, 7, hybridization of [^{35}S]-UTP labeled riboprobe without any mRNA with (lane 6) and without RNase treatment (lane 7). Lane 8, 100 fold dilution of [^{35}S]-UTP labeled riboprobe.

PCR products from cortex and cerebellum were comparable to the control RT-PCR using mRNA from the thyroid gland and blood. These data were verified by RNase protection assays which also detected the TSHR mRNA in neuronal tissues besides thyroid gland and blood.

Indirect evidence has been presented for TSHR expression in other tissues than the thyroid gland. Van Renterghem *et al.* (40) showed that the nuclear phosphoprotein Pax 8 acts as a transcription factor in positively regulating the expression of both thyroglobulin (Tg) and thyroperoxidase (TPO) by TSH, although no typical cAMP-responsive element has been detected in the promotor regions of Tg and TPO genes. Since Pax 8 has been localized in thyroid, ovary, placenta, and embryonic brain (41, 42) it is tempting to speculate that TSH could act on brain cells by binding to the TSHR and regulating the expression of other genes via the activation of Pax 8.

Another hint for a possible role of TSHR in the CNS came from Hosaka *et al.* (43). The authors investigated the expression and regulation of glucose transporter mRNAs in FRTL-5 cells by TSH. The increase in a glucose transporter isoform mRNA, GLUT1, by TSH was correlated with the increase in GLUT1 protein and the increase in 2-deoxyglucose transport activity. This effect could be mimicked by forskolin indicating that TSH acts via the TSHR on cAMP-dependent pathways. Interestingly, the cDNA of GLUT1 has been cloned from a cDNA library prepared from adult rat brain (44). Until now three brain specific GLUTs have been cloned (GLUT1, 3, 5): GLUT1 was detected in whole brain as two molecular mass forms of 55kDa in cerebral microvessels (45-47) and 45kDa in parenchymal cells (48, 49), GLUT3 in neurons (48, 50), and GLUT5 in microglia (51). In a recent publication Morgello and coworkers (52) could show by electronmicroscopy that in humans and monkeys the GLUT1 glucose transporter is localized in the CNS endothelium and in gray matter astrocytes forming part of the gray matter blood-brain barrier. By virtue of its location this transporter seems to be involved in postendothelial pathways of glucose and glucose metabolite transport both to the astrocytic and neuronal compartments. This supports the hypothesis that TSH could directly regulate and facilitate the uptake of glucose into cells of the CNS where glucose is the principal energy source. A continous supply of this substrate is essential to maintain normal neuronal function. In this respect, the proposed action of the TSHR in the CNS has to be examined by generating TSHR specific antibodies and a thorough analysis of its expression in different CNS cell types.

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REFERENCES

1. Kohn, L. D., Saji, M., Akamizu, T., Ikuyama, S., Isozaki, O., Kohn, A. D., Santisteban, P., Chan, J. Y., Bellur, S., Rotella, C. M., Alvarez, F. V., and Aloj, S. M. (1989) *in* Control of the Thyroid Gland. Advances in Experimental Medicine and Biology (Ekholm, R., Kohn, L. D., and Wollman, S. H., Eds.), Vol. 38, pp. 363–378, NATO ASI Series, Springer-Verlag, Berlin.
2. Dumont, J. E., Lamy, F., Roger, P., and Maenhaut, C. (1993) *Physiol. Rev.* **72,** 667–697.
3. Nagayama, Y., and Rapoport, B. (1992) *Mol. Endocrinol.* **6,** 145–156.
4. Yamashita, K., and Field, J. B. (1970) *Biochem. Biophys. Res. Commun.* **40,** 171–178.
5. Wolff, J., and Jones, A. B. (1971) *J. Biol. Chem.* **246,** 3939–3947.
6. Weiss, S. J., Philp, N. J., and Grollman, E. F. (1984) *Endocrinology* **114,** 1108–1116.
7. Philp, N. J., and Grollman, E. F. (1986) *FEBS Lett.* **202,** 193–196.
8. Corda, D., Marcocci, C., Kohn, L. D., Axelrod, J., and Luini, A. (1985) *J. Biol. Chem.* **260,** 9230–9236.
9. Parmentier, M., Libert, F., Maenhaut, C., Lefort, A., Gerard, C., Perret, J., Van Sande, J., Dumont, J. E., and Vassart, G. (1989) *Science* **246,** 1620–1622.
10. Nagayama, Y., Kaufman, K. D., Set, P., and Rapoport, B. (1989) *Biochem. Biophys. Res. Commun.* **165,** 1184–1190.
11. Kohn, L. D., Shimura, H., Shimura, Y., Hidaka, A., Giuliani, C., Napolitano, G., Ohmori, M., Laglia, G., and Saji, M. (1995) *Vitam. Horm.* **50,** 287–384.
12. Akamizu, T., Kohn, L. D., and Mori, T. (1995) *Endocr. J.* **42**(5), 617–627.

13. Kosugi, S., Sugawa, H., and Mori, T. (1996) *Endocr. J.* **43**(6), 595–604.
14. Francis, T., Burch, H. B., Cai, W., Lukes, Y., Peele, M., Carr, F. E., Wartofsky, L., and Burman, K. D. (1991) *Thyroid* **1**(3), 223–228.
15. Roselli-Rehfuss, L., Robbins, L. S., and Cone, R. D. (1992) *Endocrinology* **130,** 1857–1861.
16. Endo, T., Ohno, M., Kotani, S., Gunji, K., and Onaya, T. (1993) *Biochem. Biophys. Res. Commun.* **190,** 774–779.
17. Feliciello, A., Porcellini, A., Ciullo, I., Bonavolontà, G., Avvedimento, E. V., and Frenzi, G. (1993) *Lancet* **342,** 337–338.
18. Mengistu, M., Lukes, Y. G., Nagy, E. V., Burch, H. B., Carr, F. E., Latrivi, S., and Burman, K. D. (1994) *J. Endocrinol. Invest.* **17,** 437–441.
19. Drvota, V., Janson, A., Norman, C., Sylvén, C., Häggblad, J., Brönnegard, M., and Marcus, C. (1995) *Biochem. Biophys. Res. Commun.* **211**(2), 426–431.
20. Souêtre, E., Salvati, E., Wehr, T. A., Sack, D. A., Krebs, B., and Darcourt, G. (1988) *Am. J. Psychiatry* **145,** 1133–1137.
21. Lei, Z. M., Rao, Ch. V., Kornyei, J. L., Licht, P., and Hiatt, E. S. (1993) *Endocrinology* **132,** 2262–2270.
22. Lanzi, R., and Tannenbaum, G. S. (1992) *Endocrinology* **130,** 780–788.
23. Waters, M. J., Barnard, R., Lobie, P. E., Lim, L., Hamlin, G., Spencer, S. A., Hammonds, R. G., Leung, D. W., and Wood, W. I. (1990) *Acta. Paediatr. Scand.* **366,** 60–72.
24. Fraser, R. A., Attardo, D., and Harvey, S. (1990) *J. Mol. Endocrinol.* **5,** 231–238.
25. Glauser, L., and Walter, B. I. (1997) *J. Neuroendocrinol.* **9,** 217–227.
26. De Souza, E. B. (1995) *Psychoendocrinol* **20**(8), 789–819.
27. Chomczynski, P. I., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
28. Bockmann, J., Böckers, T. M., Vennemann, B., Niklowitz, P., Müller, J., Wittkowski, W., Sabel, B., and Kreutz, M. R. (1996) *Endocrinology* **137,** 1804–1813.
29. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
30. Libert, F., Lefort, A., Gerard, C., Parmentier, M., Perret, J., Ludgate, M., Dumont, J. E., and Vassart, G. (1989) *Biochem. Biophys. Res. Commun.* **165,** 1250–1255.
31. Stein, S. A., Oates, E. L., Hall, C. R., Grumbles, R. M., Fernandez, L. M., Taylor, N. A., Puett, D., and Jin, S. (1994) *Mol. Endocrinol.* **8**(2), 129–138.
32. Akamizu, T., Ikuyama, S., Saji, M., Kosugi, S., Kocak, C., McBridge, O. W., and Kohn, L. D. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5677–5681.
33. Wadsworth, H. L., Chazenbalk, G. D., Nagayama, Y., Russo, D., and Rapoport, B. (1990) *Science* **249,** 1423–1425.
34. Kosugi, S., and Mori, T. (1995) *Endocr. J.* **42**(5), 587–606.
35. Lefkowitz, R. J., and Caron, M. G. (1988) *J. Biol. Chem.* **263,** 4993–4996.
36. Kjelsberg, M. A., Cotecchia, S., Ostrowski, J., Caron, M. G., and Lefkowitz, R. J. (1992) *J. Biol. Chem.* **267,** 1430–1433.
37. Frazier, A. L., Robbins, L. S., Stork, P. J., Sprengel, R., Segaloff, D. L., and Cone, R. D. (1990) *Mol. Endocrinol.* **4**(8), 1264–1276.
38. Tomer, Y., Graves, P. N., Jin, A., Schwartz, A. E., Friedman, E. W., and Davies, T. F. (1993) *Thyroid* **3**(3), 219–224.
39. Takeshita, A., Nagayama, Y., Fujiyama, K., Yokoyama, N., Namba, H., Yamashita, S., Izumi, M., and Nagataki, S. (1992) *Biochem. Biophys. Res. Commun.* **188**(3), 1214–1219.
40. Van Renterghem, P., Vassart, G., and Christophe, D. (1996) *Biochim. Biophys. Acta.* **1307,** 97–103.
41. Poleev, A., Fickenscher, H., Mundlos, S., Winterpacht, A., Zabel, B., Fidler, A., Gruss, P., and Plachov, D. (1992) *Development* **116,** 611–623.
42. Kozmik, Z., Kurzbauer, R., Dörfler, P., and Busslinger, M. (1993) *Mol. Cell. Biol.* **13,** 6024–6035.
43. Hosaka, Y., Tawata, M., Kurihara, A., Ohtaka, M., Endo, T., and Onaya, T. (1992) *Endocrinology* **131,** 159–165.
44. Birnbaum, M. J., Howard, C. H., and Rosen, O. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5784–5788.
45. Dick, A. P. K., Harik, S. I., Klip, A., and Walker, D. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7233–7237.
46. Kasaniki, M. A., Jessen, K. R., Baldwin, S. A., Boyle, J. M., Davies, A., and Gardiner, R. M. (1989) *Histochem. J.* **21,** 47–51.
47. Gerhart, D. Z., LeVasseur, R. J., Broderius, M. A., and Drewes, L. R. (1989) *J. Neurosci. Res.* **22,** 464–472.
48. Bondy, C. A., Lee, W.-H., and Zhou, J. (1992) *Mol. Cell. Neurosci.* **3,** 305–314.
49. Lee, W.-H., and Bondy, C. A. (1993) *Endocrinology* **133,** 2540–2544.
50. Nagamatsu, S., Kornhauser, J. M., Burant, C. F., Seino, S., Mayo, K. E., and Bell, G. I. (1992) *J. Biol. Chem.* **267,** 467–472.
51. Payne, J., Mattiaci, L. A., Maher, F., Simpson, I. A., and Davies, P. (1993) *Soc. Neurosci. Abstr.* **19,** 1042.
52. Morgello, S., Uson, R. R., Schwartz, E. J., and Haber, R. S. (1995) *Glia* **14**(1), 43–54.