Expression of Somatostatin Receptors 1 and 2 in Human Choroid Plexus and Arachnoid Granulations

Implications for Idiopathic Intracranial Hypertension

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Objective: To investigate the localization of somatostatin receptor types 1 and 2 in human choroid plexus (CP) and arachnoid granulations (AGs) by immunohistochemistry.

Methods: A prospective study was performed in an institutional setting. Immunohistochemistry was performed on 15 samples of CP and 12 samples of AGs from 15 patients who died with no signs or symptoms of intracranial disease (age range, 52-81 years). The CP samples were dissected from the lateral ventricles and AG samples were dissected from the superior sagittal sinus.

Results: We demonstrated the presence of both somatostatin receptor types 1 and 2 in all samples of normal human CP and AGs.

Conclusions: Analogous to their demonstration and to their function in kidney and ocular tissues, these receptors may be involved in the processes of cerebrospinal fluid production and absorption, and may play a role in the increased intracranial pressure of idiopathic intracranial hypertension.

Clinical Relevance: Somatostatin analogues have been used to treat idiopathic intracranial hypertension, a disorder of cerebrospinal fluid homeostasis. Data are scarce regarding the cell-specific distribution of somatostatin receptors in normal human CP and AGs, the sites of cerebrospinal fluid production and egress.

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The purpose of this study was to investigate the localization of SSTR1 and SSTR2 in human CP and AGs by immunohistochemistry. These findings are discussed in the context of abnormalities associated with IIH, a disorder of CSF homeostasis.

METHODS

Fifteen samples of CP and 12 samples of AGs were obtained at autopsy from 15 patients who died with no signs of intracranial disease (age range, 52-81 years). Samples of CP were dissected from lateral ventricles, and AG samples were dissected from superior sagittal sinus. Tissue samples were obtained and processed in accordance with tissue procurement protocols from The University of Iowa, Iowa City. Tissues were fixed in 4% buffered formalin, embedded in paraffin, and serially cut on a microtome at 4 µm. Tissue sections were placed on charged slides, deparaffinized in xylene, and rehydrated. The slides were placed into preheated 10% sodium citrate (Antigen Retrieval Solution; Biogenics, Napa, Calif), micro waved for 10 minutes, and left in a sealed container for 15 minutes, then washed in phosphate-buffered saline 3 times. Afterward, the slides were washed in OptiMax Wash Buffer.
Expression of SSTR1 and SSTR2 was studied by immunohistochemistry. Newly developed antibodies raised against N-terminal 57–amino acids of SSTR1 and N-terminal 45–amino acids of SSTR2 were used. Preparation of rabbit polyclonal SSTR1 antibody (Ab) and SSTR2 Ab as well as specificity testing were previously described. The slides were incubated overnight at 4°C with primary SSTR1 Ab and SSTR2 Ab (1:1000 and 1:2000 dilution in 3% bovine serum albumin in phosphate-buffered saline, respectively). Sections were washed with phosphate-buffered saline 3 times and incubated for 1 hour at room temperature with peroxidase-labeled polyclonal anti–rabbit Ab (DAKO A/S, Glostrup, Denmark). Immunohistochemical binding was visualized by incubating the tissue sections with diaminobenzidine chromogen for up to 15 minutes. The slides were then washed, counterstained, and coverslipped. Antigen competition was performed by preabsorbing SSTR1 Ab and SSTR2 Ab with corresponding truncated SSTR1 and SSTR2 proteins that were used as antigens in antigen preparation (for 3 hours at room temperature; final concentration of peptide was 50 mg/mL). Concentrations of preabsorbed Abs used for immunohistochemistry were corrected to account for dilution with blocking peptide.

RESULTS

In all 15 CP samples, intense membrane and cytoplasmic SSTR1 and SSTR2 immunoreactivity (ir) was detected on all epithelial cells and vascular endothelial cells in capillaries within individual villi (Figure 1). Equally strong SSTR1-ir and SSTR2-ir was observed in arachnoid cells, connective tissue, and dural layer cells in all AG samples (Figure 2). No immunostaining was observed on slides where primary Ab was omitted or in the presence of competing antigen (data not shown). There was no apparent difference in the intensity of immunoreactivity for either receptor subtype in CP or AGs in regard to patient age (within the age range noted).

COMMENT

The SSTR1-ir and SSTR2-ir were observed in all human CP and AG samples. Strong SSTR1-ir and SSTR2-ir in CP are consistent with the previously published results by Thoss and colleagues. They detected binding of radioactively labeled [125I]Tyr3-octreotide (binds SSTR2 and SSTR5), [125I]CGP 23996 (binds SSTR1 and/or SSTR4), and [125I]LTT-SRIF-28 (binds all types of SSTRs) in human CP. The SST analogues used in that study bind more than 1 receptor subtype. Those results were suggestive of but not conclusive for the presence of any specific SSTRs on CP. Distribution of SSTRs was not studied previously on AGs in humans or any primate species.

The exact function of SST and its receptors in CSF homeostasis in humans is unknown at present; however, the abundance of SSTRs on CP and AGs implies their involvement in the processes of CSF production and absorption. The CSF dynamics might be affected locally at the level of CP and AGs through SSTRs or systemically by modulating the levels or activity of circulating hormones (growth hormone, insulinlike growth factor [IGF] 1, insulin, leptin, etc). In addition, SSTR1 and SSTR2...
might modulate blood flow in CP capillaries and filtration in CP epithelium.

The SST axis has a role in ion transport, extracellular fluid production, and absorption in different mammalian and human tissues. Recent work has demonstrated SST effects in kidney and in the eye. Intravenous injection of SST modulates glomerular filtration rate, renal plasma flow, urine volume, and water clearance in normal human kidney. The recent work of Balster and colleagues demonstrated SSTR1 and SSTR2 gene expression as well as orderly segmental distribution of SSTR1-ir and SSTR2-ir along the glomerular and tubular system in normal human kidney tissue. Such data offer us a reasonable theoretical model for a similar role of the SST axis in CSF dynamics.

In the rabbit eye, SST inhibits aqueous production by stimulating adenylyl cyclase activity and release of intracellular calcium in nonpigmented epithelium of ciliary processes. Using reverse transcription polymerase chain reaction, Klisovic and colleagues recently detected gene expression for SSTR1, SSTR2, and SSTR4 in ciliary body in normal human eyes. In addition, SSTR1-ir and SSTR2-ir were detected on nonpigmented epithelium of ciliary processes, in marginal capillaries in ciliary processes, and on endothelial cells in trabecular meshwork. These data again suggest an analogous role for SST in aqueous dynamics of the eye, as well as the CSF.

The best studied activity of SST and its analogues in humans is the inhibition of the release of growth hormone from pituitary, which in turn results in the reduction of circulating levels of IGF-1. This is of special interest, because it is now apparent that a side effect of recombinant IGF-1 treatment in children with growth hormone receptor deficiency is a transient increase in intracranial pressure. The increased intracranial pressure resolves with complete cessation of IGF-1 treatment or reduction of IGF-1 dose by 50%. In this regard, it is important to recognize that CP epithelium has one of the highest densities of IGF-1 receptors in the human brain. These data suggest a role for high plasma IGF-1 levels in the pathogenesis of increased intracranial pressure in children.

The IGF-1 axis also has a significant role in the pathogenesis of obesity. Obese patients often have numerous hormonal abnormalities, including glucose intolerance, insulin resistance, hyperinsulinemia, low plasma levels of growth hormone, and high levels of free IGF-1 due to overproduction of IGF-1 by adipocytes. Idiopathic intracranial hypertension is a disease that predominantly affects obese women of childbearing age. Octreotide treatment in such patients might decrease levels of circulating IGF-1 but could also counteract its biological activity at the level of CP epithelium. In human retinal pigment epithelial cells (SSTR1 and SSTR2 positive), SST and octreotide inhibit biological effects of IGF-1 by inhibition of autophosphorylation of IGF-1 receptors, thereby inhibiting the IGF-1 signaling cascade.

In conclusion, we demonstrated the presence of SSTR1 and SSTR2 in normal human CP and AGs. Analogous to their demonstration and to their function in kid-
ney and ocular tissues, these receptors may be involved in the processes of CSF production and absorption in humans and may play a role in the increased intracranial pressure in IIH.

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REFERENCES


