

EXPRESSION AND LOCALISATION OF SOMATOSTATIN RECEPTOR SUBTYPE 2a IN NEUROENDOCRINE CELLS AND CELLS OF NEUROECTODERMAL ORIGIN

Akademisk avhandling

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av

Sayed Hossein (Amir) Hashemi, M.Sc.,
Department of Anatomy and Cell Biology, Göteborg University

Fakultetsopponent:

Dr. Peter F. T. Vaughan,
Reader in Neurochemistry,
King's College London, UK

Huvudhandledare:

Prof. Annica Dahlström,
Dept. of Anat. & Cell Biol.
Göteborg University

Bihandledare:

Prof. Ola Nilsson
Dept. of Pathology
Göteborg University

Avhandlingen baseras på följande delarbeten:

- I. Expression of SSR_{2(a)} receptor in neuroendocrine tumours studied by ribonuclease protection assay related to ¹¹¹In-octreotide binding. Hashemi, S. H., Benjegård, S. A., Ahlman, H., Wängberg, B., Forssell-Aronsson, E., Billig, H., and Nilsson, O., **British Journal of Surgery** (In revision).
- II. SSR_{2(a)} receptor expression and adrenergic/cholinergic characteristics in differentiated SH-SY5Y cells. Hashemi, S. H., Li, J-Y., Ahlman, H., and Dahlström, A., **Cell & Tissue Research** (Submitted).
- III. Adrenergic differentiation and SSR_{2(a)} receptor in CAD cells cultured in serum-free medium. Hashemi, S. H., Li, J-Y., Faigle, R., and Dahlström, A., **Neurochemistry International** (Accepted).
- IV. Presence of SSR_{2(a)} receptor immunoreactivity in rat ependyma and tanocytes. Hashemi, S. H., Li, J-Y., Schindler, M., and Dahlström, A., **NeuroReport** 12(9):1793-7 (2001).

Abstract

Somatostatin (SS) is an inhibitory hormone/transmitter of the secretory and proliferative responses of the widely spread target cells. SS exerts its effects via a family of G-protein-coupled receptors termed SSR₁₋₅. SSR₂ are one of the most frequently found receptors in normal cells and in neuroendocrine tumour cells. Owing to alternative splicing, SSR₂ exist in two variants with the same binding capacity; a shorter (SSR_{2(b)}) and a longer (SSR_{2(a)}) variant. SSR_{2(a)} is much more expressed than SSR_{2(b)}. Also, SSR_{2(a)} is involved in both physiological and pathophysiological processes, which makes it of interest to study. In this thesis the expression and intracellular distribution of this receptor were investigated using tumour scintigraphy and RPA, as well as biochemical and immunocytochemical methods including Western blot and confocal laser scanning microscopy. Different cell types of neuroendocrine (NE) origin or emanating from the neuroectoderm were studied.

Human NE tumours midgut carcinoid (MC) and medullary thyroid tumour (MTC), and, for comparison, breast carcinoma (BC) were studied using RPA and ¹¹¹In-DTPA-D-Phe¹-octreotide scintigraphy. SSR_{2(a)} mRNA values were higher in MC, followed by MTC and BC, in accordance with the results (T/B values) from tumour scintigraphy using ¹¹¹In-octreotide scintigraphy. The high receptor mRNA values and T/B values were positively correlated in MC, suggesting SSR_{2(a)} as the main receptor subtype responsible for binding and uptake of the SS analogue in this tumour.

The human PNS-derived neuroblastoma cell line SH-SY5Y was studied in tissue culture. After neuronal differentiation, initiated by treatment with retinoic acid (RA), the SSR_{2(a)} was affiliated with the plasma membrane, the cytoplasm and processes. The number of SSR_{2(a)}-positive cells and SSR_{2(a)}-IR were markedly increased by RA treatment, but only minor increases in the receptor mRNA values were seen. After RA treatment, catecholaminergic markers (TH, VMAT2, NPY) were decreased whereas the cholinergic markers (ChAT, VACHT, VIP) were increased. On the other hand, the integral synaptic vesicle membrane proteins p38-IR and SV2-IR were not markedly affected by the treatment. SS was present not only in the cytoplasm, but also in the nucleus, and more strongly so after the treatment. The presence of both the receptor and its ligand in the same cells may suggest that SH-SY5Y cells exhibit autocrine regulation via this receptor/ligand system. The neuronal differentiated SH-SY5Y cells may thus be used as a model for studying turnover and transport of SSR_{2(a)} receptor in cells/neurons.

The CNS-derived mouse CAD cell line was studied *in vitro*. Neuronal differentiation, with rounded cell bodies and extension of very long neurite-like processes, was induced into this cell line after protein starvation (PFM). The SSR_{2(a)} was affiliated with the plasma membrane and the cytoplasm, concentrated in the perinuclear zone (Golgi area), both in the presence (SCM) and the absence of serum (PFM). In differentiated CAD cells SSR_{2(a)}-IR was found to be partially shifted into the long processes and markedly increased. CAD cells were positive for catecholaminergic markers (TH, PNMT, VMAT2, NPY) which were shifted into the processes after protein withdrawal. In contrast, cholinergic markers (ChAT, VACHT) were not observed in the cells. However, VIP-IR was present in the cytoplasm in SCM cultures concentrated into the perinuclear zone. After differentiation, VIP-IR was enhanced and partially shifted into the processes. Similar distribution of p38-IR and SV2-IR were seen in the cells. The marker for neuronal development and regeneration (GAP-43) was, after protein withdrawal, strongly upregulated and shifted into the processes, concentrated particularly in the distal growth cone-like swellings. CAD cells were SS-positive and showed higher SS-IR in protein starved cultures. The presence of both the receptor and its ligand in the same cells may suggest that CAD cells exhibit autocrine regulation via this receptor/ligand system. Protein-starved CAD cells may constitute an excellent model for studying the turnover and intracellular dynamics of the SSR_{2(a)} receptor and other substances.

In rat hypothalamus, ependymal cells and tanycytes of the third ventricle were studied. In tanycytes SSR_{2(a)}-IR was present and could be traced from the luminal processes all the way to the capillary plexus of the median eminence (ME). Many of the SSR_{2(a)}-positive tanycytes were also vimentin-positive, but few contained GFAP-IR. The location of S-100-IR was mainly confined to the apical tips of the tanycytes, and to the ependyma of the third ventricle. Since high levels of SS have been found in the CSF of the third ventricle, it is suggested that SS via SSR_{2(a)} receptor may regulate the physiology of tanycytes and possibly of the adenohypophysis, thereby influencing NE mechanisms.

The thesis demonstrates the presence of SSR_{2(a)} expression in four different cell types, suggesting that this receptor is of general physiological importance. In addition, several pathophysiological conditions may be influenced by this receptor and its ligands. Two new observations are reported: 1) The presence of SS in the nuclei of cultured SH-SY5Y cells, enhanced after RA-induced differentiation, which indicates that SS may serve as a transcription factor like many other smaller peptides. 2) The strong presence of the receptor protein in ependyma and tanycytes of the third ventricle, which may be influenced by the physiological levels of CSF-SS demonstrated by previous investigators.

Keywords: SSR_{2(a)} receptor, somatostatin, neuroendocrine tumours, tissue culture, immunocytochemistry, SH-SY5Y cells, CAD cell line, ependyma, tanycytes, neuronal differentiation, catecholaminergic markers, cholinergic markers, synaptic vesicle membrane proteins, intermediate filaments.