

Neuropeptide Receptor Imaging of Schwannoma

TO THE EDITOR: We read with interest the article of Reubi (1) on neuropeptide receptors in health and disease. We recently encountered a case of a 72-yr-old woman with evidence of a mediastinal tumor on CT scan. This tumor was visualized after ¹¹¹In-octreotide injection, suggesting the presence of somatostatin receptors. Histopathological examination revealed a schwannoma, a benign tumor derived from Schwann cells. Because somatostatin receptors had not yet been described in this type of tumor, we performed immunohistochemical evaluation of octreotide binding to its putative receptor in formalin-fixed, paraffin-embedded tumoral tissue to confirm the in vivo findings. In addition, formalin-fixed and paraffin-embedded tissues of four other schwannomas were also evaluated. Because anti-somatostatin receptor antibodies are not yet available, we developed an immunohistochemical technique for paraffin sections (snap-frozen tumor tissue was not available). This technique is based on preincubation of tissue sections with somatostatin that binds its receptor. An anti-somatostatin antibody is subsequently used in a classical immunohistochemical technique to detect receptor-bound somatostatin.

Five-micrometer thick paraffin sections were dried overnight at 37°C, dewaxed in Depar, followed by rehydration in descending ethanol series, water and phosphate buffered saline (PBS pH 7.4). The sections were consecutively treated with:

1. Octreotide or somatostatin in a concentration of 100 µg/ml. Incubations were carried out in a humid chamber at 4°C overnight.
2. Anti-human somatostatin rabbit polyclonal antibody for 30 min as per package insert.
3. Biotinylated mouse anti-rabbit antibody for 20 min.
4. Alkaline phosphatase-bound streptavidin was identified by incubating the sections with 5 ml naphtholphosphate in TRIS buffer supplemented with 5 mg fast red as chromogen.
5. Nuclear counterstaining with Hematoxylin Carazzi for 3 min. Sections were mounted with Imsol. Washing was carried out three times with PBS buffer after incubation steps 1, 2, 3 and 4.

Two negative control sections of each case were performed: omitting the somatostatin or octreotide incubation in the first step and replacing the anti-somatostatin antibody by another polyclonal antibody raised in the same animal species (anti-human CEA rabbit polyclonal antibody) applied in the same concentration but directed against another unrelated antigen.

Sections of an appendiceal carcinoid tumor were used as a positive control (2). Immunoreactivity was found in all five cases of schwannoma and in positive control sections as very clear dot-like signals on the cell membrane of the tumoral cells. The immunoreactivity in the tumoral tissue was inhomogeneously dispersed. Intralesional lymphocytes and vessels that were sparse displayed slight positivity with the above mentioned immunoassay thereby ruling out the possibility of false-positive somatostatin receptor status in vivo (1). No differences were found between sections incubated with octreotide or somatostatin. Negative control sections were not immunoreactive.

The immunohistochemical findings confirm the in vivo observation of octreotide binding to schwannoma and suggests that it is mediated through somatostatin receptors. This hypothesis is corroborated by the fact that binding was observed both with the complete somatostatin peptide as well as with the truncated somatostatin peptide analog octreotide. The latter is

frequently used in its radiolabeled form for nuclear imaging. The absence of immunoreactivity on the negative control sections confirms the specificity of the immunohistochemical reactions.

Although the above described technique is economically and easy to perform, these findings have to be confirmed by autoradiography or other in vitro method before the above described technique can be considered as a valuable alternative to autoradiography since specific antibodies directed against somatostatin receptors are not yet available for immunohistochemical techniques.

REFERENCES

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Origin of Janus

TO THE EDITOR: It has been a distinct pleasure to read your editorials at the beginning of each *Journal*. Your comments are often prescient, sometimes mordant, but always insightful and entertaining. I must take exception, however, to the comments in the January 1996 issue, where you mention "... the Greek god Janus" (1). The Greeks and Ionians shared many deities with other peoples, worshipped under Hellenic and other names. Herodotus, in his *Histories* (2), gives accounts of the worship of Aphrodite by Persians (as Mitra), Assyrians (as Mylitta) and Arabians (as Alilat). We may also know her by her Roman appellation, Venus.

Janus, the porter of Heaven, belongs solely in the Roman pantheon. According to Bulfinch (3), Janus was the guardian of gates, always looking in both directions. During frequent times of war, the principal gate of Janus would remain open, allowing the god to assist the Roman warriors. Janus was featured prominently on coins during Nero's reign. His temple on the Via Sacra was destroyed during the great fire of Nero (A.D. 65) and was later rebuilt by Domitian, son of Vespasian (4).

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