Multiple treatments with SRIH-14 or octreotide affect adrenal zona glomerulosa in adult male rats

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Abstract. Somatostatin analogues are currently used to treat various disorders such as hypersecretion and different neuroendocrine tumors. In this study we examined the effects on the adrenal cortex of somatostatin (SRIH-14) and octreotide administered subcutaneously twice daily for 5 days to adult male rats. Control rats received saline under the same regime. After sacrifice, the adrenal glands were removed and examined morphometrically using the M_{42} multipurpose test system. Blood samples were prepared for biochemical tests. Both SRIH-14 and octreotide induced morphofunctional changes in adrenal zona glomerulosa. We found significant decreases ($p < 0.05$) in the absolute cell and nuclear volumes of zona glomerulosa in both experimental groups in comparison to the control. The serum aldosterone level was 11% lower ($p < 0.05$) in the SRIH-14 and 13% ($p < 0.05$) lower in the octreotide-treated group in comparison with the control group.

Morphometric parameters of zona fasciculata and zona reticulata and corticosterone levels were not altered significantly ($p > 0.05$) in either treated group. It may therefore be concluded that both SRIH-14 and octreotide affected zona glomerulosa in the same manner by decreasing morphofunctional characteristics.

Key words: Adrenal cortex — SRIH-14 — Octreotide

Introduction

Somatostatin (somatotropin release-inhibiting hormone, SRIH) is a cyclic-tetradecapeptide initially discovered as an inhibitor of growth hormone release from anterior pituitary cells (Brazeau et al. 1973). Subsequently, other functions such as inhibition of hormone secretion and inhibition of cell growth have been described (Patel 1999). SRIH is synthesized as two bioactive peptides SRIH-14 and SRIH-28 which are widely distributed throughout the central nervous system, endocrine and peripheral tissues (Reichlin 1983a). SRIH was detected in different regions of the brain, in the pituitary, the gastrointestinal tract, endocrine pancreas, kidneys, the thyroid and adrenal glands (Reichlin 1983a,b; Epelbaum et al. 1994).

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SRIH acts through a family of G-protein-coupled membrane receptors containing seven transmembrane domains. Five genes encoding distinct SRIH receptor (sst) subtypes have so far been cloned and termed sst_{1-5}. All five subtypes are expressed in normal tissues (Patel 1999). Also, SRIH receptors are expressed in a large number of tumors (Kennedy and Dluhy 1997; Hofland et al. 1999; van der Harst et al. 2001).

Owing to its potent inhibitory effect on hormone secretion and cell proliferation the use of somatostatin was proposed in clinical disorders. However, the short half-life of the natural hormone made it difficult to apply. Octreotide is a cyclic octapeptide, which is more potent than endogenous SRIH because of an extended half-life (Bauer et al. 1982; Lamberts et al. 1991). Octreotide binds to three of these five receptors, sst_2, sst_3 and sst_5, which are expressed in various rat organs (Patel et al. 1995). The SRIH analogue octreotide is used clinically to control humoral hypersecretion and tumoral growth (Berlowitz 1995).

The different sst subtypes are expressed in brain, all types of the pituitary gland, gut, endocrine and exocrine pancreas,

endocrine glands, kidneys and immune cells (Patel 1999). However, sst have also been detected both in the adrenal cortex and medulla (Aguilera et al. 1982; Mauer and Reubi 1986). Moreover, Boscaro et al. (1982) showed that somatostatin significantly blocked the aldosterone response to angiotenzin II but not to adrenocorticotropic hormone (ACTH) *in vitro*.

Therefore, the primary aim of this study was to compare the effects of multiple SRIH-14 and octreotide treatments on the adrenal cortex *in vivo*, considering the reality of medical use of somatostatin analogues.

Materials and Methods

All experimental protocols were approved by the Local Animal Care Committee. They conformed to the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" (1996, National Academy Press, Washington D.C.).

Animals handling

Male Wistar rats (Institute for Biological Research, Belgrade) of mean body weight (b.w.) 240 g were housed at constant temperature (22°C) with a 12-h light, 12-h dark cycle with provided free access to food and water.

The rats were separated into three experimental groups each consisting of six animals. Rats from first group were injected subcutaneously (s.c.) twice a day with 20 μg SRIH-14/100 g b.w. (No. S 9129; Sigma, St. Louis, MO, USA) per 100 g b.w. for 5 consecutive days (between the ages of 55 and 59 days). The dose regimen selected for SRIH-14 was based on the method reported previously (Rebuffat et al. 1984), except that SRIH was administered every 12 h instead of every 8 h. Rats from second group were injected s.c. twice a day with octreotide (20 μg/100 g b.w.; Sandostatine, Novartis, Switzerland) for 5 consecutive days (between the ages of 55 and 59 days). Control group comprised rats that were treated only with saline in the same manner. All animals were killed under ether anesthesia 12 h after the last injection.

Light microscopy

The left adrenal glands were excised, fixed in Bouin's solution for 48 h, dehydrated, embedded in paraplast and serially cut into 5 μm thick sections which were stained with hematoxylin-eosin and examined under a light microscope.

Morphometric measurements in the adrenal gland

The absolute volume of the adrenal glands was calculated on the basis of their weight, assuming an average specific gravity of 1.039 $g \cdot cm^{-3}$ (Swinyard 1938). In order to evaluate the volume densities of the adrenocortical zones, every tenth section (5 μm thick) of the gland was analyzed at 125× magnification with the M_{42} multipurpose test system (Weibel 1979).

The nuclear and cytoplasmic volumes of parenchymal cells were estimated at 1000× magnification on 5 μm thick sections with the M_{42} multipurpose test system (Weibel 1979). For each adrenal gland, a single paraplast section containing the zona medullaris was chosen and 30 test areas of the zona glomerulosa (ZG) and 50 test areas of both the zona fasciculata (ZF) and zona reticulata (ZR) were analyzed. On the basis of earlier karyometric studies (Malendowicz 1974), the shape coefficient β was assumed to be 1.382 for the ZF and 1.500 for the ZG.

Hormone assays

The aldosterone and corticosterone levels in blood serum from all rats were determined by enzyme immunoassay (aldosterone ELISA and corticosterone immunoassay, respectively).

Statistics

The biochemical and morphometric data obtained for each rat were averaged per experimental group and the standard deviation (SD) of the means was calculated. The data were subjected to ANOVA followed by Duncan's multiple-range test. Values of $p < 0.05$ were considered statistically significant.

Results

Absolute and relative adrenal weights

The mean absolute weight of the adrenal glands in the octreotide-treated group was significantly ($p < 0.05$) decreased by 19% compared with the value for the control group. The SRIH-14-treated group did not differ significantly ($p > 0.05$) from the control group. The slight decrease in mean relative weight of the adrenal glands in both treated groups was not significantly different from the control value. There was no significant difference in mean absolute weight of the adrenal glands between the SRIH-14- and octreotide-treated groups (Table 1).

Table 1. Effects of multiple treatment with SRIH-14 or octreotide on absolute and relative adrenal weight in male rats

Groups	Weight	
	Absolute (mg)	Relative (%)
Controls	18.5 ± 0.7	7.7 ± 0.3
SRIH-14	17.0 ± 1.4 (–8%)	7.1 ± 0.5 (–8%)
Octreotide	15 ± 0.3 [a] (–19%)	6.7 ± 0.5 (–12%)

[a] $p < 0.05$ vs. controls; mean ± SD; $n = 5$.

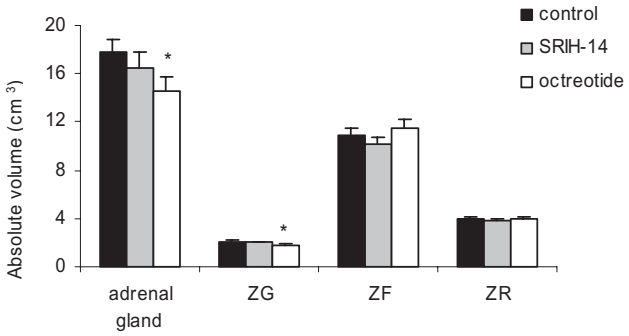

Figure 1. The absolute volume of the adrenal cortex, zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) after treatments with SRIH-14 or octreotide in male rats (mean ± SD; $n = 5$). * $p < 0.05$ vs. controls.

In comparison with the control group value, the absolute volumes of the adrenal glands decreased significantly ($p < 0.05$) in octreotide-treated group (by 19%) and in the SRIH-14-treated groups (by 8%) (Fig. 1).

Adrenal cortex

All three cortical zones of the adrenal gland (ZG, ZF and ZR) were clearly visible in all examined preparations.

Zona glomerulosa (ZG)

The cells of ZG were relatively small, columnar or pyramidal, with oval nuclei. The cells of ZG were arranged in closely packed ovoid-shaped cell cluster. The overall shape of ZG cells in both treated groups was not altered (Fig. 2).

The absolute volume of ZG was about 7% smaller ($p > 0.05$) in the SRIH-14-treated group and 12% smaller ($p < 0.05$) in octreotide-treated group. The decrease in relative volume (by 10%) of the ZG was significant ($p < 0.05$) for octreotide-treated group (Fig. 1).

Moreover, the volumes of ZG cells and their nuclei were significantly ($p < 0.05$) smaller in both SRIH-14- and octreotide-treated groups in comparison to the control group (Fig. 3).

The mean serum levels of aldosterone was significantly ($p < 0.05$) lower (by 11%) in SRIH-14-treated group compared with the control value. The decrease in mean aldosterone level (by 13%) in the octreotide-treated group was also significant ($p < 0.05$) (Fig. 4).

Zona fasciculata (ZF) and zona reticulata (ZR)

The ZF was the largest cortex zone. It consisted of polyhedral cells arranged in long straight cords, one or two cells thick,

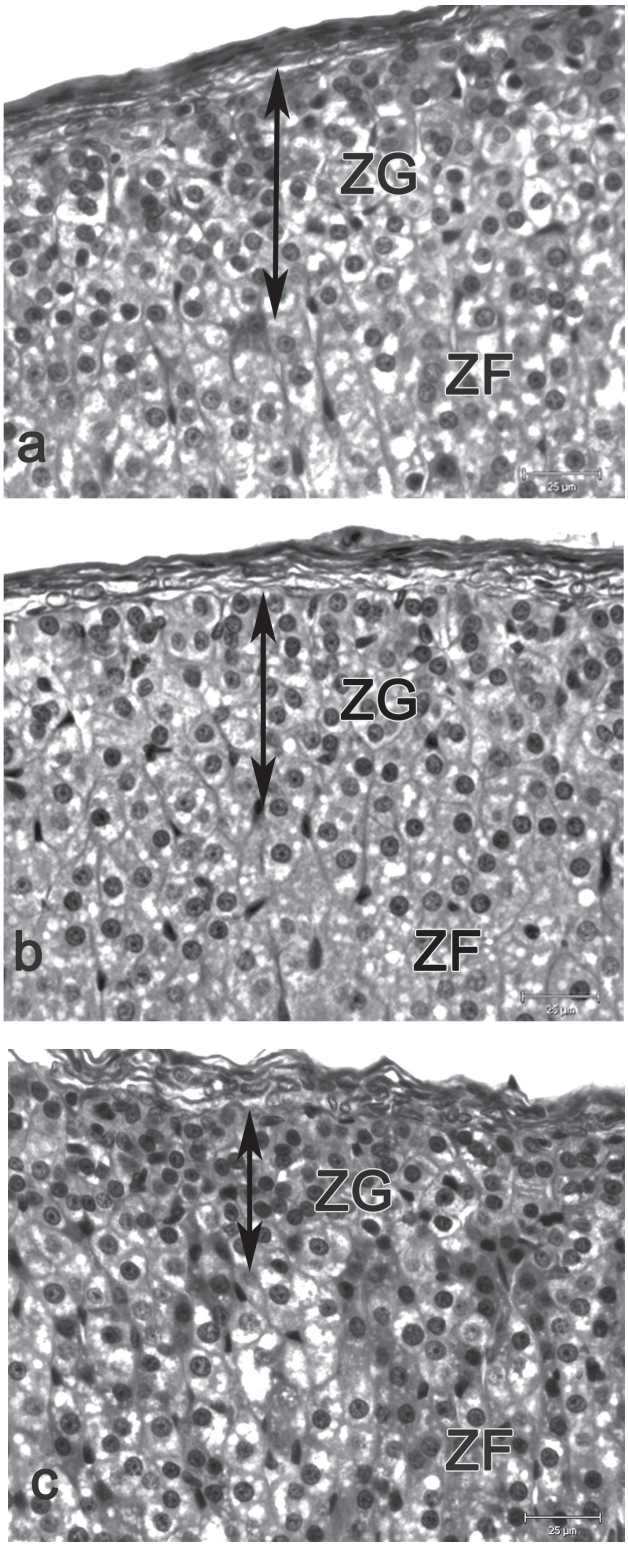

Figure 2. Zona glomerulosa (ZG) and zona fasciculata (ZF) in a) control rats; b) rats treated with SRIH-14; c) rats treated with octreotide. The decrease in volume of ZG is more notable in octreotide-treated group then in SRIH-14-treated group, in comparison with the control group. Bar, 25 µm.

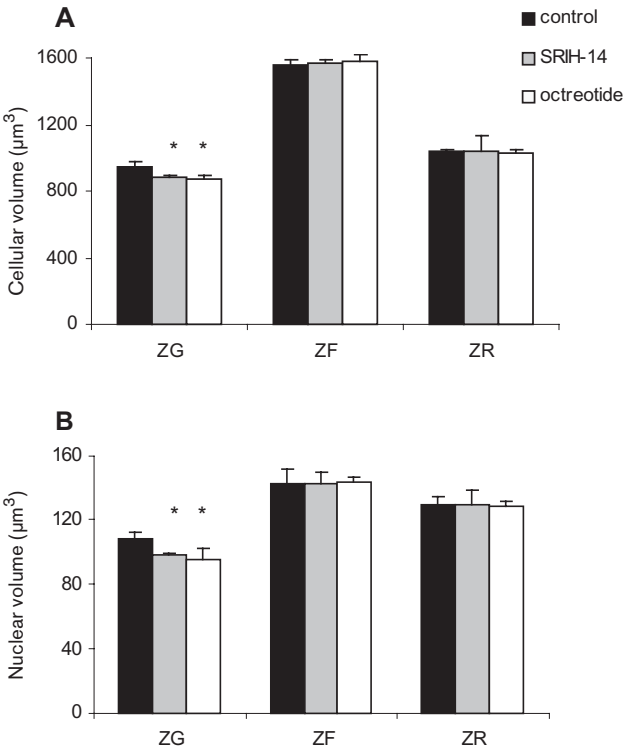

Figure 3. Cellular volume (A) and nuclear volume (B) after treatment with SRIH-14 or octreotide in male rats (mean ± SD; $n = 5$). ZG, zona glomerulosa; ZF, zona fasciculata; ZR, zona reticularis; * $p < 0.05$ vs. controls.

separated by sinusoidal capillaries. The shapes and positions of the cells did not change after multiple treatments with either SRIH-14 or octreotide (Fig. 2).

No differences in the absolute volume and stereological parameters (cell and nuclear volumes) of the ZF and ZR were detected between the groups. (Figs. 1 and 3). The serum levels of corticosterone in both treated group did not differ from the control value (Fig. 5).

Discussion

Both SRIH and its long-acting analogue octreotide exert diverse physiological actions on multiple target tissues. The present report describes the effects of multiple SRIH-14 and octreotide treatments on the structure and function of the adrenal cortex in adult male rats.

After multiple SRIH-14 or octreotide treatment we detected lowering of the absolute weight of the adrenal glands. These results confirm our previous findings obtained after intracerebroventricular (ICV) administration of SRIH and after chronic treatment with SRIH-14 and octreotide (Milošević et al. 1996; Trifunović et al. 2008).

The examined stereological parameters in the ZG showed reduction in both treated groups. Moreover, the serum levels of aldosterone were lower then the control value in both treated groups. Neither the SRIH-14-treated group nor the octreotide-treated group exhibited changes in other stereological parameters or serum levels of corticosterone.

The results observed here support previous findings after multiple ICV treatment with SRIH-14 and SRIH-28 which caused the ZG cells atrophy (Milošević et al. 1994, 1996).

SRIH immunoreactivity has been demonstrated in the ZG of the rat adrenal cortex (Aguilera et al. 1981). The presence of high-affinity binding sites for SRIH has been demonstrated in homogenates of adrenal ZG (Aguilera et al. 1982; Srikant and Patel 1985). O'Carroll (2003) showed expression of sst_1, sst_2, sst_4 and sst_5 mRNAs in the ZG while expression of sst was extremely low in the ZF and ZR.

Thus, we can assume that a direct SRIH-14 and octreotide influence *via* sst exists. Alternatively, somatostatin may exert an effect on ZG cells by influencing the rennin-angiotensin regulating system (in particular angiotensin II-induced aldosterone secretion; Aguilera et al. 1981). Moreover, it is possible that SRIH decreased the secretion of pituitary growth hormone and prolactine, the endogenous stimulators of ZG cell growth and secretion (Rebuffat et al. 1984; Mazzocchi et al. 1986). Accordingly, we assume that the findings

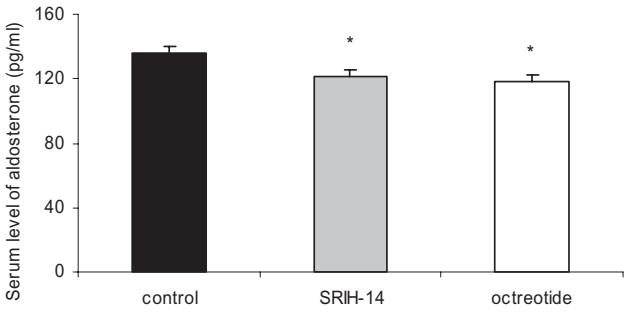

Figure 4. The effects of treatments with SRIH-14 or octreotide on aldosterone serum concentrations in male rats (mean ± SD; $n = 5$). * $p < 0.05$ vs. controls.

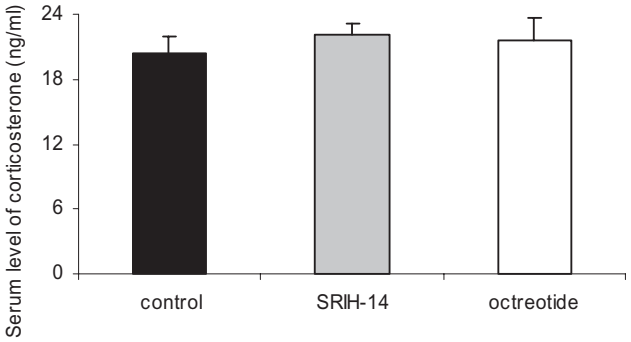

Figure 5. The effects of treatments with SRIH-14 or octreotide on corticosterone serum concentrations in male rats (mean ± SD; $n = 5$).

observed here might be the result of SRIH-14 and octreotide *via* sst plus an indirect influence.

We found no changes in morphofunctional parameters in ZF and ZR. This may be due to unchanged ACTH secretion and/or rare presence of sst in both zones (Milošević et al. 1994; Trifunović et al. 2007, 2008).

Clearly, we have demonstrated that multiple treatment with SRIH-14 and octreotide affected ZG on the same manner through reduction of morphofunctional parameters, while parameters of ZF and ZR were not altered. These results may be important considering the increasing medical administration of somatostatin analogues.

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References

Aguilera G., Harwood J. P., Catt K. J. (1981): Somatostatin modulates effects of angiotensin II in adrenal glomerulosa zone. Nature **292**, 262–263

Aguilera G., Parker D. S., Catt K. J. (1982): Characterization of somatostatin receptors in the rat adrenal glomerulosa zone. Endocrinology **111**, 1376–1384

Bauer W., Briner U., Doepfner W., Haller R., Huguenin R., Marbach P., Petcher T. J., Pless J. (1982): SMS 201-995 a very potent and selective octapeptide analogue of somatostatin with prolonged action. Life Sci. **31,** 1133–1140

Berlowitz M. (1995): Editorial: The somatostatin receptor–a window of therapeutic opportunity? Endocrinology **136**, 3695–3697

Boscaro M., Scaroni C., Edwards C. R., Mantero F. (1982): Inhibitory effect of somatostatin on the aldosterone response to angiotenzin II: *in vitro* studies. J. Endocrinol. Invest. **5**, 173–177

Brazeau P., Vale W., Burgus R., Ling N., Butcher M., Rivier J., Guillemin R. (1973): Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science **179**, 77–79

Epelbaum J., Dournaud P., Fodor M., Viollet C. (1994): The neurobiology of somatostatin. Crit. Rev. Neurobiol. **8**, 25–44

Hofland L. J., Liu Q., van Koetsveld P. M., Zuijderwijk J., van der Ham F., de Krijger R. R., Schonbrunn A., Lamberts S. W. J. (1999): Immunohistochemical detection of somatostatin receptor subtypes sst_1 and sst_{2A} in human somatostatin receptor positive tumors. J. Clin. Endocrinol. Metab. **84**, 775–780

Kennedy J. W., Dluhy R. G. (1997): The biology and clinical relevance of somatostatin receptor scintigraphy in adrenal tumor management. Yale J. Biol. Med. **70**, 565–575

Lamberts S. W., Krenning E. P., Reubi J. C. (1991): The role of somatostatin and its analogs in the diagnosis and treatment of tumors. Endocr. Rev. **12**, 450–482

Malendowicz L. K. (1974): Sex differences in adrenocortical structure and function. I. The effects of postpubertal gonadectomy and gonadal hormone replacement on nuclear volume of adrenocortical cells in the rat. Cell Tissue Res. **151**, 525–536

Maurer R., Reubi J. C. (1986): Somatostatin receptors in the adrenal. Mol. Cell. Endocrinol. **45**, 81–90

Mazzocchi G., Robba C., Rebuffat P., Nussdorfer G. C. (1986): Effects of prolactin administration on the zona glomerulosa of the rat adrenal cortex: stereology and plasma hormone concentrations. Acta Endocrinol. **111**, 101–105

Milošević V., Brkić B., Velkovski S., Starčević V. (1994): Somatostatin-induced changes of the ACTH and STH cells in the hypophysis of the male rats. Yugoslav. Med. Biohem. **13**,102–106

Milošević V., Velkovski S., Brkić B., Sekulić M., Lovren M., Starčević V., Severs W. B. (1996): Inhibitory effects of centrally administered somatostatin on the adrenal zona glomerulosa in male rats. Pharmacology **53**, 369–375

O'Carroll A. M. (2003): Localization of messenger ribonucleic acids for somatostatin receptor subtypes ($sstr_{1-5}$) in the rat adrenal gland. J. Histochem. Cytochem. **51**, 55–60

Patel Y. C. (1999): Somatostatin and its receptor family. Front. Neuroendocrinol. **20**, 157–198

Patel Y. C., Greenwood M. T., Panetta R., Demchyshyn L., Niznik H., Srikant C. B. (1995): The somatostatin receptor family. Life Sci. **57**, 1249–1265

Rebuffat P., Robba C., Mazzocchi G., Nussdorfer G. G. (1984): Inhibitory effect of somatostatin on the growth and steroidogenic capacity of rat adrenal zona glomerulosa. J. Steroid Biochem. **21**, 387–390

Reichlin S. (1983a): Somatostatin. N. Engl. J. Med. **309**, 1495–1501

Reichlin S. (1983b): Somatostatin (second of two parts). N. Engl. J. Med. **309**, 1556–1563

Srikant C. B., Patel Y. C. (1985): Somatostatin receptors in the rat adrenal cortex: characterization and comparison with brain and pituitary receptors. Endocrinology **116**, 1717–1723

Swinyard C. (1938): Methods for volumetric determination of fresh endocrine gland. Anat. Rec. **74**, 71–78

Trifunović S., Ajdžanović V., Filipović B., Sekulić M., Milošević V. (2007): Effects of SRIH-14 on ACTH cells in male rats. Arch. Biol. Sci., Belgrade **59**, 43–44

Trifunović S., Filipović B., Ajdžanović V., Šošić-Jurjević B., Manojlović-Stojanoski M., Nestorović N., Sekulić M., Milošević V. (2008): The effects of chronic SRIH-14 and octreotide administration on pituitary-adrenal axis in adult male rats. Eur. J. Histochem. **52**, 61–68

van der Harst E., de Herder W. W., Bruening H. A., Bonjer H. J., de Krijger R. R., Lamberts S. W., van de Meiracker A. H. Boomsma F., Stijnen T., Krenning E. P., Bosman F. T., Kwekkeboom D. J. (2001): [^{123}I] metaiodobenzylguanidine and [^{111}I] octreotide uptake in benign and malignant pheochromocytomas. J. Clin. Endocrinol. Metab. **86**, 685–693

Weibel E. R. (1979): Practical methods for biological morphometry. In: Stereological Methods. Vol. 1, pp. 1–415, Academic Press, New York

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