THE EFFECT OF SRIH-14 OR OCTREOTIDE ON THE MORPHOLOGICAL CHARACTERISTICS OF ADRENAL MEDULLA USING NEWCAST

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Abstract - The effects of chronic treatments with either SRIH-14 or octreotide on the adrenal medulla of male Wistar rats were examined. Adult males received subcutaneous (s.c.) injections of 20 μg/100 g body weight of either SRIH-14 or octreotide twice a day for 28 consecutive days. The absolute weights and the absolute volumes of the adrenal glands significantly ($p<0.05$) decreased after either treatment. The adrenal medulla was analyzed by histological and stereological methods using newCAST. Compared to the control, the relative volumes of the vascular tissues significantly ($p < 0.05$) decreased – by 40% and 25% in the SRIH-14- and octreotide-treated groups, respectively. In the SRIH-14- and octreotide-treated groups the relative volumes of chromaffin and interstitial tissue increased by 6% and 5% ($p < 0.05$), respectively. These findings show that both SRIH-14 and octreotide affect the morphological characteristics of the adrenal *zona medullaris* in a similar manner.

Keywords: SRIH-14, octreotid, adrenal medulla, stereology, newCAST

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INTRODUCTION

The neuropeptide somatostatin (somatostatin-release inhibitory hormone or SRIH) is involved in multiple inhibitory actions throughout the central and peripheral nervous systems (Patel, 1999). SRIH is synthesized as part of a large precursor molecule which is enzymatically cleaved into a prohormone form that is further processed to yield two active forms, SRIH 14 and SRIH 28 (Reichlin, 1983a, 1983b). Somatostatin acts through specific membrane-bound receptors (sst) that belong to the seven-transmembrane receptor super family. Five different receptor subtypes are recognized. Different experimental approaches showed that different sst receptors are expressed in normal and in tumor tissues (Patel, 1999). Because of its potent inhibitory effect on hormone secretion the use of SRIH in clinical disorders related to excessive hormone secretion has been proposed. Octreotide, a short synthetic somatostatin analogue, was introduced for clinical use in cancer therapy and treatments of gastrointestinal and hormonal hypersecretory disorders (Weckbecker et al., 1993). It is more powerful then endogenous SRIH because of its longer half-life (Bauer et al., 1982; Lamberts et al., 1991). Octreotide binds with high affinity to sst_2 and sst_5 receptors, with a moderate affinity to subtype 3 receptors, but it does not bind to either subtype 1 or 4 receptors (Rossowski & Coy, 1994).

The adrenal gland could also be a potential target for direct SRIH action. Somatostatin receptors have been detected in the rat adrenal cortex and medulla (Aguilera et al.,1982; Maurer and Reubi, 1986). However, in healthy male adult rats, intracerebroventricular (ICV) application of SRIH affects the morphometric features of the adrenal cortex and the plasma aldosterone level (Milošević et al., 1996).

The aim of this study was to investigate the effects of chronic treatments with either SRIH-14 or octreotide on the stereological parameters of the adrenal *zona medullaris* using newCAST.

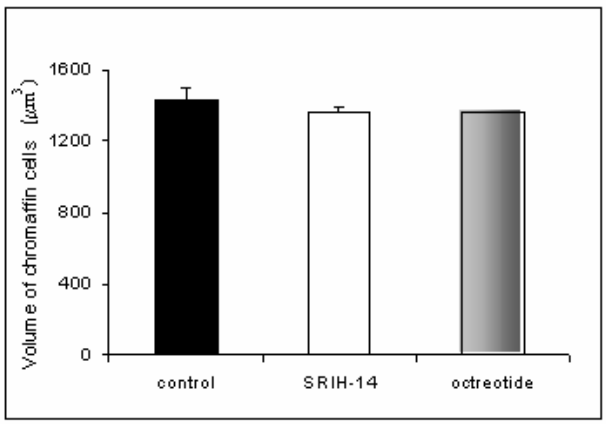

Figure 1. Effects of chronic SRIH-14 and octreotide treatments on the volume of chromaffin cells in male rats. The values are the means ± SD, n=8, [a]p<0.05 in comparison with sham operated

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.). Adult Wistar males rats were divided into three experimental groups, two treated and one control. Rats in the first and second groups were injected s.c. twice a day with 20 μg/100g b. w. of either SRIH-14 or octreotide for 28 consecutive days. There were six animals in each group. The animals were decapitated 24 h after the last injection. Adrenal glands were excised, fixed in Bouin's solution for 48 h, dehydrated and embedded in paraffin. The adrenal medulla was analyzed by histological and stereological methods using newCAST. Every tenth section (5 μm thick), was used for stereological analysis; the first section was randomly chosen. The *zona medullaris* was circumscribed and a point grid was used to estimate the relative percent changes in the volumes of chromaffin and interstitial tissues, and the volume of the vascular tissue. Two individual point grid systems were chosen: one for the chromaffin and interstitial tissues and one for the vascular tissue. About 200 matches per animal were assumed. The areas of both grids were identical, and the point areas were not. The volumes were estimated according to Cavalieri (1966). The volumes of chomaffin cells were examined with a nucleator.

The Duncan test was used for statistical comparisons between groups. Values of p less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

After 28 days of treatment, the body weights significantly decreased ($p < 0.05$) by about 19% and 16% in the SRIH-14- and octreotide-treated animals, respectively. These results are in agreement with our previous findings obtained after an ICV administration of SRIH (Milošević et al., 1996). Other authors showed that SRIH reduces food intake both in man and in rat (Scalera and Tarozzi, 1998).

The absolute weights of the adrenal glands significantly ($p<0.05$) decreased by 23% and 21% in the SRIH-14- and octreotide-treated groups, respectively. The relative weights of the adrenal glands did not differ significantly between the SRIH-14- and octreotide-treated groups. The absolute volumes of adrenal glands significantly ($p<0.05$) decreased by 24% in the SRIH-14 and by 20% in octreotide-treated groups. These results are in agreement with our previous findings observed after ICV administration of SRIH (Milošević et al., 1996).

Table 1. Effects of chronic treatment with SRIH-14 and octreotide treatments on body weight and adrenal weight in male rats

Groups	Body weight, (g)	Absolute adrenal weight (mg)	Absolute volumes of adrenal gland (mm^3)
controls	269.1 ± 22.9	18.1 ± 0.5	17.3 ± 0.5
SRIH-14	217.2 ± 19.2[a] (-19%)	13.7 ± 0.6[a] (-23%)	13.2 ± 0.7[a] (-24%)
octreotide	225.6 ± 12[a] (-16%)	14.3 ± 1.2[a] (-21%)	13.8 ± 1.1[a] (-20%)

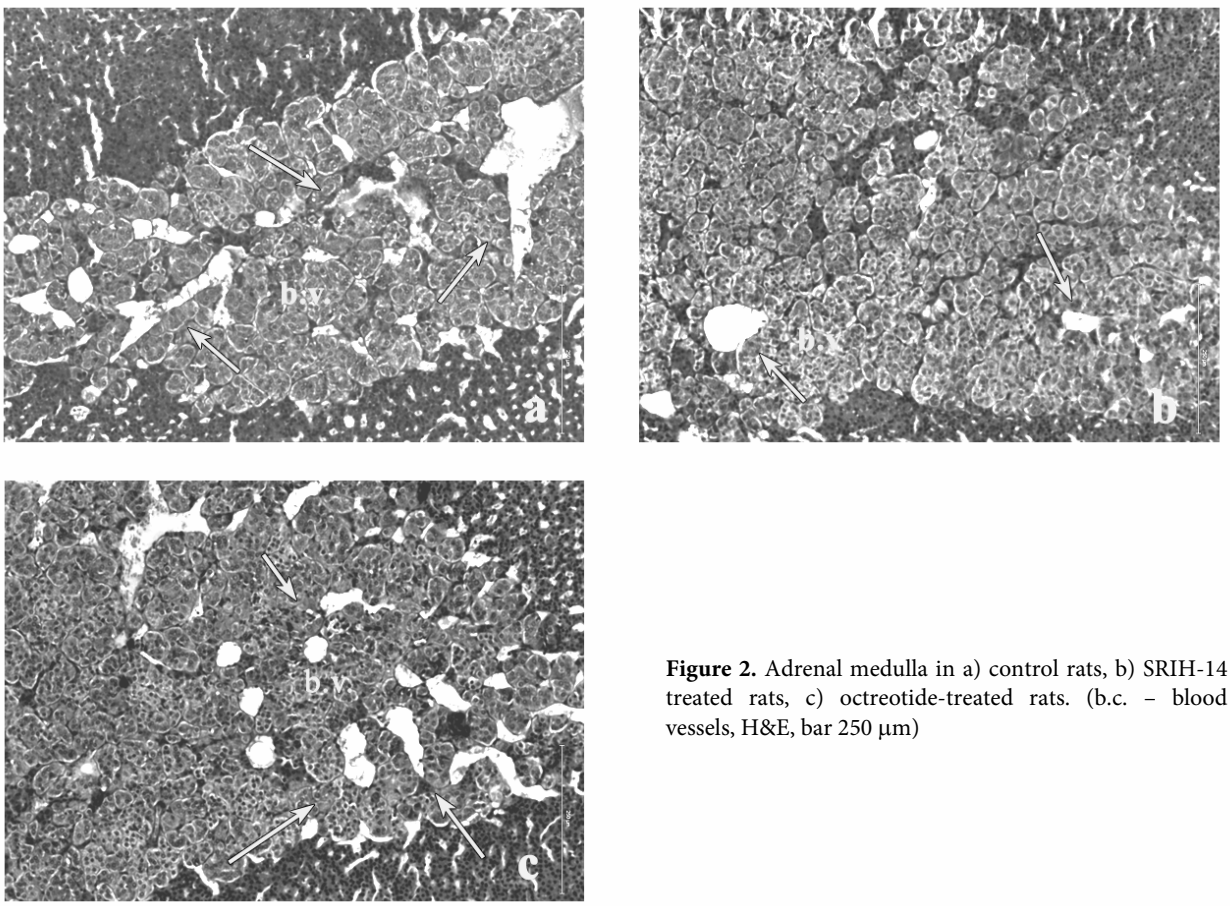

Figure 2. Adrenal medulla in a) control rats, b) SRIH-14 treated rats, c) octreotide-treated rats. (b.c. – blood vessels, H&E, bar 250 μm)

Compared to control rats, the relative volumes of the vascular tissue in the adrenal medulla decreased significantly ($p<0.05$) – by 40% and 25% in the SRIH-14 and octreotide-treated groups, respectively. Compared to the control rats, in the SRIH-14- and octreotide-treated groups the relative volumes of chromaffin and interstitial tissue increased insignificantly ($p>0.05$), by 6% and 5%, respectively. Aside from having numerous effects, SRIH possesses a vasoconstrictor activity (Patel, 1999).

The cell volumes in chromaffin tissue which is made up of adrenaline- and noradrenalin-storing cells did not differ significantly between the SRIH-14- and octreotide-treated groups, although a tendency towards decrease was observed (Table 1). Sst mRNAs expression that was detected in the adrenal medulla suggests a possible role of SRIH in catecholamine secretion (O'Carroll, 2003).

The adrenal medullae in animals treated with either SRIH-14 or octreotide have changed stereological parameters. This information is of importance with regard to the clinical applications of somatostatin analogues.

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