Acta histochemica **109** (2007) 138–153

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acta histochemica
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# Possible role of insulin-like growth factor-II C-peptide on catecholamine release and ultrastructural aspects of chromaffin cells in the adrenal gland of the frog

**Songül Süren Castillo***

*Istanbul University, Faculty of Science, Department of Biology, Zoology Section, 34459-Vezneciler, Istanbul, Turkey*

Received 5 July 2006; accepted 29 August 2006

**KEYWORDS**
Insulin-like growth factors;
Serotonin;
Dopamine $\beta$-hydroxylase;
Adrenal gland;
Amphibia

## Summary

The present study was undertaken to demonstrate that insulin-like growth factor-II C-peptide (IGF-II C-peptide) affects the function of the adrenal gland of *Rana ridibunda* (Anura, Amphibia) by stimulating chromaffin cells. Previous studies have shown that insulin-like growth factors affect adrenal gland function in mammals. On the basis of these findings, frogs were injected with IGF-II C-peptide (2.5 $\mu$g/0.2 ml), whereas control animals were injected with Ringer solution (0.2 ml). The adrenal glands were removed at 12 and 48 h after injection and fixed, embedded in paraffin wax and Epon, and examined by immunohistochemistry and transmission electron microscopy to investigate whether there were structural changes and activation of chromaffin cells in the frog adrenal gland. Sections were stained with hematoxylin and eosin for overall tissue analysis and, in parallel, serotonin was localized using the streptavidin–biotin complex technique while dopamine $\beta$-hydroxylase was shown by the peroxidase–antiperoxidase-3, 3′-diaminobenzidine tetrachloride method. After injection of IGF-II C-peptide, chromaffin cells released serotonin and synthesized dopamine $\beta$-hydroxylase. The most pronounced effect of IGF-II C-peptide on the chromaffin cells was observed at 12 h after injection. Our results indicate that there is a possible role of IGF-II C-peptide on chromaffin cell activity enhancing catecholamine release in the adrenal gland of the frog.


## Introduction

The adrenal glands of lower vertebrates (from cyclostomes to reptiles) lack the cortico-medullary

*Tel.: +90 212 455 57 00/15084; fax: +90 212 528 05 27.
*E-mail address:* songulsuren@yahoo.com.

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doi:10.1016/j.acthis.2006.08.003

organization found in higher vertebrates. In lower vertebrates, chromaffin and steroidogenic cells are present as separate groups, or as intermingled cell masses (Kawamura and Kikuyama, 1992). So far, various vertebrate species have been studied in order to determine the control of the secretory pathway of hormones in the adrenal gland. On the basis of these studies, it is assumed that sympathetic nerve stimulation (Kawamura and Kikuyama, 1992; Sato et al., 1996) and glucocorticoids (Elhamdani et al., 2000) can stimulate catecholamine secretion in chromaffin cells.

The present study focused on insulin-like growth factor-II C-peptide (IGF-II C-peptide). It is known that the insulin-like growth factors I and II (IGF-I and IGF-II) constitute a family of polypeptides that exert insulin-like metabolic and growth-promoting effects on a variety of target cells (Sara and Hall, 1990; Coulter, 2005) and act as endocrine, autocrine, or paracrine growth factors and neurotrophic factors (Gammeltoft et al., 1990; Humbel, 1990; Cohick and Clemmons, 1993). IGFs are mainly produced in the endocrine pancreas (Reinecke et al., 1995) and liver (Norman and Litwack, 1987) and also are synthesized locally in many tissues. IGF-I and IGF-II have been detected in the human adrenal medulla (Haselbacher et al., 1987) and human adrenal cortex (Baquedano et al., 2005). The level of free available IGFs in the circulation and extracellular fluids is modulated by association with IGF-binding proteins (IGFBPs) (Cohick and Clemmons, 1993), whose production in species-and tissue-specific patterns is affected by specific hormones (Jones and Clemmons, 1995; Gronning and Serck-Hanssen, 2003). IGFs interact with at least two specific cell surface receptors, the type I-IGF receptor and the type II-IGF receptor (Reinecke et al., 1995; Siddle et al., 2001). The type I-IGF receptor has been identified in sympathetic ganglia and in adrenomedullary chromaffin cells (Dahmer et al., 1989; Marley et al., 1990; Frödin and Gammeltoft, 1994; Russell and Feldman, 1999). Bovine chromaffin cells also express two types of IGF receptors (Danielsen et al., 1990). The type I-IGF receptor is homologous to the insulin receptor (IR) in structure and has tyrosine kinase activity, while the type II-IGF receptor is identical to the mannose-6-phosphate receptor and has been suggested to be implicated in the degradation of IGF-II (Shimasaki and Ling, 1991). The actions of the IGFs are thought to be mainly due to their activation of the type I-IGF receptor. However, IGF-II can also bind with high affinity to, and effectively activate, an isoform of the IR (Denley et al., 2006). The receptor binding, internalization, and tyrosine kinase activation of IGF-I and IGF-II have been investigated in cultured adult bovine chromaffin cells (Danielsen et al., 1990).

Mature IGF-I and IGF-II are composed of four domains (A, B, C, and D) and share extensive sequence and structural similarity. The IGF-II C and D domains confer high-affinity binding to the IR-A and IR-B, whereas the C and D domains of IGF-I do not (Denley et al., 2004). IGF-II and IGF-I differentially activate specific tyrosine residues on IR-A and IR-B. The sole structural determinant for the differential ability of IGF-I and IGF-II to induce autophosphorylation of specific IR tyrosine residues and activate downstream signalling molecules is the C domain (Denley et al., 2006). The studies of cell surface IR in bovine chromaffin cells has revealed that IR play pivotal roles, such as up-regulation of cell surface voltage-dependent $Na^+$ channels and enhancement of voltage-dependent $Ca^{2+}$ channel gating and of exocytotic secretion of catecholamines (Yamamoto et al., 1996; Johansson et al., 2002), as well as increased synthesis of various bioactive peptides (e.g., enkephalins) contained within chromaffin granules (Wilson et al., 1985).

In the study described here, IGF-II C-peptide was administered to the frog *Rana ridibunda*, and chromaffin cells in the adrenal glands were then examined by immunohistochemical techniques and by transmission electron microscopy. The aim of the study was to determine whether there were structural changes in the adrenal gland, and whether the IGF-II C-peptide caused activation or inactivation of chromaffin cells. In addition to this, we also studied the dopamine $\beta$-hydroxylase involved in catecholamine biosynthesis and serotonin that is present together with epinephrine in granules.

## Materials and methods

### Animals, experimental design and tissue preparation

The synthetic 8-amino acid C-peptide segment of IGF-II (fragment 33–40; (Ser–Arg–Val–Ser–Arg–Arg–Ser–Arg), FW 1003.1) was purchased from Sigma, St. Louis, MO, USA (I-3263). Frogs (*R. ridibunda*) weighing 30–40 g were used in the study ($n = 20$). Animals were kept before and during experimental periods in aquaria at 24 °C under natural daylight. Frogs were divided into two groups and all the injections were administered to the dorsal lymph sacs. Group 1 ($n = 12$) was injected with IGF-II C-peptide (2.5 μg/0.2 ml) and subsequently the group