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Neuroscience Letters 385 (2005) 110–113
Neuroscience Letters
www.elsevier.com/locate/neulet

Coexistence of salusin and vasopressin in the rat hypothalamo-hypophyseal system

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Received 12 January 2005; received in revised form 28 April 2005; accepted 24 May 2005

Abstract

Salusins are two newly discovered TOR-related peptides consisting of 28 and 20 amino acids and designated salusin-alpha and salusin-beta, respectively. Using immunohistochemistry techniques, salusin-like immunoreactivity was detected in the rat hypothalamo-neurohypophyseal tract and immunopositive cells were distributed in the suprachiasmatic, supraoptic and paraventricular nucleus. In the paraventricular nucleus, salusin-like immunoreactivity was observed both in parvocellular and magnocellular neurons. Many salusin-positive nerve fibers and their terminals were identified in the internal layer of the median eminence and posterior pituitary. Less intense salusin-positive staining of fibers and terminals was found in the suprachiasmatic nucleus and external layer of the median eminence. Dual immunostaining was performed to determine if salusin coexisted with vasopressin or oxytocin in the hypothalamus. Most of the salusin-like immunoreactivity was detected in vasopressin- but not in oxytocin-containing neurons in these nuclei. The functional significance of the coexistence of salusin with vasopressin is discussed, including the possibility that salusin participates in the regulation of blood pressure.

Keywords: Salusin; Immunohistochemistry; Hypothalamus; Hypophysis; Vasopressin

Salusins are a recently identified class of neuropeptides consisting of two related peptides of 28 and 20 amino acids designated salusin-α and salusin-β, respectively [9]. Salusins are expressed and synthesized in human tissues including blood vessels and kidney [9]. They are responsible for causing rapid and profound decreases in blood pressure and heart rate [9], and have recently been shown to promote cardiocyte growth [14], and to depress cardiac contractility by a cholinergic mechanism [5]. These data suggest that salusins may form a new class of regulatory peptides which affect the cardiovascular system.

Studies on the rat have shown that preprosalusin expression was most abundant in bone marrow, moderate in endocrine glands (adrenal, pancreas, thyroid, testis and pituitary) and brain and nearly undetectable in cardiac and skeletal muscle [9]. Immunohistochemical analysis highlighted the presence of salusin proteins in surgically resected cardiovascular and renal tissues. Both salusin-α- and salusin-β-like immunoreactivities were reported to be localized in renal glomeruli and tubules as well as in the endothelium of renal interlobular arteries and saphenous vein tissue, but were not detected in cardiac tissue [9]. The hypothalamus and pituitary express prosalusin, in addition to which, salusin-β, but not salusin-α, stimulated the release of arginin–vasopressin (AVP) from the posterior pituitary in a concentration-dependent manner [9].

These results suggest a potential role for salusin-β as a neuropeptide that regulates water homeostasis by regulating vasopressin release. Because the distribution and localization of salusins in the rat brain are not well known, we have used immunohistochemistry techniques in the present study

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0304-3940/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2005.05.061

to determine these properties and to examine the coexistence of salusins with posterior pituitary hormones such as AVP and oxytocin (OT).

Adult male Sprague–Dawley rats (250–300 g body weight; $n = 7$: Saitama Experimental Animal Center, Saitama, Japan) were maintained on a 12-h light/12-h dark cycle and supplied with standard laboratory chow and water ad libitum. For detection of salusin-immunoreactive cell bodies and fibers in the hypothalamus, rats were perfused through the ascending aorta with 50 ml of saline (37 °C), followed by 250–300 ml of 2% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4) for 20 min. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Showa University. Brains were removed, trimmed, and immersed in the same fixative for 12 h at 48 °C. After washing, the fixed brains were transferred to a solution containing 20% sucrose in 0.1 M PB for 2 days at 4 °C. Brains were then embedded in a solution of O.C.T. compound (Sakura Finetechnical, Tokyo, Japan) and were quickly snap frozen in isopentane cooled with liquid nitrogen.

The frozen brain tissues were cut into 7-μm-thick coronal sections on a cryostat (MICROM HM 500; MICROM, Heidelberg, Germany), and mounted on glass slides coated with 3% poly-L-lysine solution (Sigma, St. Louis, USA). The sections were washed in phosphate-buffered saline (PBS) for 15 min and incubated overnight with 0.3% Triton X-100 in PBS at 4 °C. The sections were subsequently rinsed with PBS for 15 min and then blocked with 10% normal horse serum in PBS for 1 h at room temperature. The sections were then incubated with rabbit polyclonal anti-salusin-beta antibody (1/15000) overnight at room temperature. This was followed by washing in PBS before incubation with Alexa 546-labeled goat anti-rabbit IgG antibody (1/400, Molecular Probes, Eugene, OR, USA) again overnight at room temperature. For dual immunostaining, the coronal sections were first incubated with guinea pig anti-AVP antiserum (1/4000, Peninsula Laboratories, Inc., USA) or mouse anti-OT antiserum (1/15000, Chemicon International, Inc., USA) for 24 h at room temperature. These sections were then incubated with Alexa 488-labeled anti-mouse antibody or anti-guinea pig antibody (1/400, Molecular Probes) for 2 h at room temperature. Sections were examined with the use of an Olympus AX-70 fluorescence microscope (Olympus, Tokyo, Japan). The anti-salusin-β (1–8) antibody does not recognize salusin-α or larger proteins such as prosalusin [9]. Control experiments were carried out by preabsorption of the 1:15,000 diluted anti-salusin antibody with salusin-β at a concentration of 10 nM. Immunostaining was completely abolished by absorbing the anti-salusin antibody with antigen. Some sections in which no anti-salusin antiserum was used in the incubation process were used as controls. No evidence of immunofluorescence was observed in these sections.

Salusin-like immunoreactivity (LI) in the rat hypothalamus was identified in *some* neurons of the magnocellular hypothalamo-neurohypophyseal system (Fig. 1). In addition, salusin-LI was also detected in *some* neurons of the suprachiasmatic nucleus (SCN) (Fig. 1A) and parvocellular neurons of the paraventricular nucleus (PVN) (Fig. 1B). Cell bodies exhibiting salusin-LI were observed ventrally located in the supraoptic nucleus (SON) (Fig. 1A) and centrally located in the PVN. Many nerve fibers and terminals with salusin-LI were found in the internal layer of the median eminence (Fig. 1F and G) and posterior pituitary (Fig. 1H and I). Moderate salusin-LI was observed in the external layer of the median eminence (Fig. 1G) and in the SCN (Fig. 1A and D). In the dual immunofluorescence experiments, antisera against salusin and AVP or OT were used in this study. The coexistence of salusin- and AVP-LI neuronal cell bodies in the SON (Fig. 2A) and PVN (Fig. 2C) was observed. Approximately 90% of AVP-positive magnocellular neurons were found to coexist with salusin-LI in the SON (Fig. 2A) and PVN (Fig. 2C). Moreover, there is co-existence of salusin-LI with AVP in the magnocellular dendritic processes (Fig. 2C). However, salusin-LI was not detected in OT-containing neurons in the SON (Fig. 2B) and PVN (Fig. 2D).

The present study is the first to detect the presence of salusin in the hypothalamo-hypophyseal system but also it would appear in the AVP parvocellular neurons of the PVN projecting to the external zone of the median eminence. The magnocellular neurons of the SON and PVN synthesize AVP and OT, and axonally transport these neuropeptides to the posterior pituitary where they are released and subsequently elicit their actions on vascular tissue. It is generally accepted that the posterior pituitary hormones are secreted from axon terminals in the posterior pituitary. AVP and OT are released from their dendrites [7,12]. It is also demonstrated here that salusin-LI coexists with AVP-containing magnocellular neurons and dendritic processes of the hypothalamus, suggesting that salusin may be secreted from magnocellular dendrites. The coexistence of salusin with AVP in the posterior pituitary suggests that salusin may be co-secreted with AVP into general circulation. The coexistence of corticotropin-releasing hormone (CRH) with OT and AVP has been demonstrated in neurons [1,13]. Parvocellular CRH neurons express AVP when particularly stimulated by adrenalectomy [8,13]. Robust coexistence of salusin and AVP in parvocellular PVN neurons was demonstrated in this study, suggesting that salusin may be expressed in the CRH neurons. Thus, it is needed to make experiments whether salusin is co-existed and co-released with CRH.

The physiological significance of salusin in the hypothalamo-neurohypophyseal system is not fully understood. Salusin-α and salusin-β cause rapid and profound decreases in blood pressure and heart rate [9], and it is possible that the hypotensive potency of salusin-β in rats is greater than that of the most potent endogeneous hypotensive peptides thus far identified such as calcitonin gene-related peptide [2,3,11] or adrenomedullin [4,6,10]. Salusin-β stimulates the release of AVP from perfused rat pituitaries, demonstrating its potent role as a neural hemodynamic regulator not only via systemic cardiovascular