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Identification of orexin-A immunoreactivity in ostrich (*Struthio camelus camelus*) kidneys

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ABSTRACT

Orexin-A (hypocretin-1), a neuropeptide, was originally shown to be specifically expressed in the hypothalamus. We studied the presence of orexin-A immunoreactivity in ostrich kidneys. Immunocytochemistry showed that orexin-A immunoreactivity was localized in the tubules of the ostrich kidneys. Immunoreactivity was found both in the renal cortex and the medulla. Immunostaining was mainly localized in the apical border and lumen of tubules.

Key words: orexin-A, ostrich, kidney, immunoreactivity

Introduction

Orexins (hypocretins) are neuropeptides that were originally shown to be specifically expressed in the hypothalamus. Orexins consist of orexin A (OXA; hypocretin 1) and orexin B (OXB; hypocretin 2) which are 33- and 28-amino acid peptides originating from a single precursor, produced by prepro-orexin (PPO) gene (DE LECEA et al., 1998; SAKURAI et al., 1998). There are at least two types of orexin receptors: orexin receptors 1 and 2, which are both G protein-coupled receptors and display different affinities for the two orexin peptides. Orexin receptor 2 binds orexin-A and -B with the similar affinities, whereas orexin receptor 1 is 30-100 times more responsive to orexin-A than orexin-B (SAKURAI et al., 1998).

Orexins are thought to be involved in the control mechanisms for food intake (WILLIE et al., 2001), sleep (MONDA et al., 2003), thermoregulation and autonomic and cardiovascular functions (SHIRASAKA et al., 1999). Although orexins and their

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functions have been mainly described in the central nervous system (CNS), there are substantial data implying that orexins may also function outside the CNS. Orexins and their receptors have been detected in a wide range of tissues, including the intestine, pancreas, adrenals, kidney, adipose tissue and reproductive tract in mammals (JOHREN et al., 2001; KIRCHGESSNER and LIU, 1999; NAKABAYASHI et al., 2003; SILVEYRA et al., 2007a; SILVEYRA et al., 2007b). Also, in chickens OXR is cloned widely in the brain and abundant expression is observed in the cerebrum, hypothalamus (OHKUBO et al., 2003).

Although the precise functions of orexins still remain unclear in many tissues, it has been reported that orexin A, but not orexin B, penetrates the blood-brain barrier by simple diffusion (KASTIN and AKERSTROM, 1999). Orexins also affect insulin release, intestinal motility and secretion (NOWAK et al., 2000). Furthermore, orexins have anti-growth effects in cultured colon cancer and neuroblastoma cells (ROUET-BENZINEB et al., 2004). Orexin A-like immunoreactivity is present in human plasma (ARIHARA et al., 2001) and plasma concentrations of orexinA-LI were elevated in patients with chronic renal failure (SUGIMOTO et al., 2002).

In the kidneys, orexin receptor 1 has been found in the rat (JOHREN et al., 2001), and orexin receptor 1 and orexin receptor 2 mRNA in humans (NAKABAYASHI et al., 2003; TAKAHASHI et al., 2006). In addition to these reports, TAKAHASHI et al. (2006) reported localization of orexin A immunoreactivity in human kidney tubules.

Although orexins have been studied in some detail in mammal kidneys, no information is so far available about the occurrence and or distribution of orexins in the kidney of non-mammals. Thus, in order to help completion of phylogenetic analysis in the expression of this neuropeptides in the kidney of vertebrates, the presence and localization of orexin A immunoreactivity are reported here in avian kidneys.

Materials and methods

Animals and tissue samples. Five adult male ostriches were used. Birds with a body mass of 45-60 kg were anaesthetized by injecting pentobarbitone sodium. The left carotid artery was cannulated at the base of the neck and allowed to exsanguinate. Tissue samples were immediately collected from the kidneys and fixed in 4% neutral-buffered formalin for 24 h. They were then dehydrated through graded ethanol and embedded in paraffin. Seven µm-thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemistry. Immunohistochemical staining was carried out using the peroxidase-antiperoxidase (PAP) method. Blocking of endogenous peroxidase was carried out with 3% hydrogen peroxidase (H_2O_2) in methanol for 10 minutes (STERNBERGER, 1979). In order to block unspecific binding, incubation with normal goat serum in 0.1 M phosphate buffered saline (PBS), pH 7.2 (Dilution 1:10) was performed. Sections were incubated for 16-20 hours at 4 °C with rabbit IgG antibodies against Orexin-A (Millipore,

AB3704). The primary antibodies were diluted to 1:3000 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin (BSA). Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421, Denmark) followed by rabbit peroxidase anti-peroxidase complex (Zymed Lab., 61.2003, San Francisco), both at a dilution of 1:50 in PBS, for 1 hour at room temperature. Sections were washed in PBS for 30 minutes after each incubation and finally immersed in glucose oxidise-DAB-nickel ammonium sulphate substrate (SHU et al., 1988) for 10 minutes. After washing in distilled water and counterstaining with hematoxylin, sections were dehydrated and coverslips mounted with aqueous permanent mounting medium.

The controls for the specificity of immunhistochemical reactions were performed by the pre-absorption of each antiserum with the corresponding antigen (STERNBERGER, 1986). Sections were examined with an Olympus BX-51 microscope and photographs were taken.

Results

Immunohistochemistry showed that orexin-A immunoreactivity was localized in the renal tubular cells of the ostrich kidneys. Immunoreactive tubules were localized both in the renal cortex (Fig. 1A) and the medulla (Fig. 1B). In the renal tubules, immunostaining was observed generally in the apical surface of the cells. Proximal convoluted tubules were showed more dense immunoreactivity, and staining was localized mainly in the microvillus and lumen (Fig. 1A). No positive immunostaining was found in the glomerulus or in the vasculature.

The negative control using non-immune rabbit serum gave no positive immunostaining.

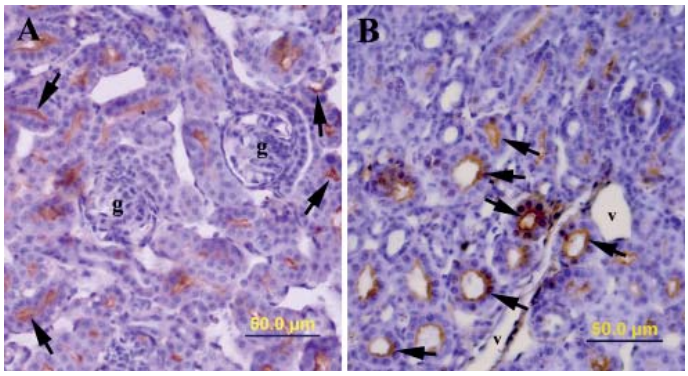

Fig. 1. Orexin A immunoreactive tubules in the renal cortexs (Fig. 1A arrows) and medulla (Fig. 1B arrows) of ostrich kidney. Positive immunoreactivity in the microvillus and lumen of proximal convoluted tubules (Fig. 1A arrows). Glomerulus (g) and vascularite (v) were not showed immunoreactivity (Fig. 1A and B).

Discussion

Orexins and their receptors have been detected in a wide range of organs outside the CNS and several groups have demonstrated their involvement in a number of physiological processes (JOHREN et al., 2001; KIRCHGESSNER and LIU, 1999; NAKABAYASHI et al., 2003; SILVEYRA et al., 2007a; SILVEYRA et al., 2007b). Orexins stimulate arousal and appetite in the brain (CHEMELLI et al., 1999; HAGAN et al., 1999; SAKURAI et al., 1998). Orexins may stimulate insulin secretion (NOWAK et al., 2000) and adrenocortical hormones (JOHREN et al., 2004).

On the other hand, the precise mechanism of the action of orexins has not been clarified in the kidney. However, TAKAHASHİ et al. (2006) reported that orexin-A is produced by human kidneys, particularly the renal tubular cells and they raised the possibility that orexin-A may regulate tubular function and renal circulation as an autocrine or paracrine regulator. They also revealed that orexin-A is secreted to the urine by renal tubular cells, and they suggested that it may regulate renal reabsorbtion and secretion as an urocrine mediator.

In the present study, orexin-A immunoreactivity was examined and detected in the ostrich kidney. This is the first report of the localization of orexin-A in the avian kidney. Immunocytochemistry showed that orexin-A immunoreactivity was localized in the tubules. Renal tubular cells were diffusely immunostained with orexin-A, both in the renal cortex and the medulla. No positive immunostaining was found in the glomerulus or in the vasculature.

In conclusion, the results obtained indicated that there are no clear differences between the ostrich and human concerning the existence and localization of orexin-A in the kidney. Thus, we can suggest that orexin-A might be involved in a similar function in avian kidneys as reported in mammalian kidneys (TAKAHASHI et al., 2006). On the other hand, our results raised the possibility that orexin-A may regulate the tubular function in the kidney as an autocrine or paracrine mediator. Further study, however, is required to reveal the precise effects of orexin-A in avian kidneys.

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SAŽETAK

Neuropeptid oreksin-A (hypokretin-1) izvorno je bio dokazan u hipotalamusu. U ovom je radu njegova imunoreaktivnost istražena u bubregu noja. Imunohistokemijski je dokazana njegova imunoreaktivnost u bubrežnim tubulima. Dokazana je također u kori i srži bubrega. Aktivnost je pretežito bila lokalizirana u apikalnom području i lumenu bubrega.

Ključne riječi: oreksin A, noj, bubreg, imunoreaktivnost, kora, srž
