Neuroscience 238 (2013) 297–304

CENTRAL PACAP MEDIATES THE SYMPATHETIC EFFECTS OF LEPTIN IN A TISSUE-SPECIFIC MANNER

M. TANIDA,[a,b]* A. HAYATA,[c] N. SHINTANI,[c] N. YAMAMOTO,[d] Y. KURATA,[b] T. SHIBAMOTO,[b] D. A. MORGAN,[e] K. RAHMOUNI[e,f] AND H. HASHIMOTO[c,g]

[a] *Department of Biomedical Sciences, College of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan*

[b] *Department of Physiology II, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan*

[c] *Laboratory of Molecular Neuropharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan*

[d] *College of Pharmacology, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan*

[e] *Departments of Pharmacology, University of Iowa Carver, College of Medicine, Iowa City, IA 52242, USA*

[f] *Internal Medicine, University of Iowa Carver, College of Medicine, Iowa City, IA 52242, USA*

[g] *United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*

Abstract—We previously demonstrated that the peptidergic neurotransmitter pituitary adenylate cyclase-activating polypeptide (PACAP) affects the autonomic system and contributes to the control of metabolic and cardiovascular functions. Previous studies have demonstrated the importance of centrally-mediated sympathetic effects of leptin for obesity-related hypertension. Here we tested whether PACAP signaling in the brain is implicated in leptin-induced sympathetic excitation and appetite suppression. In anesthetized mice, intracerebroventricular (ICV) pre-treatment with $PACAP_{6-38}$, an antagonist of the PACAP receptors (PAC1-R and VPAC2), inhibited the increase in white adipose tissue sympathetic nerve activity (WAT-SNA) produced by ICV leptin (2 μg). In contrast, leptin-induced stimulation of renal sympathetic nerve activity (RSNA) was not affected by ICV pre-treatment with $PACAP_{6-38}$. Moreover, in PACAP-deficient (*Adcyap1*−/−) mice, ICV leptin-induced WAT-SNA increase was impaired, whereas RSNA response was preserved. The reductions in food intake and body weight evoked by ICV leptin were attenuated in *Adcyap1*−/− mice. Our data suggest that hypothalamic PACAP signaling plays a key role in the control by leptin of feeding behavior and lipocatabolic sympathetic outflow, but spares the renal sympathetic traffic.

Key words: leptin, autonomic nerve, kidney, white adipose tissue, feeding behavior, pituitary adenylate cyclase-activating polypeptide.

INTRODUCTION

The hormone leptin is produced mainly in the white adipose tissue (WAT), released into the circulation, and acts on the central nervous system. Leptin is involved in appetite suppression, thermogenesis, and lipolysis acceleration (Shen et al., 2007; Kalil and Haynes, 2012). Leptin stimulation of the sympathetic nerve activity (SNA) to brown adipose tissue, and WAT is important for the metabolic regulation. In addition, leptin causes sympathetic activation to the kidney, resulting in blood pressure elevation (Shen et al., 2007; Rahmouni, 2010). In obese hypertensive animals, the high levels of plasma leptin are associated with the inability of leptin to properly regulate thermogenic sympathetic traffic and feeding behavior, but intact renal sympathetic and arterial pressure responses to leptin (Rahmouni et al., 2005; Tanida et al., 2006), implicating selective leptin resistance as a major mechanism in obesity-associated hypertension.

The pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide and member of the vasoactive intestinal polypeptide (VIP)/secretin/glucagon family (Miyata et al., 1989; Hashimoto et al., 2001), was recently identified by immunohistochemical study in the hypothalamus (Hannibal, 2002), which is major site of leptin action (Kim et al., 2011). Central PACAP was found to regulate homeostatic functions such as appetite (Mounien et al., 2009), body temperature (Hawke et al., 2009; Resch et al., 2011), energy metabolism (Inglott et al., 2011), the cardiovascular system (Tanida et al., 2010; Farnham et al., 2012), and the autonomic nervous system (Tanida et al., 2010). In particular, we recently found that intracerebroventricular (ICV) injection of PACAP stimulated SNA to the kidney and adipose tissue, and raised arterial pressure in anesthetized rats (Tanida et al., 2010). Furthermore, sympathetic and hyperglycemic responses to some stressful stimuli including strong light exposure (Hatanaka et al., 2008), immobilization, and/or ether exposure (Tanida et al., 2010) were disrupted in *Adcyap1*−/− mice.

With regard to PACAP neurotransmission in the brain, it is considered that PACAP-induced responses result

*Correspondence to: M. Tanida, Department of Physiology II, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan. Tel: +81-76-218-8105; fax: +81-76-286-8010.
E-mail address: mtanida@kanazawa-med.ac.jp (M. Tanida).

Abbreviations: BP, blood pressure; CART, cocaine- and amphetamine-regulated transcript; HR, heart rate; ICV, intracerebroventricular; IP, intraperitoneal; MAP, mean arterial pressure; PAC1-R, PACAP-specific receptor; PACAP, pituitary adenylate cyclase-activating polypeptide; RSNA, renal sympathetic nerve activity; SEM, standard error of the mean; SNA, sympathetic nerve activity; VIP, vasoactive intestinal polypeptide; WAT-SNA, white adipose tissue sympathetic nerve activity.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.02.016

from interaction with three receptor types expressed in the hypothalamus: the PACAP-specific receptor (PAC1-R); the PACAP/VIP mutual receptors, VPAC1-R, and VPAC2-R (Ishihara et al., 1992; Lutz et al., 1993; Spengler et al., 1993). Of note, in mice ICV PACAP-evoked suppression of appetite and elevation of body temperature were abolished by pre-injection of $PACAP_{6-38}$, an antagonist of PAC1-R and VPAC2 (Hawke et al., 2009). In addition, light-induced phase delay was also attenuated by ICV $PACAP_{6-38}$ (Bergström et al., 2003).

There is evidence for the regulation of PACAP signaling by leptin in the brain: ICV injection of leptin increased the expression of PACAP mRNA in the ventromedial hypothalamus, and leptin-induced appetite suppression and body temperature elevation were eliminated by pre-treatment with $PACAP_{6-38}$ (Hawke et al., 2009). In addition, neuroanatomical studies showed that PACAP receptors (Usdin et al., 1994; Hashimoto et al., 1996; Vertongen et al., 1997) or leptin receptor-containing hypothalamic nuclei (Elmquist et al., 1998) are expressed in the hypothalamus that innervates the kidney and WAT through the sympathetic nerves (Bamshad et al., 1998; Cano et al., 2004). These lines of evidence suggested that hypothalamic PACAP signaling may be an important mediator of central leptin actions on food intake, cardiovascular, and metabolic regulation through the autonomic nervous system; however, it remains unclear whether leptin-induced sympathetic activation is mediated by the hypothalamic PACAP pathway. Thus, in this study, we evaluated the effects of ICV injection of $PACAP_{6-38}$ on leptin-induced SNA subserving the kidney and WAT. Moreover, we compared regional sympathetic responses to ICV injection of leptin in the *Adcyap1*−/− mice and wild-type mice.

EXPERIMENTAL PROCEDURES

Animals

We used male CD1 (ICR) mice (10 weeks, $n = 34$) for electrophysiological experiment. Generation of *Adcyap1*−/− mice by a gene targeting technique has been reported previously (Hashimoto et al., 1996). The null mutation was backcrossed onto the genetic background of Crlj:CD1 (Institute of Cancer Research, Charles River, Tokyo, Japan) at least 10 times. All wild-type control ($n = 29$) and *Adcyap1*−/− mice ($n = 17$) used were obtained from the intercross of animals heterozygous for the mutant PACAP gene, and experiments were conducted with naive male mice 10 weeks of age. Animals were housed in a temperature-controlled room with a 12-h light–dark cycle. Food and water were freely available. Animals were adapted to the experimental environment for at least 1 week prior to the experiment. All animal care and handling procedures were approved by the Institutional Animal Care and Use Committee of the Ritsumeikan University.

Recording of sympathetic nerve discharge and cardiovascular parameters

On the day of the experiment, food was removed 5 h prior to surgery. Under anesthesia induced by intraperitoneal (IP) injection of 1 g/kg urethane (when it was insufficient, 0.2–0.3 g/kg of urethane was added), a polyethylene catheter was inserted into the jugular vein for intravenous injection, and another catheter was inserted into the carotid artery for blood pressure (BP) determination. The mice were then cannulated through the trachea and fixed in a stereotaxic apparatus. Body temperature was maintained at 36.5–37.0 °C using a heating pad and monitored with a thermometer inserted into the rectum. Using a dissecting microscope, the left sympathetic nerve innervating the kidney was exposed through an incision to the left flank and a recording of the renal SNA (RSNA) was made. For recording WAT-SNA, an abdominal testicular blood vessel supplying the testis and adipose tissue of the epididymis was located and the nerve bundle was exposed. The nerve was attached to a pair of stainless-steel electrodes, and then hooked up to electrodes for recording. The electrodes were fixed with a silicon gel (liquid A & liquid B, Kagawa kikai Co., JAPAN) to prevent dehydration and for electrical insulation. The mouse was allowed to stabilize for 10–20 min after being placed on the recording electrodes.

Electrical changes in RSNA and WAT-SNA were amplified 2000–5000 times with a band path of 100–1000 kHz, and monitored by an oscilloscope as described previously (Tanida et al., 2008). Raw data of nerve activity were converted to standard pulses by a window discriminator, which separated discharge from electrical background noise which remained post-mortem. Both the discharge rates and the neurogram were sampled with a Power-Lab analog-to-digital converter for recording and data analysis on a computer. Background noise, which was determined 30–60 min after the animal was euthanized, was subtracted. Nerve activity was rectified and integrated, and baseline nerve activity was normalized to 100%. A catheter inserted in the carotid artery was connected to a blood pressure transducer (DX-100, Nihon Kohden, Japan), and the output signal of the transducer amplified (AP641G, Nihon Kohden, Japan), monitored with an oscilloscope, sampled with the Power-Lab, and stored on a hard disk for off-line analysis to calculate mean arterial pressure (MAP) and heart rate (HR).

Baseline measurements of RSNA, WAT-SNA MAP and HR were made 5 min prior to ICV injection of leptin (2 μg/2 μl vehicle) or vehicle (Phosphate Buffered Saline 2 μl). The dose of leptin was that used for effect in a previous study (Rahmouni et al., 2003). After the start of the injection, these parameters were recorded for 240 min. The effects of $PACAP_{6-38}$ (2.4 nmol/2 μl), antagonist of a specific PAC-1R, on changes in sympathetic activities and cardiovascular parameters by leptin were examined. At the end of the experiment, hexamethonium chloride (10 mg/kg) was administered intravenously to ensure that post-ganglionic efferent sympathetic nerve activity had been recorded.

ICV cannulation

One week before the experiment, a 25-gauge stainless steel guide cannula with a stylet was implanted into the third ventricle area by using a stereotaxic apparatus (coordinates: AP, 0.7 mm posterior to the bregma; L, 0 mm; V, 3.0 mm) under anesthesia (IP injection of ketamine/xylazine) as previously described (Rahmouni et al., 2003). After recovery, the stylet was removed from the guide cannula, and in turn, a 33-gauge injection cannula attached to a 25 μl Hamilton syringe was inserted. The injection cannula was designed to be 1.0 mm longer than the guide cannula in order to reach the floor of the third ventricle area. Subsequently, 2 μl solution was injected during 1 min using a micro syringe pump. After the experiment, in order to check correct cannula placement, the brain was removed and histologically examined by Cresyl Violet staining (Fig. 5).

Determination of plasma leptin, glucose and triglyceride

Food was removed 5 h prior to blood sampling, and under anesthesia induced by IP injection of ketamine/xylazine, blood samples were withdrawn from the celiac vein using the syringe