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Arginine vasopressin inhibits adipogenesis in human adipose-derived stem cells

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ABSTRACT

Intracellular Ca^{2+} signaling is important for stem cell differentiation and there is evidence it may coordinate the process. Arginine vasopressin (AVP) is a neuropeptide hormone secreted mostly from the posterior pituitary gland and increases Ca^{2+} signals mainly via V1 receptors. However, the role of AVP in adipogenesis of human adipose-derived stem cells (hASCs) is unknown. In this study, we identified the V1a receptor gene in hASCs and demonstrated that AVP stimulation increased intracellular Ca^{2+} concentration during adipogenesis. This effect was mediated via V1a receptors, Gq-proteins and the PLC-IP_3 pathway. These Ca^{2+} signals were due to endoplasmic reticulum release and influx from the extracellular space. Furthermore, AVP supplementation to the adipogenic medium decreased the number of adipocytes and adipocyte marker genes during differentiation. The effect of AVP on adipocyte formation was reversed by the V1a receptor blocker V2255. These findings suggested that AVP may function to inhibit adipocyte differentiation.

1. Introduction

Arginine vasopressin (AVP) is synthesized in the paraventricular and supraoptic nucleus of the hypothalamus and is secreted from the posterior pituitary gland mainly in response to dehydration. It is also detected in ovaries, adrenal gland, testis, thymus and pancreas (Ang and Jenkins, 1984; Kasson et al., 1985; Markwick et al., 1986; Schaeffer et al., 1984; Yibchok-anun et al., 2004). AVP mediates its effects via three G protein-coupled receptors, the V1a, V1b, and V2. The V1a receptor can be found in lymphocytes, smooth muscle and mesangial cells (Jessop et al., 1995; Serradeil-Le Gal et al., 1995; Tahara et al., 2008b). The V1b receptor is present in pancreatic α and β cells, astrocytes and adrenal medulla (Grazzini et al., 1996; Lee et al., 1995; Syed et al., 2007; Yibchok-Anun et al., 1999). In addition, AVP binds to V2 receptors in the renal distal tubule and collecting duct cells to promote water reabsorption during dehydration by activating the cAMP pathway (Laycock, 2010). Important physiological roles for AVP include platelet aggregation, liver glycogenolysis, uterine motility, vasoconstriction, cell proliferation and growth. It stimulates protein synthesis via V1a receptors and insulin, glucagon, catecholamine, ACTH secretion via V1b receptors (Koshimizu et al., 2012). Activation of V1 receptors has a significant impact on intracellular Ca^{2+} signals because they are coupled to Gq-proteins and the phospholipase C-β (PLC-β) and inositol triphosphate (IP_3) pathway. Increases in IP_3 result in Ca^{2+} release from the endoplasmic reticulum (ER) and influx from the extracellular space (Thibonnier et al., 1998). Calcium oscillations are required for cell proliferation and differentiation, because it activates transcription factors, such as nuclear factor of activated T-cells (NFAT) (Dolmetsch et al., 1998). Translocation of NFAT into the nucleus is observed during stem cell differentiation, but ends at terminal stages of adipogenic and osteogenic differentiation (Kawano et al., 2006; Sun et al., 2007). Elevation in intracellular Ca^{2+} inhibits adipocyte formation during early stages of differentiation, but facilitates it at later stages (Draznin et al., 1988; Shi et al., 2000). An increase in Ca^{2+} influx activates the mitogen-activated protein kinase (MAPK) pathway to stimulate osteogenic differentiation in mesenchymal stem cells (Sun et al., 2007). Calcium signaling is essential for embryonic and mesenchymal stem cell cycle progression via the G_1/S phase (Kapur et al., 2007; Resende et al., 2010; Todorova et al., 2009). They

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Table 1
List of primers for RT-PCR.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Size (bp)
AVP V1a receptor	CAGGTGTTCGGCATGTTTG	ACCAGATGTTGTAGCAGATGAA	343
AVP V1b receptor	CTCATCTGCCATGAGATCTGTAA	CCACATCTGGACACTGAAGAA	249
AVP V2 receptor	ATTCATGCCAGTCTGGTGC	TCACGATGAAGTGTCCTTGG	422
GAPDH	AACAGCGACACCCACTCCTC	GGAGGGGAGATTCAGTGTGGT	258

up-regulate peroxisome proliferator-activated receptor γ (PPARγ) gene expression, a master transcription factor for adipogenesis. PPARγ increases fatty acid synthase activity and triacylglycerol production in lipid droplets (Shi et al., 2000). Other studies revealed a stimulatory effect of AVP on myoblast and T-cell differentiation via V1 receptors (Gutkowska et al., 2007; Jessop et al., 1995; Toschi et al., 2011). However, AVP inhibits osteoclast differentiation (Lagumdzija et al., 2004).

Other physiological functions for AVP include control of thermogenesis by increasing UCP-1 protein expression for uncoupling oxidative phosphorylation during heat production from brown fat (Kuchler et al., 2010). Studies in V1a receptor knockout mice show enhanced lipid metabolism, muscle proteolysis and insulin signaling suppression that is supported by observations of impaired glucose tolerance in these animals (Aoyagi et al., 2007; Hiroyama et al., 2007; Nakamura et al., 2009). The anti-lipolytic effect is mediated by Ca^{2+} signals and the MAPK pathway (Boston and Cone, 1996; Kuchler et al., 2010). A reduction in adiponectin mRNA is detected after adipocyte stimulation with AVP (Kuchler et al., 2010). This hormone also inhibits ketogenesis by suppressing β-oxidation of fatty acid (Sugden et al., 1980). Pathophysiological conditions such as hypertension and obesity are associated with reduced plasma AVP (Kotchen, 2008; Sukhonthachit et al., 2014). Obese and sedentary individuals have lower AVP levels compared to lean ones, whereas exercise increases hormone secretion (Hew-Butler et al., 2010; Inder et al., 1998). This observation is confirmed in patients before and after weight loss (Coiro and Chiodera, 1987). The same study reported an inverse correlation between AVP and insulin that is consistent with the knowledge that obese non-diabetic individuals have increased insulin and adipose tissue but low AVP levels. Patients with multiple symmetric lipomatosis due to alcohol abuse exhibit large symmetrical accumulation of non-capsulated fat tissue that is associated with reduced AVP secretion caused by alcohol (Angelini, 2014). Interestingly, nicotine stimulates AVP secretion and may in part explain why cigarette smokers gain weight after they stop smoking (Thorgeirsson et al., 2013). Although Ca^{2+} signals are reported in hASCs (Kotova et al., 2014), the mechanism by which AVP increases intracellular Ca^{2+} and its role on adipogenesis is unknown. In this study, we characterized the AVP receptor subtype, its signaling pathway and impact on adipocyte differentiation.

2. Materials and methods

2.1. Reagents

All reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA), except that fura-2 acetoxymethyl ester (Fura-2AM) was from Anaspec (Fremont, CA, USA), d-calcium pantothenate was from Fisher Scientific (Pittsburgh, PA, USA), [Arg8] vasopressin (AVP) from American Peptide Co. (Sunnyvale, CA, USA) and 2-APB from Cayman Chemical Co. (Ann Arbor, MI, USA).

2.2. Cell culture

Human adipose-derived stem cells were isolated from lipoaspirates of abdomen, breast adipose tissues, right knee and right scapula donated by consenting two Caucasian females and a male, age from 34 to 66 years old, BMI from 23.5 to 33.78 under a protocol reviewed and approved by the Pennington Biomedical Institutional Review Board (#PBRC23040) and maintained in Dulbecco's modified Eagles medium (DMEM)/Ham's F-12 medium with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, GA, USA) and aerated with 5% CO_2 at 37 °C. All experiments were performed with cells from passages 3–8.

2.3. RT-PCR

Total RNA was extracted using the RNAqueous-4PCR® kit according to the manufacturer's instructions (Ambion, Austin, TX, USA). The RNA was treated with DNase 1 to remove DNA contamination. Reverse transcription and PCR were performed using Ambion's RETROscript® kit. The human PCR primers (forward/reverse [5′–3′]) are listed in Table 1. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and dH_2O were used as positive and negative controls, respectively.

2.4. Quantitative RT-PCR

From each sample, 2 μg of total RNA was reverse-transcribed into 20 μl of cDNA. Each PCR was prepared by mixing 2 μl of the cDNA with 2× SYBR Green PCR master mix (Bio-Rad, Hercules, CA, USA) and gene-specific primers (Table 2). The PCR was carried out with

Table 2
List of primers for qRT-PCR.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Size (bp)
C/EBPα	CGGTGGACAAGAACAGCAAC	CGGAATCTCCTAGTCCTGGC	365
C/EBPβ	CACAGCGACGACTGCAAGATCC	CTTGAACAAGTTCCGCAGGGTG	188
PPARγ2	GCTGTTATGGGTGAAACTCTG	ATAAGGTGGAGATGCAGGTTC	325
aP2	TGGTTGATTTTCCATCCCAT	TACTGGGCCAGGAATTTGAT	150
Adiponectin	GGCCGTGATGGCAGAGAT	TTTCACCGATGTCTCCCTTAGG	88
LPL	GAGATTTCTCTGTATGGCACC	CTGCAAATGAGACACTTTCTC	276
Leptin	GGCTTTGGCCCTATCTTTTC	GCTCTTAGAGAAGGCCAGCA	325
Cyclophilin B	GGAGATGGCACAGGAGGAAA	CGTAGTGCTTCAGTTTGAAGTTCTCA	72