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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-IP₃ 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.

Keywords

Human adipose-derived stem cells; Arginine vasopressin; Ca^{2+} signalling; Differentiation

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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 G₁/S phase (Kapur et al., 2007; Resende et al., 2010; Todorova et al., 2009). They up-regulate peroxisome proliferator-activated receptor γ (PPAR γ) gene expression, a master transcription factor for adipogenesis. PPAR γ 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; Hiroshima 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 RNeasy-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 dH₂O 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 ABI 7300 real-time PCR system (Life Technologies, Grand Island, NY, USA) to obtain the C_T value. Relative gene expression (RGE) was calculated by the delta C_T method using β -actin as the endogenous control for normalization and cyclophilin B as the reference control with an RGE of 1.

2.5. Calcium imaging analysis

Cells were loaded with 2 µM Fura-2AM for 30 min at 37 °C. The imaging buffer containing (in mM): NaCl 136, KCl 4.8, CaCl₂ 1.2, MgSO₄ 1.2, HEPES 10, glucose 4, and 0.1% BSA, pH 7.3 was used for Fura-2AM loading and perfusion throughout the experiments. Calcium measurements were obtained using a dual excitation fluorometric imaging system (TILL-Photonics, Gräfelfingen, Germany) controlled by TILLvisION software. Fura-2AM loaded cells in perfusion chamber were excited by 340 nm and 380 nm wavelengths. Fluorescence emissions were sampled at a frequency of 1 Hz and computed into relative ratio units of the fluorescence intensity of the difference of wavelengths (F340/F380). Data were expressed as averages from several cells from three separate experiments.

2.6. Induction and detection of lipid droplets

Cells reaching between 80% and 90% were induced with differentiation medium containing DMEM/Ham's F-12 supplemented with 3% FBS, 0.25 mM 3-isobutyl-1-methylxanthine (IBMX), 33 µM biotin, 17 µM d-calcium pantothenate, 100 nM human insulin, 1 µM dexamethasone and 5 µM rosiglitazone for 3 days. Adipocyte medium having the same composition as differentiation medium except for IBMX and rosiglitazone was changed every 3 days from day 3 to day 21 (Yu et al., 2011). To test the effects of AVP and V2255 on adipogenesis, 100 nM AVP and/or 1 µM V2255 was supplemented to the adipogenic medium and changed every 2 days. Adipogenesis was determined by Oil Red O (ORO) staining. Briefly, cells were washed with PBS, fixed with 10% formalin for 10 min, washed twice with dH₂O, and incubated with 60% isopropanol for 5 min. Cells were then stained with ORO solution for 5 min and washed again to remove excess dye. The presence of lipid droplets was visualized by phase contrast microscopy using an inverted microscope (Zeiss, Thornwood, NY, USA).

2.7. Data analysis

Adipocyte count was performed from a total of 36 or 18 frames taken at 10× magnification from each group using software ImageJ (1.47v; <http://imagej.nih.gov/ij>). The peak Ca²⁺ signals and adipocyte counts are shown as means ± S.E.M. and were analyzed using a twotailed and unpaired Student's *t*-test (GraphPad Software Inc., LaJolla, CA, USA). Statistical significance was established at $P < 0.05$.

3. Results

3.1. hASCs differentiate into adipocytes and express the V1a receptor gene

In order to demonstrate that hASCs can differentiate into adipocytes, we cultured the cells in adipogenic medium and examined the differentiated cells with ORO staining at the end of 14 days. Adipogenesis was confirmed by the presence of lipid droplet accumulation (Fig. 1A). Next, we examined the type of AVP receptor in hASCs at day 0 and 14 of differentiation. RT-PCR analysis revealed V1a receptor gene expression, but not V1b or V2 (Fig. 1B).

3.2. AVP increases intracellular Ca^{2+} in hASCs under growth and adipogenic conditions

Since hASCs expressed the V1a receptor before and after differentiation, we performed real-time Ca^{2+} imaging analysis to investigate the responses to AVP. Stimulation of cells with 1 μM AVP increased intracellular Ca^{2+} at days 0, 7, 14 and 21 of differentiation (Fig. 2A). However, there was a greater response to AVP at day 14 compared to day 21 of differentiation (Fig. 2B). Therefore, we chose day 14 for our experiments since at this time period, we also detected adipocyte differentiation. To confirm the AVP responses in hASCs, cells were stimulated with 0.003–1 μM AVP that resulted in a concentration-dependent increase in intracellular Ca^{2+} with a peak at 1 μM concentration (Fig. 2C and D). In order to test whether the V1a receptor indeed mediated the effect of AVP, cells were pretreated for 5 min with 0.0001–1 μM V2255, a selective V1a receptor blocker. V2255 inhibited the Ca^{2+} signals in a concentration-dependent manner in both undifferentiated and differentiated cells (Fig. 3A–D).

3.3. AVP increases intracellular Ca^{2+} via the PLC- IP_3 pathway

AVP stimulates Ca^{2+} signals via the V1a receptor in smooth muscle and intestinal epithelial cells by activating the phospholipase C enzyme (PLC) (Chiu et al., 2002; Thibonnier et al., 1991). Therefore, we utilized the PLC blocker, U73122 to test whether it could inhibit AVP signaling. U73122 (2–12 μM) pretreatment for 5 min inhibited the responses to AVP in a concentration-dependent manner (Fig. 4A and B). Pretreatment of cells with U73343, an inactive analog of U73122 did not alter the responses to AVP (Fig. 4C and D). Since IP_3 is a downstream second messenger to PLC, we utilized 2-APB, an IP_3 receptor blocker. Pretreatment of cells for 5 min with 10–300 μM 2-APB inhibited the responses to AVP in a concentration-dependent manner (Fig. 5A and B). In addition, we compared the maximum AVP response in hASCs to those of 2 μM ionomycin and 100 μM ATP, another Gq-coupled receptor agonist (Fig. 5C and D).

3.4. Sources of Ca^{2+} for AVP signals

In bone marrow-derived stem cells, Ca^{2+} influx and release from the ER generate the Ca^{2+} signals (Kawano et al., 2002). Hence, we tested in hASCs the contribution of extracellular Ca^{2+} on AVP signaling by performing experiments under Ca^{2+} free buffer condition. Removal of extracellular Ca^{2+} significantly reduced the Ca^{2+} signals compared to control cells in Ca^{2+} containing buffer (Fig. 6A). Next, we pretreated cells with thapsigargin (TG) for 20 min, a Ca^{2+} -ATPase pump inhibitor in the ER to investigate the impact of

intracellular Ca^{2+} on AVP signals. Depletion of Ca^{2+} stores with TG completely abolished the responses to AVP (Fig. 6B). Under extracellular free Ca^{2+} and TG conditions, AVP also failed to increase intracellular Ca^{2+} (Fig. 6C and D).

3.5. AVP inhibits adipogenesis during hASC differentiation

To investigate the effect of AVP on adipogenesis, we supplemented the differentiation medium with 100 nM AVP which enhances differentiation of other cell types (Gassanov et al., 2007; Gutkowska et al., 2007; Nervi et al., 1995). AVP supplementation reduced lipid droplet accumulation as determined by ORO staining (Fig. 7A–C) and decreased adipocyte formation compared to control cells without AVP (Fig. 7D). The hormone also decreased the expression of adipocyte marker genes: CCAAT/enhancer-binding protein α (C/EBP α), CCAAT/enhancer-binding protein β (C/EBP β), peroxisome proliferator-activated receptor γ 2 (PPAR γ 2), adipocyte protein 2 (aP2), adiponectin, lipoprotein lipase (LPL) and leptin compared to control group without AVP after 14 days of differentiation (Fig. 8).

3.6. V1a receptor blocker V2255 reverses the effect of AVP on adipogenesis

Finally, to confirm the effect of AVP on adipocyte differentiation we performed adipogenic experiments with the V1a receptor blocker V2255. AVP (100 nM) supplementation significantly reduced the number of adipocytes as indicated by lipid droplet accumulation and ORO staining. When V2255 (100 μM) was added to the differentiation medium along with AVP, it reversed the inhibitory effect of AVP on adipogenesis (Fig. 9A and B). In the study, V2255 alone did not alter adipocyte formation.

4. Discussion

The role of AVP (a.k.a. anti-diuretic hormone) in water conservation during dehydration is well established. It acts by promoting Aquaporin channel translocation into the plasma membrane of tubular cells to allow water reabsorption (Yu et al., 2014). Other studies revealed that the hormone is capable of stimulating cellular growth and proliferation. Despite the identification of AVP receptors in stem cells (Gassanov et al., 2007), its function in Ca^{2+} signaling and differentiation remains largely unexplored. We investigated the mechanism of AVP signaling and its impact on adipocyte differentiation in hASCs, which is a well-accepted stem cell type for adipogenic studies (Bourin et al., 2013). When placed in adipogenic medium, accumulation of lipid droplets can be evidenced within the cell population that resembles the characteristics of mature adipocytes. We demonstrated that undifferentiated hASCs and differentiated adipocytes express the V1a receptor, but not the V1b or V2. This observation differs from mouse embryonic stem cells that express all three receptor subtypes (Gassanov et al., 2007). It is possible that the pluripotent nature of embryonic stem cells compared to multipotent adult stem cells may be accountable for this variation. The possibility of AVP receptor variation among species also needs to be considered. Since V1a receptors are coupled to Gq-proteins that utilize the PLC-IP $_3$ pathway and Ca^{2+} signaling (Nakamura et al., 2000; Pequeux et al., 2002; Tahara et al., 2008a), we examined whether the Ca^{2+} signals observed in hASCs during AVP stimulation was a result of this mechanism. In addition, we investigated the impact of the hormone on adipocyte differentiation.

Initial experiments demonstrated that hASCs are responsive to AVP throughout the differentiation process, with a peak Ca^{2+} increase at day 14, but decreases at day 21. Other types of adult stem cells (e.g. bone marrow derived) exhibit similar Ca^{2+} signaling pattern during the terminal stages of osteoblast and adipocyte differentiation (Kawano et al., 2006; Sun et al., 2007). It is reasoned that these signals are important for directing and terminating the differentiation process. Furthermore, we confirmed the V1a receptor as the type mediating the effect of AVP before and after differentiation. The V1a receptor blocker V2255 inhibited the responses to AVP in a concentration-dependent manner, which is consistent with the RTPCR data showing V1a gene expression in hASCs. Since receptors coupled to Gq proteins in hASCs activate the PLC enzyme (Kotova et al., 2014), we utilized U73122, a PLC inhibitor to test whether it mediated the effects of AVP. Indeed, pretreatment of cells with U73122 inhibited the Ca^{2+} signals, but not U73343, an inactive analog. Next, we examined the effect of IP_3 a downstream second messenger to PLC on ER Ca^{2+} release. Pretreatment of cells with 2-APB, an IP_3 receptor blocker inhibited the AVP responses in a concentration-dependent manner. These findings revealed that the mechanism by which AVP signals in hASCs is by activating V1a receptors and the PLC- IP_3 pathway. In view that 2-APB abolished the AVP response, it indicated that ER Ca^{2+} release was a source for the Ca^{2+} signals. To test this hypothesis and to determine whether extracellular Ca^{2+} was involved, we performed experiments with TG and under extracellular Ca^{2+} free conditions. Depletion of Ca^{2+} stores by TG resulted in a complete suppression of the AVP responses, but not in the absence of extracellular Ca^{2+} . Under both conditions, AVP also failed to increase intracellular Ca^{2+} . From these results, it is clear that both Ca^{2+} release and influx are required for AVP signaling in hASCs. While most of the Ca^{2+} appears to come from the extracellular space, the ER release is needed to initiate the process. These observations are consistent with those reported for Gq-coupled receptor hormones that utilize the PLC- IP_3 pathway. This mechanism involves both Ca^{2+} release from the ER followed by the opening of store operated Ca^{2+} channels in the plasma membrane via the Ca^{2+} sensor STIM-1 and Orai1 (Peinelt et al., 2006; Prakriya et al., 2006).

One important question that needed to be addressed was the effect of AVP on adipogenesis. This was determined by supplementing the adipogenic differentiation medium with AVP followed by adipocyte quantification and marker gene expression in the cell population. The data demonstrated an inhibitory effect of AVP on adipogenesis since the number of adipocytes was significantly reduced. In addition, key adipogenic marker genes (e.g. C/EBP α , C/EBP β , PPAR γ) were down-regulated. It is possible that AVP's effect on adipogenesis may involve MAPK/Erk signaling since this pathway is activated by the hormone and V1a receptors (He et al., 2008; Keegan et al., 2006). This is supported by findings in bone marrow-derived stem cells where inhibition of MAPK/Erk facilitates adipocyte differentiation (Jaiswal et al., 2000; Liu et al., 2009). Furthermore, MAPK/Erk signaling phosphorylates PPAR γ leading to decreased transcriptional activity and suppression of adipogenesis (Camp and Tafuri, 1997). Most importantly, Ca^{2+} signals inhibit adipogenesis by down-regulating C/EBP α , C/EBP β , C/EBP γ and PPAR γ genes in preadipocytes via MAPK/Erk (Lin et al., 2011).

Based on our results and those presented by others, it appears that AVP exerts mainly an inhibitory effect on adipocyte metabolism and differentiation. This is based on the fact that

the absence of V1a receptor in knockout mice enhances lipolysis but decreases glucose uptake by adipocytes (Hiroyama et al., 2007; Nakamura et al., 2009). AVP also inhibits β -oxidation of fatty acid as an alternative energy source for the body (Sugden et al., 1980). The inhibitory effect of AVP on adipocyte differentiation could be reversed by the V1a receptor blocker V2255 which confirms its role on adipogenesis. It is tempting to speculate that the reduction in plasma AVP levels during obesity or multiple symmetric lipomatosis might be one of the factors responsible for adipose tissue accumulation/formation. Additional studies in V1a receptor knockout mice and humans with V1a receptor mutation show a tendency to develop obesity and diabetes (Aoyagi et al., 2007; Enhorning et al., 2009). A link between AVP and body weight gain in cigarette smokers after quitting has been established due to the stimulatory action of nicotine on AVP secretion (Thorgeirsson et al., 2013). These observations together with our findings in hASCs could provide insights into new therapies for obesity.

In conclusion, we have identified the V1a receptor as the only type for AVP in hASCs. This receptor mediates the effect of AVP on intracellular Ca^{2+} signaling by activating Gq-proteins and the PLCIP₃ pathway. This mechanism appears to exert an inhibitory effect on adipogenesis by down-regulating the expression of key adipogenic genes.

References

- Ang VT, Jenkins JS. Neurohypophysial hormones in the adrenal medulla. *J Clin Endocrinol Metab.* 1984; 58:688–691. [PubMed: 6699132]
- Angelini, C. Multiple Symmetric Lipomatosis, *Genetic Neuromuscular Disorders*. Springer; 2014. p. 251-254.
- Aoyagi T, Birumachi J, Hiroyama M, Fujiwara Y, Sanbe A, Yamauchi J, et al. Alteration of glucose homeostasis in V1a vasopressin receptor-deficient mice. *Endocrinology.* 2007; 148:2075–2084. [PubMed: 17303660]
- Boston BA, Cone RD. Characterization of melanocortin receptor subtype expression in murine adipose tissues and in the 3T3-L1 cell line. *Endocrinology.* 1996; 137:2043–2050. [PubMed: 8612546]
- Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy.* 2013; 15:641–648. [PubMed: 23570660]
- Camp HS, Tafuri SR. Regulation of peroxisome proliferator-activated receptor gamma activity by mitogen-activated protein kinase. *J Biol Chem.* 1997; 272:10811–10816. [PubMed: 9099735]
- Chiu T, Wu SS, Santiskulvong C, Tangkijvanich P, Yee HF Jr, Rozengurt E. Vasopressin-mediated mitogenic signaling in intestinal epithelial cells. *Am J Physiol Cell Physiol.* 2002; 282:C434–C450. [PubMed: 11832328]
- Coiro V, Chiodera P. Effect of obesity and weight loss on the arginine vasopressin response to insulin-induced hypoglycaemia. *Clin Endocrinol (Oxf).* 1987; 27:253–258. [PubMed: 3311483]
- Dolmetsch RE, Xu K, Lewis RS. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature.* 1998; 392:933–936. [PubMed: 9582075]
- Draznin B, Sussman KE, Eckel RH, Kao M, Yost T, Sherman NA. Possible role of cytosolic free calcium concentrations in mediating insulin resistance of obesity and hyperinsulinemia. *J Clin Invest.* 1988; 82:1848–1852. [PubMed: 3143744]
- Enhorning S, Leosdottir M, Wallstrom P, Gullberg B, Berglund G, Wirfalt E, et al. Relation between human vasopressin 1a gene variance, fat intake, and diabetes. *Am J Clin Nutr.* 2009; 89:400–406. [PubMed: 19056558]

- Gassanov N, Jankowski M, Danalache B, Wang D, Grygorczyk R, Hoppe UC, et al. Arginine vasopressin-mediated cardiac differentiation: insights into the role of its receptors and nitric oxide signaling. *J Biol Chem*. 2007; 282:11255–11265. [PubMed: 17298949]
- Grazzini E, Lodboerer AM, Perez-Martin A, Joubert D, Guillon G. Molecular and functional characterization of V1b vasopressin receptor in rat adrenal medulla. *Endocrinology*. 1996; 137:3906–3914. [PubMed: 8756565]
- Gutkowska J, Miszkurka M, Danalache B, Gassanov N, Wang D, Jankowski M. Functional arginine vasopressin system in early heart maturation. *Am J Physiol Heart Circ Physiol*. 2007; 293:H2262–H2270. [PubMed: 17630342]
- He YP, Zhao LY, Zheng QS, Liu SW, Zhao XY, Lu XL, et al. Arginine vasopressin stimulates proliferation of adult rat cardiac fibroblasts via protein kinase C-extracellular signal-regulated kinase 1/2 pathway. *Sheng Li Xue Bao*. 2008; 60:333–340. [PubMed: 18560723]
- Hew-Butler T, Noakes TD, Soldin SJ, Verbalis JG. Acute changes in arginine vasopressin, sweat, urine and serum sodium concentrations in exercising humans: does a coordinated homeostatic relationship exist? *Br J Sports Med*. 2010; 44:710–715. [PubMed: 18801773]
- Hiroyama M, Aoyagi T, Fujiwara Y, Birumachi J, Shigematsu Y, Kiwaki K, et al. Hypermetabolism of fat in V1a vasopressin receptor knockout mice. *Mol Endocrinol*. 2007; 21:247–258. [PubMed: 17021052]
- Inder WJ, Hellemans J, Swanney MP, Prickett TC, Donald RA. Prolonged exercise increases peripheral plasma ACTH, CRH, and AVP in male athletes. *J Appl Physiol*. 1998; 85:835–841. [PubMed: 9729555]
- Jaiswal RK, Jaiswal N, Bruder SP, Mbalaviele G, Marshak DR, Pittenger MF. Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase. *J Biol Chem*. 2000; 275:9645–9652. [PubMed: 10734116]
- Jessop DS, Murphy D, Larsen PJ. Thymic vasopressin (AVP) transgene expression in rats: a model for the study of thymic AVP hyper-expression in T cell differentiation. *J Neuroimmunol*. 1995; 62:85–90. [PubMed: 7499496]
- Kapur N, Mignery GA, Banach K. Cell cycle-dependent calcium oscillations in mouse embryonic stem cells. *Am J Physiol Cell Physiol*. 2007; 292:C1510–C1518. [PubMed: 17092997]
- Kasson BG, Meidan R, Hsueh AJ. Identification and characterization of arginine vasopressin-like substances in the rat testis. *J Biol Chem*. 1985; 260:5302–5307. [PubMed: 3988755]
- Kawano S, Shoji S, Ichinose S, Yamagata K, Tagami M, Hiraoka M. Characterization of Ca(2+) signaling pathways in human mesenchymal stem cells. *Cell Calcium*. 2002; 32:165–174. [PubMed: 12379176]
- Kawano S, Otsu K, Kuruma A, Shoji S, Yanagida E, Muto Y, et al. ATP autocrine/paracrine signaling induces calcium oscillations and NFAT activation in human mesenchymal stem cells. *Cell Calcium*. 2006; 39:313–324. [PubMed: 16445977]
- Keegan BP, Akerman BL, Pequeux C, North WG. Provasopressin expression by breast cancer cells: implications for growth and novel treatment strategies. *Breast Cancer Res Treat*. 2006; 95:265–277. [PubMed: 16331351]
- Koshimizu TA, Nakamura K, Egashira N, Hiroyama M, Nonoguchi H, Tanoue A. Vasopressin V1a and V1b receptors: from molecules to physiological systems. *Physiol Rev*. 2012; 92:1813–1864. [PubMed: 23073632]
- Kotchen TA. Obesity-related hypertension?: weighing the evidence *Hypertension*. 2008; 52:801–802. [PubMed: 18852380]
- Kotova PD, Syssoeva VY, Rogachevskaja OA, Bystrova MF, Kolesnikova AS, Tyurin-Kuzmin PA, et al. Functional expression of adrenoreceptors in mesenchymal stromal cells derived from the human adipose tissue. *Biochim Biophys Acta*. 2014; 1843(9):1899–1908. [PubMed: 24841820]
- Kuchler S, Perwitz N, Schick RR, Klein J, Westphal S. Arginine-vasopressin directly promotes a thermogenic and pro-inflammatory adipokine expression profile in brown adipocytes. *Regul Pept*. 2010; 164:126–132. [PubMed: 20594991]
- Lagumdzija A, Bucht E, Stark A, Hulting AL, Petersson M. Arg-vasopressin increases proliferation of human osteoblast-like cells and decreases production of interleukin-6 and macrophage colony-stimulating factor. *Regul Pept*. 2004; 121:41–48. [PubMed: 15256272]

- Laycock, JF. Perspectives on Vasopressin. Imperial College Press; 2010.
- Lee B, Yang C, Chen TH, al-Azawi N, Hsu WH. Effect of AVP and oxytocin on insulin release: involvement of V1b receptors. *Am J Physiol*. 1995; 269:E1095–E1100. [PubMed: 8572202]
- Lin F, Ribar TJ, Means AR. The Ca²⁺/calmodulin-dependent protein kinase kinase, CaMKK2, inhibits preadipocyte differentiation. *Endocrinology*. 2011; 152:3668–3679. [PubMed: 21862616]
- Liu Q, Cen L, Zhou H, Yin S, Liu G, Liu W, et al. The role of the extracellular signal-related kinase signaling pathway in osteogenic differentiation of human adipose-derived stem cells and in adipogenic transition initiated by dexamethasone. *Tissue Eng Part A*. 2009; 15:3487–3497. [PubMed: 19438323]
- Markwick AJ, Lolait SJ, Funder JW. Immunoreactive arginine vasopressin in the rat thymus. *Endocrinology*. 1986; 119:1690–1696. [PubMed: 3530722]
- Nakamura K, Aoyagi T, Hiroyama M, Kusakawa S, Mizutani R, Sanbe A, et al. Both V(1A) and V(1B) vasopressin receptors deficiency result in impaired glucose tolerance. *Eur J Pharmacol*. 2009; 613:182–188. [PubMed: 19375419]
- Nakamura Y, Haneda T, Osaki J, Miyata S, Kikuchi K. Hypertrophic growth of cultured neonatal rat heart cells mediated by vasopressin V(1A) receptor. *Eur J Pharmacol*. 2000; 391:39–48. [PubMed: 10720633]
- Nervi C, Benedetti L, Minasi A, Molinaro M, Adamo S. Arginine-vasopressin induces differentiation of skeletal myogenic cells and up-regulation of myogenin and Myf-5. *Cell Growth Differ*. 1995; 6:81–89. [PubMed: 7718487]
- Peinelt C, Vig M, Koomoa DL, Beck A, Nadler MJ, Koblan-Huberson M, et al. Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat Cell Biol*. 2006; 8:771–773. [PubMed: 16733527]
- Pequeux C, Breton C, Hendrick JC, Hagelstein MT, Martens H, Winkler R, et al. Oxytocin synthesis and oxytocin receptor expression by cell lines of human small cell carcinoma of the lung stimulate tumor growth through autocrine/paracrine signaling. *Cancer Res*. 2002; 62:4623–4629. [PubMed: 12183418]
- Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature*. 2006; 443:230–233. [PubMed: 16921383]
- Resende RR, Adhikari A, da Costa JL, Lorencon E, Ladeira MS, Guatimosim S, et al. Influence of spontaneous calcium events on cell-cycle progression in embryonal carcinoma and adult stem cells. *Biochim Biophys Acta*. 2010; 1803:246–260. [PubMed: 19958796]
- Schaeffer JM, Liu J, Hsueh AJ, Yen SS. Presence of oxytocin and arginine vasopressin in human ovary, oviduct, and follicular fluid. *J Clin Endocrinol Metab*. 1984; 59:970–973. [PubMed: 6480815]
- Serradeil-Le Gal C, Herbert JM, Delisee C, Schaeffer P, Raufaste D, Garcia C, et al. Effect of SR-49059, a vasopressin V1a antagonist, on human vascular smooth muscle cells. *Am J Physiol*. 1995; 268:H404–H410. [PubMed: 7840290]
- Shi H, Halvorsen YD, Ellis PN, Wilkison WO, Zemel MB. Role of intracellular calcium in human adipocyte differentiation. *Physiol Genomics*. 2000; 3:75–82. [PubMed: 11015602]
- Sugden MC, Ball AJ, Ilic V, Williamson DH. Stimulation of [1-¹⁴C]oleate oxidation to ¹⁴CO₂ in isolated rat hepatocytes by vasopressin: effects of Ca²⁺. *FEBS Lett*. 1980; 116:37–40. [PubMed: 7409134]
- Sukhonthachit P, Aekplakorn W, Hudthagosol C, Sirikulchayanonta C. The association between obesity and blood pressure in Thai public school children. *BMC Public Health*. 2014; 14:729. [PubMed: 25034700]
- Sun S, Liu Y, Lipsky S, Cho M. Physical manipulation of calcium oscillations facilitates osteodifferentiation of human mesenchymal stem cells. *FASEB J*. 2007; 21:1472–1480. [PubMed: 17264165]
- Syed N, Martens CA, Hsu WH. Arginine vasopressin increases glutamate release and intracellular Ca²⁺ concentration in hippocampal and cortical astrocytes through two distinct receptors. *J Neurochem*. 2007; 103:229–237. [PubMed: 17877638]

- Tahara A, Tsukada J, Tomura Y, Suzuki T, Yatsu T, Shibasaki M. Effect of vasopressin on type IV collagen production in human mesangial cells. *Regul Pept.* 2008a; 147:60–66. [PubMed: 18258315]
- Tahara A, Tsukada J, Tomura Y, Yatsu T, Shibasaki M. Vasopressin increases type IV collagen production through the induction of transforming growth factor-beta secretion in rat mesangial cells. *Pharmacol Res.* 2008b; 57:142–150. [PubMed: 18299204]
- Thibonnier M, Bayer AL, Simonson MS, Kester M. Multiple signaling pathways of V1-vascular vasopressin receptors of A7r5 cells. *Endocrinology.* 1991; 129:2845–2856. [PubMed: 1659517]
- Thibonnier M, Berti-Mattera LN, Dulin N, Conarty DM, Mattera R. Signal transduction pathways of the human V1-vascular, V2-renal, V3-pituitary vasopressin and oxytocin receptors. *Prog Brain Res.* 1998; 119:147–161. [PubMed: 10074787]
- Thorgeirsson TE, Gudbjartsson DF, Sulem P, Besenbacher S, Styrkarsdottir U, Thorleifsson G, et al. A common biological basis of obesity and nicotine addiction. *Transl Psychiatry.* 2013; 3:e308. [PubMed: 24084939]
- Todorova MG, Fuentes E, Soria B, Nadal A, Quesada I. Lysophosphatidic acid induces Ca²⁺ mobilization and c-Myc expression in mouse embryonic stem cells via the phospholipase C pathway. *Cell Signal.* 2009; 21:523–528. [PubMed: 19136060]
- Toschi A, Severi A, Coletti D, Catizone A, Musaro A, Molinaro M, et al. Skeletal muscle regeneration in mice is stimulated by local overexpression of V1a-vasopressin receptor. *Mol Endocrinol.* 2011; 25:1661–1673. [PubMed: 21816902]
- Yibchok-anun S, Abu-Basha EA, Yao CY, Panichkriangkrai W, Hsu WH. The role of arginine vasopressin in diabetes-associated increase in glucagon secretion. *Regul Pept.* 2004; 122:157–162. [PubMed: 15491785]
- Yibchok-Anun S, Cheng H, Heine PA, Hsu WH. Characterization of receptors mediating AVP- and OT-induced glucagon release from the rat pancreas. *Am J Physiol.* 1999; 277:E56–E62. [PubMed: 10409128]
- Yu G, Floyd ZE, Wu X, Hebert T, Halvorsen YD, Buehrer BM, et al. Adipogenic differentiation of adipose-derived stem cells. *Methods Mol Biol.* 2011; 702:193–200. [PubMed: 21082403]
- Yu L, Moriguchi T, Souma T, Takai J, Satoh H, Morito N, et al. GATA2 regulates body water homeostasis through maintaining aquaporin 2 expression in renal collecting ducts. *Mol Cell Biol.* 2014; 34:1929–1941. [PubMed: 24636993]

Fig. 1. hASCs differentiation into adipocytes and expression of the V1a receptor gene. (A) hASCs were differentiated into adipocytes for 14 days and stained with Oil Red O (ORO) for the presence of lipid droplets (20× magnification). (B) hASCs expressed the V1a receptor gene at day 0 and day 14 of differentiation. RT-PCR was performed with specific human V1a, V1b, V2 primers. dH₂O and GAPDH served as negative and positive controls, respectively.

Fig. 2. hASCs are responsive to AVP prior to and during adipogenesis. (A) Average Ca^{2+} signals in response to AVP from day 0 to day 21 of differentiation. (B) The AVP response increased until day 14, but decreased at day 21. (C) Average Ca^{2+} signals in response to increasing AVP concentration. (D) Average peak responses to different AVP concentrations. Values are means $\pm$ S.E.M.; $n = 86\text{--}705$ cells; $*P < 0.0001$ compared to day 0.

Fig. 3.

The V1a receptor mediates AVP signaling in hASCs. (A, B) Average traces and peak Ca^{2+} signals generated by AVP after V2255, a selective V1a blocker pretreatment at day 0. (C, D) Same experiments as in A and B, except at day 14 of adipogenic differentiation. Values are means $\pm$ S.E.M.; $n = 51$ –433 cells/concentration.

Fig. 4. Involvement of PLC in the AVP mechanism in hASCs. (A, B) Averages and peak Ca^{2+} signals generated by AVP after pretreatment of cells with the PLC inhibitor, U73122. (C, D) Averages and peak Ca^{2+} signals after pretreatment with U73343, an inactive analog of U73122. Values are means $\pm$ S.E.M.; n = 59–81 cells/concentration; * $P < 0.0001$.

Fig. 5.

IP₃ binding to its receptor initiates the Ca²⁺ signals. (A, B) Average Ca²⁺ signals and peak responses after pretreatment of cells with 2-APB, a selective IP₃ receptor blocker. Values are means ± S.E.M.; n = 77–705 cells/concentration. (C, D) Average Ca²⁺ signals and peak responses induced by ionomycin, AVP and ATP in hASCs. Values are means ± S.E.M.; n = 102–131 cells/concentration.

Fig. 6. Intracellular Ca^{2+} release and influx are sources for the Ca^{2+} signals. (A) Average Ca^{2+} signals during AVP stimulation under extracellular Ca^{2+} and Ca^{2+} free conditions. (B) Depletion of ER Ca^{2+} stores with thapsigargin (TG) abolished the AVP responses. (C) Experiments performed under extracellular Ca^{2+} free condition and TG simultaneously. (D) Average peak responses from experiments in A–C. Values are means $\pm$ S.E.M; $n = 63$ –705 cells/treatment; * $P < 0.0001$ compared to peak control group.

Fig. 7.

AVP inhibits adipogenesis during hASC differentiation. (A, B) Bright-field and ORO staining images taken at day 14 of differentiation from AVP treated and control group without AVP (10 $\times$ magnification). (C) Adipocyte morphology shown at 20 $\times$ magnification from cells in A and B. (D) Adipocyte count per frame from a total of 36 frames from each group. Values are means $\pm$ S.E.M; $n = 471$ (+AVP) and 1213 (–AVP) cells; * $P < 0.0001$.

Fig. 8.

AVP down-regulates adipocyte marker genes in hASCs. Stimulation of cells with AVP during 14 days of adipogenic differentiation decreased the expression of adipocyte marker genes compared to control cells in the absence of AVP.

Fig. 9.

V1a receptor blocker reverses the effect of AVP on adipogenesis. (A) Bright-field and ORO staining images taken at day 10 of differentiation from control adipogenic medium alone and AVP, AVP+V2255 or V2255 supplemented groups (10× magnification). (B) Adipocyte count per frame from a total of 18 frames from each group. Values are means±S.E.M; n = 1869 (control adipogenic medium), 725 (+AVP), 1805 (+AVP and V2255), and 1723 (+V2255) cells; * $P < 0.001$.

Table 1

List of primers for RT-PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)
AVP V1a receptor	CAGGTGTTTCGGCATGTTTG	ACCAGATGTTGTAGCAGATGAA	343
AVP V1b receptor	CTCATCTGCCATGAGATCTGTAA	CCACATCTGGACACTGAAGAA	249
AVP V2 receptor	ATTCATGCCAGTCTGGTGC	TCACGATGAAGTGTCTTGG	422
GAPDH	AACAGCGACACCCACTCCTC	GGAGGGGAGATTCAGTGTGGT	258

Table 2

List of primers for qRT-PCR.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')	Size (bp)
C/EBP α	CGGTGGACAAGAACAGCAAC	CGGAATCTCCTAGTCCTGGC	365
C/EBP β	CACAGCGACGACTGCAAGATCC	CTTGAACAAGTTCCGCAGGGTG	188
PPAR γ 2	GCTGTTATGGGTGAAACTCTG	ATAAGGTGGAGATGCAGGTTC	325
aP2	TGGTTGATTTTCCATCCCAT	TACTGGGCCAGGAATTTGAT	150
Adiponectin	GGCCGTGATGGCAGAGAT	TTTCACCGATGTCTCCCTTAGG	88
LPL	GAGATTTCTCTGTATGGCACC	CTGCAAATGAGACACTTTCTC	276
Leptin	GGCTTTGGCCCTATCTTTTC	GCTCTTAGAGAAGGCCAGCA	325
Cyclophilin B	GGAGATGGCACAGGAGGAAA	CGTAGTGCTTCAGTTTGAAGTTCTCA	72