

Neurohypophyseal hormones and skeletal muscle: a tale of two faces

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*Histology and Embryology Section, Dept. AHFOS, Sapienza University of Rome, Italy**This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (CC BY-NC 4.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.***Abstract**

The neurohypophyseal hormones vasopressin and oxytocin were invested, in recent years, with novel functions upon striated muscle, regulating its differentiation, trophism, and homeostasis. Recent studies highlight that these hormones not only target skeletal muscle but represent novel myokines. We discuss the possibility of exploiting the muscle hypertrophying activity of oxytocin to revert muscle atrophy, including cancer cachexia muscle wasting. Furthermore, the role of oxytocin in cardiac homeostasis and the possible role of cardiac atrophy as a consequence of death in cachectic patients is discussed.

Key Words: Vasopressin, Oxytocin, Muscle, Homeostasis.*Eur J Transl Myol 30 (1): 53-57, 2020***Neurohypophyseal Hormones and Skeletal Muscle Homeostasis**

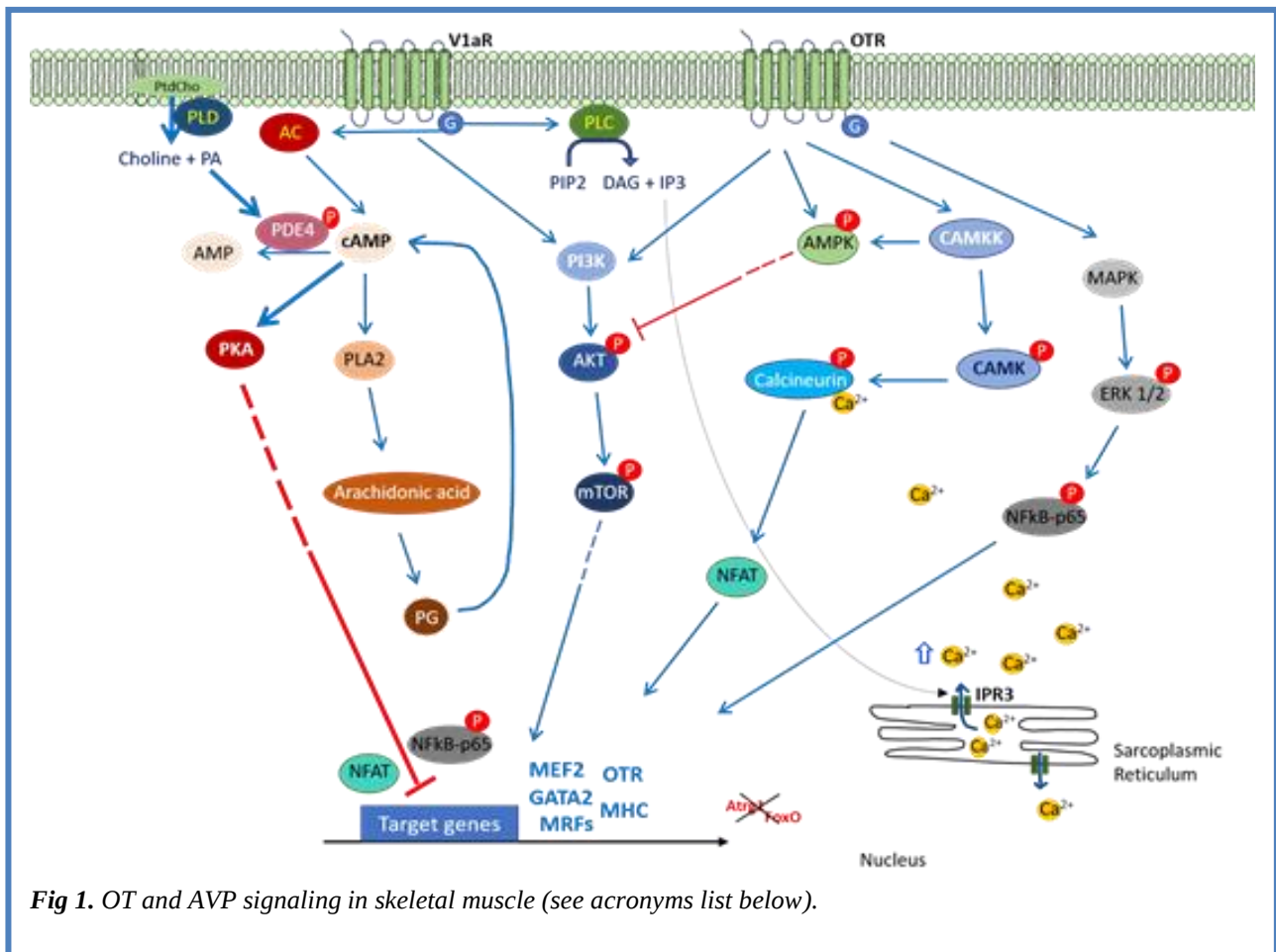
In the past few decades, the neurohypophyseal hormones (NH) vasopressin (AVP) and oxytocin (OT) emerged as novel myogenic regulators.¹⁻⁵ In fact, besides their classical roles (uterus, mammary gland, vascular smooth muscle, kidney tubules, CNS),² they were shown to target skeletal muscle. OT and AVP are mainly produced in the hypothalamus, transported to the neurohypophysis, and secreted into circulation. Seven-transmembrane-domain G-protein-coupled receptors (GPCR) and complex downstream signaling processes mediate their pleiotropic physiological functions.⁵ Functional receptors for NH mediate AVP and OT signals in myogenic cells (V1aR in the murine myogenic cell line L6, OTR in human satellite cells) and muscle fibers.^{3,6} Interestingly, despite possessing individual receptors, AVP and OT have been shown to cross-activate each other's receptors.⁷

Skeletal muscle is a target of AVP and OT

In vitro studies indicated that OT and AVP treatments dramatically increased the fusion of murine and avian myoblasts and human satellite cells.^{1,2,3} The addition of AVP to the culture medium of murine myoblasts induces the expression of Myf-5 and myogenin.¹ Interaction of AVP and the V1a receptor rapidly increases intracellular $[Ca^{2+}]$ and stimulates the phospholipases C (PLC), D (PLD) and A2 (PLA2), producing intracellular messengers such as phosphatidic acid (PA), diacylglycerol (DAG) and InsP3 (Figure 1). As shown in Figure 1, PA (produced by the PLD-dependent

hydrolysis of PtdCho) activates PDE4, which in turn promotes cAMP breakdown, thus inhibiting cAMP-dependent activation of PKA, a kinase known to negatively regulate myogenic differentiation.⁹ The increase in the intracellular level of Ca^{2+} interferes with the Calcium calmodulin Kinase (CaMK)/Calcineurin pathway inducing upregulation of skeletal muscle differentiation markers myogenin, MEF2 and GATA2 (zinc finger proteins) expression.¹⁰ The PI3K/Akt/mTOR pathway is well known for its role in cell growth, proliferation, differentiation, and muscle homeostasis. This pathway is recruited in response to NH in myoblasts, and its inhibition hampered the AVP-stimulated expression of both Akt and mTOR and increased the expression of the ubiquitin ligase atrogen-1 and FoxO (Figure 1).¹¹

Satellite cells obtained from aged mice display lower OT receptor expression and myogenic potential when compared to satellite cells from young animals. By treating the "old" and "young" satellite cells with exogenous OT or with a selective oxytocin antagonist, respectively, OT was demonstrated to be required for effective muscle regeneration.¹² In vivo, AVP signaling accelerates the regeneration of injured skeletal muscle and reverts TNF-induced muscle atrophy: overexpressing V1aR in the tibialis anterior decreased the p-NF κ B-p65 protein expression, increased Pax7, myogenin and embryonic myosin heavy chain (eMHC) expressions after cardiotoxin injury, even in the presence of TNF.⁶ In summary, OT and AVP activate a complex array of signaling pathways to regulate skeletal muscle homeostasis (modulating different kinases, modifying the calcium level, through the CaMK/Calcineurin



signaling to activate the myogenic regulatory factors expressions, and by stimulating the Akt/mTOR or the MAPK pathway to induce muscle cell proliferation, differentiation, contraction, and regeneration.

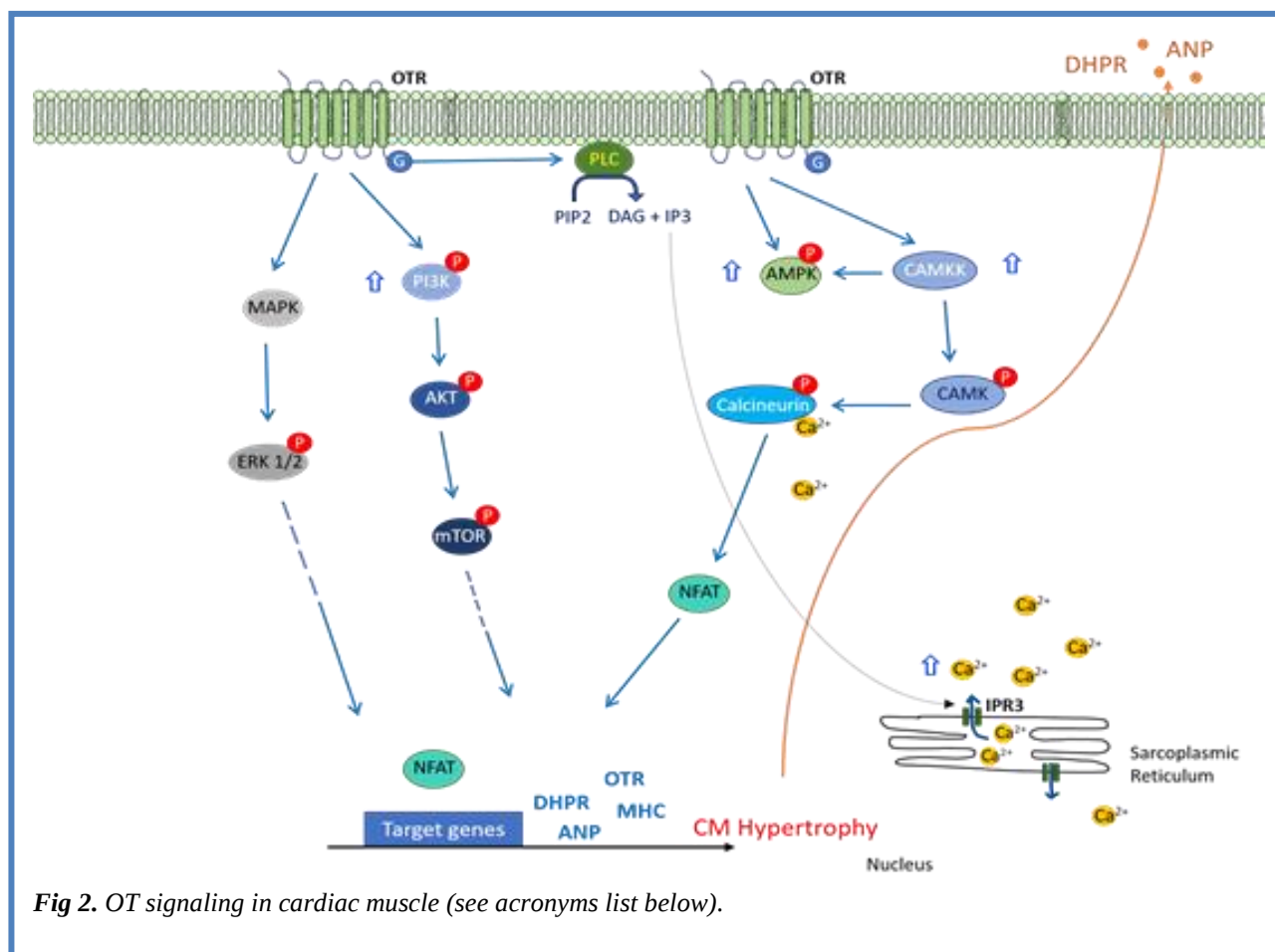
Skeletal muscle is a source of AVP and OT

Skeletal muscle is not only a target of neurohypophyseal hormones but also a source, acting in this way, as an autocrine/paracrine gland for its regulation. OT was demonstrated to be expressed by cultured human myoblasts.³ De Jager and collaborators revealed that cattle administrated continuously with anabolic steroids featured an augmented OT mRNA expression in skeletal muscle, followed by a ~ 50-fold higher plasma level of OT.¹³ More recently, C2C12 myogenic cells were shown to express and secrete OT in the culture medium at a low level under basal conditions.⁸ These authors demonstrated that 17β-estradiol, which stimulates the differentiation of these myoblasts, transiently up-regulates OT gene expression and secretion rapidly after treatment. Increased expression of OT in livestock as a result of anabolizing treatments was reported by other groups.¹⁴ Interestingly, the estrogen control of OT expression appears to be mediated by ERα, an effect which can be further amplified by an OT/OT-R loop.¹⁴

Furthermore, physical activity was shown to physiologically stimulate AVP and OT secretion. Several groups demonstrated that exercise results in increased levels of AVP and OT in the hypothalamus as well as in plasma.¹⁴ Exercise plays beneficial effects on muscle physiology. The exercise-mediated stimulation of AVP and OT suggests that physical activity participates in their autocrine effect upon skeletal muscle triggering NH secretion by muscle and brain, thus regulating their receptors and contributing to muscle homeostasis.

OT, a potential therapeutic agent against muscle atrophy

Investigations have been conducted in aged mice to examine the potential of OT to reverse sarcopenia, which involves impaired skeletal muscle regeneration. A significant decrease of OT expression in aged mice in comparison to young mice has been reported.¹² Indeed, a low level of OTR on satellite cells accompanies the decrease of OT circulating levels in the plasma. Moreover, exogenous OT administration in aged mice significantly increases muscle regeneration after cardiotoxin injury by increasing both the expansion and the differentiation of satellite cells through the MAPK/ERK pathway.¹² Analysis of OT-knock out (KO) mice was conducted to better understand the OT role in regeneration and sarcopenia. Muscle regeneration was



strongly impaired in OT-KO mice. 12-month-old KO mice, compared to wild type, displayed premature aging with muscle fibrosis and fat infiltration, a pattern characteristic of sarcopenia.¹²

As in sarcopenia, cancer-cachexia (CC) is characterized by muscle wasting, loss of fat and lean mass, and is associated with an adverse prognosis. Our group demonstrated that physical activity counteracts CC *in vivo*, increasing the survival of tumor-bearing mice and counteracting muscle atrophy.¹⁵ The effects of CC are dependent upon the presence of pro-inflammatory cytokines, among these TNF, which has been shown to hamper the differentiation potential of myogenic cells, ultimately leading to cachexia.¹⁶ Since NH have been demonstrated to stimulate myogenic differentiation, muscle trophism and regeneration, we believe that the use of OT may counteract CC-dependent skeletal muscle atrophy and thus, ameliorate the quality of life and response to treatment of cancer patients.

Role of Oxytocin in Cardiovascular Regulation

In the 90s, a group showed that OT is synthesized and released also by the heart. They found the concentration of OT higher in the atria and in the ventricles than in the uterus of rats.¹⁷ Furthermore, as in skeletal muscle, OT induces cardiomyocytes differentiation *in vitro*. Its addition to the culture medium of P19 cells (mouse

embryonal carcinoma cells) increased the expression of MHC, and cardiac markers DHPR- $\alpha 1$ (dihydropyridine receptor $\alpha 1$) and ANP (atrial natriuretic peptide) (Figure 2). OT up-regulated OTR expression by 5–10%.¹⁸ Later,¹⁹ the establishment of the PI3K, Ca-CAMKK and AMPK pathways involvement in OT-dependent stimulation of glucose uptake was demonstrated (Figure 2). Therefore, OT contributes to cardiomyogenesis and CM differentiation. However, a recent study showed that overexpressing OTR specifically in the heart led to a decreased motor activity, irregular respiration and death because of severe heart failure.²⁰ Indeed, chronic heart failure that affects more than 20 millions of people in the world, has been reported to induce cachexia. Conversely, it is possible to hypothesize that cardiac atrophy takes place in cachexia, and that heart failure may play a role in cachexia-associated mortality. This suggests a new therapeutic approach, using OT to ameliorate the cachectic patients' quality of life and survival. In conclusion, OT could be a potential candidate to counteract muscle wasting implicated in cachexia.

List of acronyms

AKT – Serine-threonine Protein Kinase
 AMPK – AMP-activated Protein Kinase
 ANP - Atrial Natriuretic Peptide
 AVP – Vasopressin

Ca-CAMKK – Ca²⁺/Calmodulin-dependent Protein Kinase
 CaMK – Calcium Calmodulin Kinase
 cAMP – Cyclic Adenosine Monophosphate
 CC – Cancer Cachexia
 CNS – Central Neural System
 DAG - Diacylglycerol
 DHPR- α 1 – Dihydropyridine Receptor α 1
 eMHC - embryonic Myosin Heavy Chain
 ERK – Extracellular signal-regulated Kinase
 ERR α – Estrogen-related Receptor α
 FoxO – Forkhead box O
 G – G-protein
 GATA2 – GATA Binding Protein 2
 GPCR - G-protein-coupled receptors
 InsP3 – Inositol Triphosphate
 MAPK – Mitogen-activated Protein Kinase
 MEF – Myocyte Enhancer Factor-2
 MHC – Major Histocompatibility Complex
 mTOR – mammalian Target of Rapamycin
 Myf5 – Myogenic Factor 5
 NH - Neurohypophyseal Hormones
 OT – Oxytocin
 OTR – Oxytocin Receptor
 PA – Phosphatidic Acid
 Pax7 – Paired box protein 7
 PDE4 – Phosphodiesterase-4
 PI3K – Phosphoinositide 3-kinase
 PKA – Protein Kinase A
 p-NF κ B – phosphorylated Nuclear Factor-kappa B
 PLA2 – Phospholipases A2
 PLC – Phospholipases C
 PLD – Phospholipases D
 PtdCho – Phosphatidylcholine
 TNF – Tumor Necrosis Factor
 V1aR - Vasopressin Receptor 1a

Authors contributions

AB and SA played a main role in the conception, study design, and data acquisition, while AR and GC participated in analyses of data and in drafting and finalizing the manuscript.

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Conflict of Interest

The authors declare they have no financial, personal, or other conflicts of interest.

Ethical Publication Statement

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References

1. Nervi C, Benedetti L, Minasi A, et al. Arginine-Vasopressin Induces Differentiation of Skeletal Myogenic Cells and up-Regulation of Myogenin and Myf-5. *Cell Growth Differ* 1995;6:81–9.
2. Minotti S, Scicchitano BM, Nervi C, et al. Vasopressin and Insulin-like Growth Factors Synergistically Induce Myogenesis in Serum-Free Medium. *Cell Growth Differ* 1998;9:155–63.
3. Breton C, Haenggeli C, Barberis C, et al. Presence of Functional Oxytocin Receptors in Cultured Human Myoblasts. *J Clin Endocrinol Metab* 2002; 87:1415–18. doi: 10.1210/jcem.87.3.8537.
4. Costa A, Rossi E, Scicchitano BM, et. Neurohypophyseal Hormones: Novel Actors of Striated Muscle Development and Homeostasis. *Eur J Transl Myol* 2014;24(3):3790. doi: 10.4081/ejtm.2014.3790.
5. Barberis C, Mouillac B, Durroux T. Structural Bases of Vasopressin/Oxytocin Receptor Function. *J Endocrinol* 1998;156:223–9. doi: 10.1677/joe.0.1560223.
6. Costa A, Toschi A, Murfuni I, Pelosi L, et al. M. Local Overexpression of V1a-Vasopressin Receptor Enhances Regeneration in Tumor Necrosis Factor-Induced Muscle Atrophy. *Biomed Res Int* 2014;2014:235426. doi: 10.1155/2014/235426.
7. Song Z, Albers H. E. Cross-Talk among Oxytocin and Arginine-Vasopressin Receptors: Relevance for Basic and Clinical Studies of the Brain and Periphery. *Front Neuroendocrinol* 2015;14:14–24. doi:10.1016/j.yfrne.2017.10.004.
8. Berio E, Divari S, Cucuzza LS, et al. 17 β -Estradiol Upregulates Oxytocin and the Oxytocin Receptor in C2C12 Myotubes. *PeerJ* 2017;2017:(3). doi: 10.7717/peerj.3124.
9. Naro F, De Arcangelis V, Sette C, et al. A Bimodal Modulation of the CAMP Pathway Is Involved in the Control of Myogenic Differentiation in L6 Cells. *J Biol Chem* 2003;278:49308–15. doi: 10.1074/jbc.M306941200.
10. Scicchitano B. M, Spath L, Musarò A, et al. Vasopressin-Dependent Myogenic Cell Differentiation Is Mediated by Both Ca²⁺/Calmodulin-Dependent Kinase and Calcineurin Pathways. *Mol. Biol. Cell* 2005;16:3632–41. doi: 10.1091/mbc.E05-01-0055.
11. Sorrentino S, Barbiera A, Proietti G, et al. Inhibition of Phosphoinositide 3-Kinase/Protein Kinase B Signaling Hampers the Vasopressin-Dependent

- Stimulation of Myogenic Differentiation. *Int J Mol Sci* 2019;20:(17). doi: 10.3390/ijms20174188.
12. Elabd C, Cousin W, Upadhyayula P, et al. Oxytocin Is an Age-Specific Circulating Hormone That Is Necessary for Muscle Maintenance and Regeneration. *Nat Commun* 2014;5:4082. doi: 10.1038/ncomms5082.
13. de Jager N, Hudson NJ, Reverter A, et al. Chronic Exposure to Anabolic Steroids Induces the Muscle Expression of Oxytocin and a More than Fifty Fold Increase in Circulating Oxytocin in Cattle. *Physiol Genomics* 2011;43: 467–78. doi: 10.1152/physiolgenomics.00226.2010.
14. Adamo S, Pigna E, Lugarà Ret al. Skeletal Muscle: A Significant Novel Neurohypophyseal Hormone-Secreting Organ. *Front Physiol* 2019;9: doi: 10.3389/fphys.2018.01885.
15. Pigna E, Berardi E, Aulino P, et al. Aerobic Exercise and Pharmacological Treatments Counteract Cachexia by Modulating Autophagy in Colon Cancer. *Sci Rep* 2016;6. doi: 10.1038/srep26991.
16. Moresi V, Pristerà A, Scicchitano B. et al. Tumor Necrosis Factor- α Inhibition of Skeletal Muscle Regeneration Is Mediated by a Caspase-Dependent Stem Cell Response. *Stem Cells* 2008;26:997–1008. doi: 10.1634/stemcells.2007-0493.
17. Jankowski M, Hajjar F, Al Kawas S, et al. Rat Heart: A Site of Oxytocin Production and Action. *Proc Natl Acad Sci USA*. 1998;95:14558–63. doi: 10.1073/pnas.95.24.14558.
18. Paquin J, Danalache BA, Jankowski M, et al. Oxytocin Induces Differentiation of P19 Embryonic Stem Cells to Cardiomyocytes. *Proc Natl Acad Sci USA* 2002;99: 9550–5. doi:10.1073/pnas.152302499.
19. Florian M, Jankowski M, Gutkowska J. Oxytocin Increases Glucose Uptake in Neonatal Rat Cardiomyocytes. *Endocrinology* 2010;151:482–91. doi: 10.1210/en.2009-0624.
20. Jung C, Wernly B, Bjursell M, et al. Cardiac-Specific Overexpression of Oxytocin Receptor Leads to Cardiomyopathy in Mice. *J Card Fail* 2018;24:470–8. doi: 10.1016/j.cardfail.2018.05.004.

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