OSMOREGULATORY ROLE OF VASOTOCINERGIC

AND ISOTOCINERGIC SYSTEMS IN THE GILTHEAD SEA BREAM (*Sparus aurata* L)

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THE MENU

- A small introduction
- Getting molecular tools
- AVT/IT systems and environmental salinity
- AVT/IT receptors and environmental salinity
- AVT/IT: direct osmoregulatory effects
- AVT/IT: metabolism
- Conclusions
- Quo Vadis?

GENERAL ASPECTS OF NEUROPEPTIDES

ARGININE VASOTOCIN Cys – <i>Tyr</i> – <i>Ile</i> – <i>Gln</i> – Asn – Cys – Pro – <i>Arg</i> – Gly – NH ₂ 1 2 3 4 5 6 7 8 9	MESOTOCIN Cys – <i>Tyr</i> – <i>Ile</i> – <i>Gln</i> – Asn – Cys – Pro – <i>Ile</i> – Gly – NH ₂ 1 2 3 4 5 6 7 8 9
ARGININE VASOPRESSIN Cys – <i>Tyr</i> – <i>Phe</i> – <i>Gln</i> – Asn – Cys – Pro – <i>Arg</i> – Gly – NH ₂	VALITOCIN Cys – <i>Tyr</i> – <i>Ile</i> – <i>Gln</i> – Asn – Cys – Pro – <i>Val</i> – Gly – NH ₂
LYSINE VASOPRESSIN Cys – <i>Tyr</i> – <i>Phe</i> – <i>Gln</i> – Asn – Cys – Pro – <i>Lys</i> – Gly – NH ₂	GLUMITOCIN Cys – <i>Tyr</i> – <i>Ile</i> – <i>Ser</i> – Asn – Cys – Pro – <i>Gln</i> – Gly – NH ₂
PHENYPRESSIN Cys – <i>Phe</i> – <i>Phe</i> – <i>Gln</i> – Asn – Cys – Pro – <i>Arg</i> – Gly – NH ₂	ASPARGOTOCIN Cys – <i>Tyr</i> – <i>Ile</i> – <i>Asn</i> – Asn – Cys – Pro – <i>Leu</i> – Gly – NH ₂
ISOTOCIN Cys – <i>Tyr</i> – <i>Ile</i> – <i>Ser</i> – Asn – Cys – Pro – <i>Ile</i> – Gly – NH ₂	ASVATOCIN Cys – <i>Tyr</i> – <i>Ile</i> – <i>Asn</i> – Asn – Cys – Pro – <i>Val</i> – Gly – NH ₂
OXITOCIN Cys – <i>Tyr</i> – <i>Ile</i> – <i>Gln</i> – Asn – Cys – Pro – <i>Leu</i> – Gly – NH ₂	PHASVATOCIN Cys – <i>Tyr</i> – <i>Phe</i> – <i>Asn</i> – Asn – Cys – Pro – <i>Val</i> – Gly – NH ₂

VASOTOCINERGIC AND ISOTOCINERGIC SYSTEMS

ARGININE VASOTOCIN (AVT) AND ISOTOCIN (IT)

AVT family receptors

- a) **V1**: associated with vascular smooth muscle
- b) **V1b**: associated with pituitary corticotrophe cells
- c) **V2**: linked to renal hydrosmotic actions

IT family receptors

- a) **ITR1**
- b) **ITR2**

Physiological functions

- Metabolism and stress
- Reproductive behaviour
- Cardiovascular activity
- Brain neurotransmission and pituitary endocrine activity
- Osmoregulation

GETTING MOLECULAR TOOLS: CLONING OF pro-VT AND pro-IT

GETTING MOLECULAR TOOLS: CLONING OF AVTRS AND ITR

AVTR V1a2-type

```
5' - tggagagacacttggctccctctgtgagaccggttagctccatatacgggt 53
tggagagacacttcaagacccctctggcgtgtatctgtcggcgacacaggggg 110
agtcacagacactcccgactgcgtctgtctgagcggaggagacgggtctctg 167
M G T F G N D S V R 10
ggttccagctccactgatgatttctggggaacccctggaaagagaggtccac 224
Q W S D P P F Q G N E E V A Q 229
cagaagagatccatctctctcaggggaacagagagatgacacacacagagc 281
cagctgaacacttctctgagctgctgtgctcggagagctgagcctcctgga 338
N T K K K S R M 67
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cctgctgaacttctgctgactctctcagagctgctgagcagctgctgagagc 452
T F A T Y G D F L C 105
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R Y I 124
atagagcttctcagacacacacagagagagagagagagagagagagagag 566
A I C H P L K T L Q Q P T Q R S Y 143
gacctctgacacccctgaaacccctctcagagacacacacacacacacacac 623
F 162
atatctcaactgagctgagagagagagagagagagagagagagagagagag 680
S L S E I K N G S D V D C M A Y F I 181
tccctgagagagagagagagagagagagagagagagagagagagagagagag 737
E P W G K A Y 200
gagcactggggccacacagagctgacacacacacacacacacacacacacac 794
I W K N 219
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G K N S V S S V T T I S R A K L R T 257
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A V T L S A L L A 314
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F S C 333
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L R M W P F R D D D S I R R T 352
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T L L T K M T N Q S P T G S S A N W R 371
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E L D N S P K S S I Q P E E N H S 388
gagctgagagagagagagagagagagagagagagagagagagagagagagag 1364
tcttttcaagggggtggaggtttagaagagagagagagagagagagagagag 1421
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cccaatgagagagagagagagagagagagagagagagagagagagagagagag 2333
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caagagagagagagagagagagagagagagagagagagagagagagagagag 2447
tgctgtctctgagagagagagagagagagagagagagagagagagagagagag 2504
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gacatgagagagagagagagagagagagagagagagagagagagagagagag 2618
gtatatactgagagagagagagagagagagagagagagagagagagagagag 2675
atgagagagagagagagagagagagagagagagagagagagagagagagagag 2732
-polyA tail -3'
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GenBank
KC195974

GenBank
KC960488

AVTR V2-type

```
5' - agatacagctgagctgacacacacagagctgacacacacacttggcaactg 53
acagttaaagagagagagagagagagagagagagagagagagagagagagag 110
cagatgagagagagagagagagagagagagagagagagagagagagagagag 167
tgtttggggagctgtctctgacacacacagagagagagagagagagagagag 224
gcaaaatcacacacagagagagagagagagagagagagagagagagagagag 281
M E S I S L E T D W D G L A L S 16
cacaagagagagagagagagagagagagagagagagagagagagagagagag 281
S L A A C R N N F S S S S L F V S E 35
tctctgctgctgagagagagagagagagagagagagagagagagagagagag 338
L M S L N S S H G G G S P F F G I P P E 54
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N S T T T P H T L P Q P R V R D L G 73
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L A 92
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asagattctgctgctgagagagagagagagagagagagagagagagagagag 623
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W D I T E R F Q G P D L 149
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D R H A C C P L Q V R O G 181
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A M S R W N 206
gaaatgtctcctgagagagagagagagagagagagagagagagagagagagag 263
aagatcacagagagagagagagagagagagagagagagagagagagagagag 320
F S R T E V N H G E Y E 225
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C W G H F A E P N G L K A 244
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R E I H N N I Y L K S E R M V T A E M 282
cagagagagagagagagagagagagagagagagagagagagagagagagagag 339
K K N E I L F R F H G F K K E D E R A 301
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R E R G T R A S G G G G G R G G Q L L 320
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K E V W M H P H V H H O V R E F 339
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I Q Y N S C C G E H D T T A S P T O Q 358
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Q I L S S S D C H E S Y T S Y E L N S 377
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G S P R C S L D Y A P L P P A T P P 491
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P S I T K M S R T V 507
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A A W D 434
gtctatactactctgctgctgctgctgctgctgctgctgctgctgctgctgctg 535
P N P P N Q G V 453
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ITR

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5' - tgggggctgagaggttcagaacacacttccggagagctgcttttaagacat 53
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aagcagacccagactctgtgtgtgatgagcagctgtgctgcccagagtgagctgt 224
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W S N I S R G N E S H L G N T T V M P 35
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L K R N E E V A K 54
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A T K H 73
cgtgctgctgagcggacacttctgctgctgaggttcaacacacagagagagag 452
S Q S R M 92
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D I T T F R Y G 111
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P D L L 130
cagaggttctgctgagagagagagagagagagagagagagagagagagagagag 623
R C L A I C Q P L 149
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R S V H K R N 168
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Y I P S L K E V D G 181
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K W Q N F N L K T R R E H I 344
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V R L I S K A K I R 282
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D P A A P R E D M A 320
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L P Q 339
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gagagagagagagagagagagagagagagagagagagagagagagagagagag 1820
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gagagagagagagagagagagagagagagagagagagagagagagagagagag 1991
gagagagagagagagagagagagagagagagagagagagagagagagagagag 2048
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gagagagagagagagagagagagagagagagagagagagagagagagagagag 2732
-polyA tail -3'
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GenBank
KC195973

AVTRs AND ITR: TISSUE DISTRIBUTION

DIFFERENT PATTERN EXPRESSION

Sub-funcialization depending on the tissue and physiological action

AVT/IT SYSTEMS AND SALINITY: FIRST EVIDENCES

A steady increase in plasma AVT correlates with increasing water salinity (Kleszczyńska et al., 2006)

Sea bream treated with AVT enhanced gill Na⁺,K⁺-ATPase activity (Sangiao-Alvarellos et al., 2006)

AVT/IT SYSTEMS AND ENVIRONMENTAL SALINITY

Two different periods:

- Adaptive period: 12 h to 3 days
- Chronic regulatory period: 3 days onwards

(Martos-Sitcha et al., 2013)

AVT SYSTEM AND ENVIRONMENTAL SALINITY

During the adaptative period, pro-VT cDNA increased in both hypo- and hyperosmotic challenges suggests a relation between pro-VT expression and the initial stress.

(Martos-Sitcha et al., 2013)

IT SYSTEM AND ENVIRONMENTAL SALINITY

These results suggest a role of the isotocinergic system in the stress response that is linked to hyperosmotic, but not to hypoosmotic, transfer during the adaptative period.

(Martos-Sitcha et al., 2013)

AVT/IT RECEPTORS AND ENVIRONMENTAL SALINITY

OSMOREGULATORY TISSUES

Gills

Kidney

Changes in both AVTRs mRNA expression after osmotic challenges are salinity and tissue dependent.

Their variations seem associated with control of different proteins to produce the physiological effect required.

ITR seems to be undisturbed by osmotic challenge

(Martos-Sitcha et al., 2014)

AVT RECEPTORS AND ENVIRONMENTAL SALINITY

Relationship with ion transporters in gills

Changes in both AVTR V1a2-type and AVTR V2-type mRNAs could be associated and correlated with different ion transporters or channels to produce the necessary osmoregulatory effects.

(Martos-Sitcha et al., 2014)

ARE AVT AND IT RECEPTORS PRESENTS IN THE OPERCULAR EPITHELIUM?

(Martos-Sitcha et al., 2015)

YES!!

AVT AND IT IN THE SEA BREAM OPERCULAR EPITHELIUM

The basolateral addition of AVT (10^{-6} M) significantly stimulated Cl^- secretion, while IT (10^{-6} M) was without effect.

(Martos-Sitcha et al., 2015)

AVTR V1a2-type DISTRIBUTION AND SALINITY DEPENDENCE IN THE INTESTINE

(Martos-Sitcha et al., 2013)

AVTR V1a2-type: INTESTINAL ELECTROPHYSIOLOGY

The magnitude of absorption stimulation by AVT is higher in those portions and salinities with the highest secretory ($I_{sc} > 0$) at basal level.

Threshold significant effects of AVT were produced at concentrations similar to normal AVT circulating levels in plasma.

(Martos-Sitcha et al., 2013)

AVT/IT AND METABOLISM

Treatment with AVT induced several metabolic changes related to the energy repartitioning process occurring in this species during acclimation to extreme environmental salinities:

- hepatic glycogenolic potential
- hepatic glucose release capacity

This could be attributed to:

- a) the increased cortisol levels also elicited by AVT treatment and salinity transfer (Sangiao-Alvarellos et al., 2006)
- b) the AVTRs enhancement observed after salinity transfer from SW to LSW and HSW (Martos-Sitcha et al., 2013)

AVT/IT AND METABOLISM

Liver

AVTRs expression enhancement could be related to the energy repartitioning process occurring during osmotic adaptation of *S. aurata* to extreme environmental salinities, which could be mediated not only by plasma cortisol but also by AVT hormone.

ITR seems to be undisturbed by osmotic challenge

(Martos-Sitcha et al., 2013)

CONCLUSIONS

1. Changes in hypothalamic mRNA expressions, storage of mature hormones in pituitary and plasma levels after hypoosmotic and hyperosmotic challenges confirm the hypoosmoregulatory role of the vasotocinergic, but not of the isotocinergic system in *Sparus aurata*.

In addition, a role of AVT and/or IT in acute stress response associated to plasma cortisol values enhancement is also suggested.

2. Changes in both AVTRs (V1a2-type and V2-type) mRNA expression after osmotic challenges are salinity and tissue dependent, and their variations seem associated with control of different proteins (enzymes, ion transporters and/or water channels) to produce the physiological effect required.

However, ITR seems to be undisturbed by osmotic challenge, potentially ruling out an important role of IT related to osmoregulatory process.

CONCLUSIONS

3. AVT is involved in intestinal ionic transport regulation in *S. aurata*. This fact is supported not only by the presence and different mRNA levels of AVT receptors along the whole gastrointestinal tract after osmotic challenges, but also by the *in vitro* stimulation of AVT at physiological levels depending on the intestinal region and environmental salinity.

4. Vasotocinergic and isotocinergic receptors are present in the operculum, being responsible for the modulation of chloride secretion. AVT presents a double mechanism, involving two different AVTR types, where hormone modulates the chloride secretion depending on the osmoregulatory requirements of the specimens. However, ITR mediates a single stimulatory effect of chloride secretion.

QUO VADIS?

STRESS

- High stocking density and food deprivation affect AVT, IT and stress pathways in the gilthead sea bream (*Sparus aurata*). Skrzynska A.K. et al. (2015). 9th International Congress of Comparative Physiology and Biochemistry. Kraków (Poland)
- Acute stress regulates vasotocinergic and isotocinergic system in the gilthead sea bream (*Sparus aurata*). Skrzynska A.K. et al. (2015). 10^o Congreso de la Asociación Ibérica de Endocrinología Comparada. Castellón (Spain).
- Aroclor 1254 inhibit vasotocinergic pathways related to osmoregulation. Skrzynska A.K. et al. (2016). 11th International Congress on the Biology of Fish. Texas (USA).

**SPECIAL THANKS
TO MY COLLEGES**

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**Thank you
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attention**