P3-12

Effects of salinity challenge on the endocrine control of osmoregulation and calcium homeostasis in the sea bream

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The gilthead sea bream (*Sparus aurata*) is a marine species often found in coastal lagoons, experimenting episodic exposures to both brackish and hypersaline environments. However, little is known about the underlying endocrine mechanisms controlling osmoregulation in this and in most marine species. This study aimed at characterising some of the endocrine basis of sea bream osmoregulation, with emphasis on calcium homeostasis. Juvenile fish were exposed to different salinities, either by direct transfer or continuous adaptation over a short period of time. Salinities ranged from 0 to 55 p.p.t. and sampling was carried out 4, 24, 96 and 192 h after transfer. Six fish per group and per time point were sacrificed and plasma and tissue samples were collected. Osmolarity, osmolites and cortisol were measured in plasma. Prolactin, growth hormone, stanniocalcin, and calcitonin mRNA expressions were determined by PCR and northern blot. Mortality occurred after 4 hours in FW. Sea bream fry (2 month old, 20-60 mg) were exposed to hypersaline and dilute seawater loaded with ^{45}Ca and calcium fluxes were determined. Exposure of fry to lowered external salinity (50 and 25% SW) resulted in no mortality within 24 h and significantly decreased whole body calcium influx. Results will be discussed in relation to gene expression.

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P3-13

The effects of cAMP stimulation on secretion rate and ultrastructure of the Malpighian tubules of *Acheta domesticus*.

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Rapid fluid secretion in the Malpighian tubules is controlled by several factors, including the corticotropin releasing factor related diuretic peptides (CRF- DPs). In insects, CRF- DPs are believed to act via cAMP. We mimicked the action of CRF-DPs on the Malpighian tubules by adding dibutyryl cAMP, which caused a doubling in secretion rate. In the mid-tubule, this increased fluid secretion rate was correlated ultrastructurally with a number of changes, including increased elongation of basolateral infoldings and >12% vacuolation (cf <1% vacuolation in controls). It is this striking degree of vacuolation that is most intriguing. Vesicles have been linked to the process of transcytosis of fluid in vertebrates. However, we determined that vesicles formed by cAMP stimulation did not occur by endocytosis. Although transcytosis may be occurring in the tubules in a constituitive manner, when stimulated, the transcytotic component is greatly reduced. The role of these vesicles in rapid fluid transport will be discussed. Supported by NSF grant IBN-9807948.

P3-14

Expression of arginine kinase mRNA and protein in gills of the green shore crab *Carcinus maenas*.

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Arginine kinase (AK) belongs to an evolutionarily conserved class of phosphagen kinases that play an important role in maintaining intracellular ATP during periods of high-energy demand. The role of this ATP buffering system has been primarily ascribed as a phosphate reserve in cardiac and striated muscle. Here we report the complete cDNA sequence for AK isolated from gills of the green shore crab *C. maenas*. Oligo-nucleotide primers were designed on the basis of conserved regions within published AK sequences and were used to amplify cDNA transcribed from gill mRNA. Direct sequencing of polymerase chain reaction products generated a 1,164 nucleotide sequence with an open reading frame coding for a 357 amino acid protein showing 88% homology with AK sequences from *Homarus vulgaris* (88%), *Penaeus japonicus* (85%), and *Limulus polyphemus* (78%). Measurements of AK enzyme activity in gill homogenates of *C. maenas* reveal that posterior gills exhibit significantly higher specific activities compared with anterior gills. Arginine kinase may be instrumental in providing energy for ATP-dependent transport processes within the gill, facilitating osmoregulatory responses to a wide range of environmental salinities. Supported by the National Science Foundation (IBN-9807539).

P3-15

Branchial and cutaneous mitochondria-rich cells in primitive bony fishes

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Mitochondria-rich cells, often indicated as ionocytes or chloride cells are involved in osmoregulation in fresh-water fish.
Aim of the present work was to investigate on the presence and distribution of mitochondria-rich cells in air-breathing fishes by means of transmission and scanning electron microscopy, fluorophore marker and immunohistochemistry using Ab to mitochondrial membrane and Ab to Ca^{2+} ATPase. Mitochondria-rich cells were found in the primary and secondary gill epithelium. Two types of cells, flask-shaped and oval, were observed with a different location in the two regions of the gills. In the skin only one type of cells were identified with an elongated shape. The mitochondria-rich cells reacted with Ab to Ca^{2+} ATPase.
From our results, in lungfishes, during the aquatic phase, gills and skin are involved in ionoregulation and calcium transport. The mitochondria-rich cells can therefore be considered similar to the chloride cells of fresh-water teleosts.