Comparative Biochemistry and Physiology, Part A 167 (2014) 52–58

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

In vitro characterization of acid secretion in the gilthead sea bream (*Sparus aurata*) stomach

Lorenzo Márquez [a,*], Juan Fuentes [b]

[a] Núcleo de Investigación en Producción Alimentaria/Escuela de Acuicultura, Facultad de Recursos Naturales, Universidad Católica de Temuco, Avda. Rudecindo Ortega 02950, PO Box 15-D, Temuco, Chile
[b] Centre of Marine Sciences (CCMar), CIMAR-Laboratório Associado, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 6 October 2013
Accepted 7 October 2013
Available online 12 October 2013

Keywords:
Calcium-sensing receptor agonists
cAMP promoters
Gastric acid secretion
HK-ATPase
Muscarinic agonists
Sparus aurata
V-ATPase

ABSTRACT

The gastric acid secretion of juvenile *Sparus aurata* was characterized in Ussing chambers; secretion rates were determined by a pH-stat method at pH 5.50 and bioelectrical parameters were measured in current-clamped tissues. The basal secretion equaled to 535 ± 87 nmol·cm^{-2}·h^{-1}. Serosal carbachol 100 μM produced an increase (ΔJ_{H^+}) of 725 ± 133 nmol·cm^{-2}·h^{-1} from basal secretion, this effect being inhibited by mucosal omeprazole 100 μM. Basal secretion was also sensitive to the combination of serosal forskolin (FK) 10 μM + serosal isobutylmethylxanthine (IBMX) 100 μM ($\Delta J_{H^+} = 793 \pm 239$ nmol·cm^{-2}·h^{-1}); this effect was insensitive to mucosal omeprazole 100 mM but inhibited by mucosal bafilomycin A1 100 nM. The effect of carbachol proceeded within a few minutes (<10 min), whereas the effect of FK + IBMX was gradual, taking 40 min to reach the maximum. The addition of mucosal gadolinium (Gd^{3+}) 100 μM, a potent calcium-sensing receptor (CaR) agonist, stimulated the basal secretion ($\Delta J_{H^+} = 340 \pm 81$ nmol·cm^{-2}·h^{-1}). The present results indicate that the acid secretion mechanism in the sea bream stomach is regulated by muscarinic and CaR-like receptors, cAMP is implicated in the signal transduction, and at least two proton pumps, a HK-ATPase and a V-ATPase contribute to acid secretion.

1. Introduction

The structure and function of fish gastric epithelium share a number of basic characteristics with those of the rest of vertebrates, the most outstanding of them being the presence of gastric glands and H^+/K^+ ATPases (hereafter HK-ATPase) in apical membranes of acid-secreting cells (Wilson and Castro, 2011). On the other hand, it is also possible to find differences in the organization of gastric glands (Lindström et al., 2001) and in the regulation of gastric acid secretion (Welsh et al., 1993; Holmgren and Olsson, 2011) among vertebrates. More particularly, differences at tissue and cellular levels have been reported among fish species (Michelangeli et al., 1988; Bomgren et al., 1998). Much more difficult is to ascertain whether morphological variations in the distribution of gastric gland cellular types among major vertebrate taxa (Lindström et al., 2001) are associated with specific regulation mechanisms; for example the distribution of ECL cells and D cells in the vicinity of oxynticopeptic cells could theoretically determine the net effect of gastrin on the secretion of acid; in this regard, the accumulated knowledge suggests that the digestive hormone gastrin exerts opposite effects in mammalian and fish stomachs (Holmgren and Olsson, 2011).

The regulation of gastric acid secretion is better understood in mammals, where three main cellular components of the gastric gland participate in the activation of HCl-secreting parietal cells: ECL cells capable of secreting histamine, G cells capable of secreting gastrin and vagal terminations releasing acetylcholine (Samuelson and Hinkle, 2003; Chu and Schubert, 2012). These three effector molecules act on different receptors located in the basolateral membrane of parietal cells: i) histamine upon H2 receptors in a paracrine way, ii) acetylcholine upon M3 receptors, and iii) gastrin upon CCK2 receptors in an endocrine way (Schubert and Peura, 2008). Experiments on rodents and rabbits have demonstrated a dual histamine action, through elevations of cytoplasmic cAMP and Ca^{2+}, whereas acetylcholine and gastrin exert their actions through augmentations of cytoplasmic Ca^{2+} (Chew et al., 1992; Athmann et al., 2000). The implication of cAMP in the signal transduction is also supported by the effects of the adenylyl cyclase-enhancing and phosphodiesterase-inhibiting drugs, forskolin (FK) and isobutylmethylxanthine (IBMX) respectively, on isolated parietal cells of pig (Mårdh et al., 1987). Therefore, at least three stimulating effectors and two signaling pathways seem implicated in the regulation of acid secretion by the stomach.

According to the cellular circuitry described in mammals, a number of drugs have been used to investigate the regulation of acid secretion in fish, for example: in *Anguilla anguilla*, basolateral histamine and carbachol induce HCl secretion (Trischitta et al., 1998); in *Gadus morhua*, basolateral histamine induces HCl secretion and cimetidine

* Corresponding author: Tel.: +56 45 2205520; fax: +56 45 2205503.
E-mail address: marquez.lorenzo728@gmail.com (L. Márquez).

1095-6433/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbpa.2013.10.016

abolishes the effect of histamine, but also partially the basal secretion (Bomgren and Jönsson, 1996); apical omeprazole inhibits basal, histamine-induced and carbachol-induced secretions in *A. anguilla* (Trischitta et al., 1998) and increases the postprandial pH of gastric chyme in the shark *Squalus acanthias* (Wood et al., 2009). Nevertheless, the pharmacology of gastric acid secretion has only been investigated in a small number of fish species, with special emphasis on cod (Holmgren and Olsson, 2009). The regulatory mechanisms described above receive inputs not only from internal sources, like the vagal tonus, but also from external sources such as the volume of the dietary bolus, the composition of the diet or the pH of the gastric fluid (Holmgren and Olsson, 2011). In this respect, it has been recently reported the implication of the calcium-sensing receptor, CaR, in modulating the gastric acid secretion in response to gastric luminal pH and concentrations of calcium and aromatic amino acids in mammals (Quinn et al., 2004; Feng et al., 2010; Goo et al., 2010). Different molecular approaches have also shown the expression of CaR in the gastric mucosa of a number of fish species, including *Onchorhyncus mykiss* (Randman et al., 2002), *Oreochromis mossambicus* (Loretz et al., 2004), *Pleuronectes americanus*, *Salmo salar* and *S. acanthias* (Nearing et al., 2002).

In experiments carried out with living fish or, more frequently, with fish newly dissected digesting-stomachs, the dynamics of gastric pH has been investigated in connection with the digestive process (Deguara et al., 2003; Yúfera et al., 2004; Papastamatiou and Lowe, 2005; Nikolopoulou et al., 2011; Márquez et al., 2012), the general acid–base equilibrium (Wood et al., 2009) or during inter-digestive periods (Papastamatiou, 2007). Data so accumulated indicate that the cycle of feeding and digestion is the most important process affecting the temporal rhythm of fish gastric acidity, so that feeding is associated with a transiently high intragastric pH and followed by a progressive decrease until it levels off, making it clear that some kind of negative feedback between actual pH and acid secretion is at work *in vivo*. On the other hand, a research gap continues to exist in the field of pharmacology and mechanisms underlying gastric acid secretion in fish, including otherwise well-known commercial species such as *Sparus aurata*. This kind of data would contribute not only to basic physiology but also to applied research in aquaculture nutrition; for example, the efficiency of commercial phytases used as additives in fish diets, is strongly dependent on pH values reached during gastric digestion (Morales et al., 2011). Therefore, the main goal of the present work is to provide the first data on the basal parameters, and the effects of usual activators and inhibitors of the gastric acid secretion in juveniles of *S. aurata* assayed in Ussing chambers combined with a pH-stat method (Clarke, 2009).

2. Materials and methods

2.1. Animal husbandry and tissue collection

Juvenile gilthead sea bream (*S. aurata*) weighing 136 ± 11 g (mean ± SEM) were reared in 1000 L tanks connected to a seawater flow-through system located at the Ramalhete Marine Station (University of Algarve, Faro, Portugal); fish density was <5 kg m^{-3} and the daily ration was set to 2% wet body weight (diet Balance 3, Sorgal S.A., Portugal). Previous to the experimental procedures, fish were transferred and let to acclimate to 500 L tanks connected to a closed seawater circuit maintained at 21 °C and natural photoperiod.

To obtain experimental tissues, animals were fasted for 36 h, anesthetized with 2-phenoxyethanol (1:2000 vol/vol, Sigma, Madrid) and euthanized by decapitation. The abdominal cavity was immediately opened and the stomach was clamped at the cardiac and pyloric sphincters with mosquito forceps, finally the stomach was dissected and used to obtain samples of gastric fluid or in Ussing chamber experiments.

All animal manipulations were carried out according to the Guidelines of the European Union Council (86/609/EU) and Portuguese legislation. Protocols for animal manipulation were performed under a license of Group-1 from the Direcção Geral de Veterinária, Ministerio da Agricultura, do Desevolvimiento Rural e das Pescas (Portugal).

2.2. Chemicals

Chemicals of analytical grade were obtained from Sigma-Aldrich (Spain). Final concentrations in Ussing chambers for the several chemicals used were: mucosal omeprazole 100 μM, mucosal bafilomycin A1 100 nM, serosal carbamyl-choline (carbachol) 100 μM, serosal forskolin (FK) 10 μM in combination with serosal 3-isobutyl-1-methylxanthine (IBMX) 100 μM and mucosal gadolinium ions (Gd^{3+}) 100 μM. Gd^{3+} was added as an aqueous solution of $GdCl_3$; the rest of the chemicals were added as DMSO solutions (≤0.1% of final volume in the Ussing chamber).

2.3. Characterization of gastric fluids

Gastric fluid was gently suctioned through the cardiac sphincter by means of a syringe endowed with a 200 μL plastic tip. The volume obtained was usually <50 μL. Afterwards, gastric fluid samples were centrifuged at 16,000 *g* for 5 min and the supernatant was transferred to vials stored in ice. The quantity of fluid collected was determined gravimetrically to the nearest 0.0001 g. The pH was measured with a narrow-tipped pH probe (HI1330B, Hanna Instruments) attached to a pHM 84 Research pH-meter (Radiometer, Copenhagen, Denmark). Samples of 10 μL were used to determine the osmolality of gastric fluids and seawater with a Vapro 5520 osmometer (Wescor, USA). The gastric fluid concentrations of ions Na^+, Cl^-, Ca^{2+} and Mg^{2+} were determined in duplicate using appropriate colorimetric assays (Spinreact kits, Barcelona, Spain) adapted to a microplate reader (Benchmark, BioRad).

2.4. Ussing chamber experiments

Newly dissected stomachs were placed in pre-gassed serosal saline (99.7% O_2 + 0.3% CO_2), and cut open along the dorsal line from the cardiac to the pyloric sphincter. The stomach tissue was extended (the mucosal side was flushed with apical saline), mounted on a tissue holder with an insert area of 0.71 cm^2 (P2413, Physiologic Instruments, San Diego, CA, USA) and inserted in between two half-chambers (P2400, Physiologic Instruments). Each half-chamber was filled with 1.5 mL of the appropriate saline. The temperature of the whole setup was maintained at 22.0 ± 0.1 °C (mean ± SEM) in all experiments.

The composition of the serosal saline followed those that were used by other authors in experiments with *S. aurata* (Carvalho et al., 2012): 160 mM NaCl, 1 mM $MgSO_4$, 2 mM NaH_2PO_4, 1.5 mM $CaCl_2$, 5 mM $NaHCO_3$, 3 mM KCl, 5.5 mM glucose and 5 mM HEPES, with pH = 7.800. The composition of the mucosal saline was set according to the concentrations of Na^+, Cl^-, Ca^{2+} and Mg^{2+} found in the gastric fluid (Table 1): 128 mM NaCl, 12 mM $CaCl_2$, 18.5 mM $MgCl_2$, 16.5 mM $MgSO_4$, 3 mM KCl, and 73 mM mannitol; empirical osmolality equaled to 406 ± 3 mOsm/kg (mean ± SEM); pH was corrected to 5.500. Before

Table 1
Characterization of the gastric fluid in juveniles of *Sparus aurata* fasted for 36 h.

Fluid mass (mg)	40 ± 12
pH	3.27 ± 0.34
Osmolality (mOsm/kg)	411 ± 7
[Na^+] (mM)	128 ± 6
[Cl^-] (mM)	194 ± 6
[Mg^{2+} (mM)]	34 ± 5
[Ca^{2+}] (mM)	12 ± 2

Results are shown as mean ± SEM (n = 4–7).