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Adrenergic signaling in teleost fish liver, a challenging path☆

Elena Fabbri [a,*], Thomas W. Moon [b]

[a] University of Bologna, Department of Biological, Geological and Environmental Sciences Unit of Ravenna, via S. Alberto 163, 48124 Ravenna, Italy
[b] University of Ottawa, Department of Biology and the Centre for Advance Research in Environmental Genomics, 30 Marie Curie, K1N 6N5 Ottawa, Canada

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ABSTRACT

Adrenergic receptors or adrenoceptors (ARs) belong to the huge family of G-protein coupled receptors (GPCRs) that have been well characterized in mammals primarily because of their importance as therapeutic drug targets. ARs are found across vertebrates and this review examines the path to identify and characterize these receptors in fish with emphasis on hepatic metabolism. The absence of reliable and specific pharmacological agents led investigators to define the fish hepatic AR system as relying solely on a β_2-AR, cAMP-dependent signaling transduction pathway. The use of calcium-radiometric imaging, purified membranes for ligand-binding studies, and perifused rather than static cultured fish hepatocytes, unequivocally demonstrated that both α_1- and β_2-AR signaling systems existed in the fish liver consistent with studies in mammals. Additionally, the use of molecular tools and phylogenetic analysis clearly demonstrated the existence of multiple AR-types and -subtypes in hepatic and other tissues of a number of fish species. This review also examines the use of β-blockers as pharmaceuticals and how these drugs that are now in the aquatic environment may be impacting aquatic species including fish and some invertebrates. Clearly there is a large conservation of structure and function within the AR system of vertebrates but there remain a number of key questions that need to be addressed before a clear understanding of these systems can be resolved.

1. Introduction

Catecholamines (CAs) enable vertebrates, including fish, to initiate the "fight-or-flight" response, ultimately allowing organisms to survive and reproduce in a challenging environment (Fig. 1). CAs are also vital in non-stress conditions, modulating, among other physiological processes, energy resource availability. That CAs interact with specific membrane localized G-protein coupled receptors called adrenergic receptors or adrenoceptors (ARs) is well known in mammals, and both receptor structures and downstream signaling processes are well established (Audet and Bouvier, 2012; Cotecchia et al., 2012; Katritch et al., 2013).

The information available for mammals was exploited for understanding the regulation of liver metabolism in lower vertebrates; however the characterization of adrenergic signaling in non-mammalian animals was rather difficult, as it was initially biased by the use of inadequate pharmacological tools and finally improved when gene sequences and molecular probes became available.

This paper will provide an overview of the evolution of our knowledge regarding ARs and the adrenergic control of fish metabolism and in particular the liver, including unanswered questions. Recent reports concerning the interaction between ARs and emerging contaminants in the aquatic environment, including adrenergic pharmaceuticals are also provided. These data will be discussed to develop an updated scenario regarding the threat represented to vertebrates and invertebrates with exposure to β-AR blockers.

2. Catecholamine effects on fish liver

Catecholamines in vitro induce glucose release by the livers of many fish species, including salmonids (e.g. *Oncorhynchus mykiss and Oncorhynchus tshawytscha*), carp (*Cyprinus carpio*), catfish (*Ictalurus melas* and *Ictalurus nebulosus*), eels (*Anguilla anguilla* and *Anguilla rostrata*), goldfish (*Carassius auratus*), rockfish (*Sebastes caurinus*), and toadfish (*Opsanus beta*) (for a review see Fabbri et al., 1998). Glucose release resulting from CA application has been reported using a variety of tissue preparations, initially in whole liver (Ottolenghi et al., 1984) or slices and cultured pieces (Janssens and Lowrey, 1987; Janssens and Waterman, 1988; Michelsen and Sheridan, 1990), then isolated hepatocytes (Birnbaum et al., 1976; Mommsen et al., 1988; Danulat and Mommsen, 1990; Reid et al., 1992; Vijayan et al., 1994; Manzl et al., 2002; Van Heeswijk et al., 2006). Epinephrine (EPI) was up to 100-times more potent than equivalent concentrations of norepinephrine (NEPI) in causing glucose release from liver slices or pieces of most fish species (carp, Janssens and Lowrey, 1987; Janssens and Waterman, 1988; trout, Michelsen and Sheridan, 1990), and from isolated hepatocytes (catfish, Brighenti et al., 1987; trout, Mommsen et al., 1988; trout,

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* Corresponding author at: University of Bologna, Campus of Ravenna, Via S. Alberto 163, 48123 Ravenna, Italy.
E-mail address: elena.fabbri@unibo.it (E. Fabbri).

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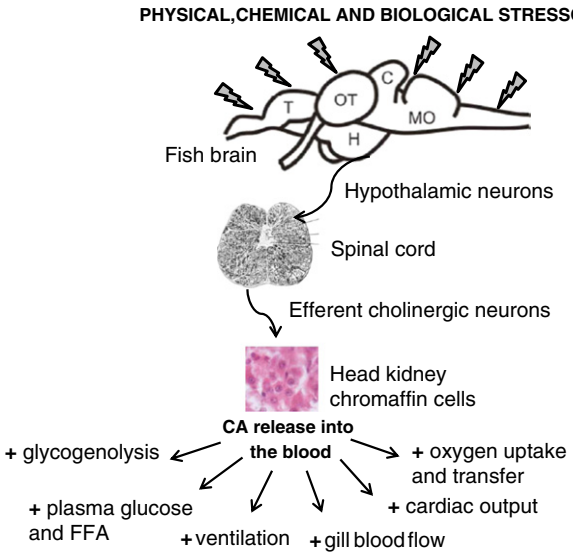

Fig. 1. Main neuroendocrine elements involved in the acute response to stress in fish. Physical (e.g. capture, handling, temperature), chemical (e.g. high ammonia or nitrite, low oxygen, changes in pH and osmolarity, pollution), and biological (e.g. predation, territorial disputes, food deprivation) stressors activate brain centers and catecholaminergic fibers modulate EPI and NEPI release from chromaffin cells associated with the head kidney. A set of beneficial (or adaptive) short term responses take place enabling the animal to overcome the threat. H, hypothalamus; T, telencephalon; OT, optical tectum; C, cerebellum; MO, medulla oblongata; CAs, catecholamines.

Vijayan et al., 1994; bullhead and eel, Fabbri et al., 1995; goldfish, Manzl et al., 2002). In contrast, EPI and NEPI were found to be equally potent in inducing glucose release from Chinook salmon (*O. tshawytscha*) (Sheridan, 1988) and catfish (*I. melas*) (Brighenti et al., 1987) hepatocytes, while the response of rockfish (*S. caurinus*) hepatocytes was much greater toward NEPI than EPI, with EC50 values of about 6 and 320 nM, respectively (Danulat and Mommsen, 1990). On average, maximum stimulation of glucose release by EPI is induced at 1–10 μM when fish hepatocytes are challenged under static incubation conditions (Fabbri et al., 1995), although perifused hepatocytes respond at lower concentrations (Caselli et al., 2002).

The effects of CAs on hepatic glucose release are primarily the result of the activation of glycogenolysis. Glycogenolysis is the result of activating glycogen phosphorylase (GPase) and mobilizing hepatic glycogen stores, processes that are significantly enhanced by CAs (Ottolenghi et al., 1986). GPase activation is the final step in a complex phosphorylation cascade. It has been recognized for many years in mammals that the two second messengers, cAMP and calcium (Ca^{2+}), are both capable of modulating the liver enzyme, while in fish the understanding of this process has been a major challenge.

3. Adrenergic signaling involved in glucose release from fish hepatocytes

Danulat and Mommsen (1990) detected a robust increase in glucose release from rockfish hepatocytes with CA stimulation (NEPI > EPI); however they were unable to demonstrate simultaneous changes in cAMP intracellular levels. Previously, Birnbaum et al. (1976) using goldfish hepatocytes were unable to detect increases in cAMP levels with CA application. Additional studies hypothesized a role for a cAMP-independent pathway in the regulation of fish hepatic glucose production. Michelsen and Sheridan (1990) reported that verapamil (a Ca^{2+} channel blocker) was able to block EPI-induced glucose release while the Ca^{2+} ionophore A23187 stimulated glucose release from trout liver cells in the presence of free Ca^{2+}. Despite this evidence, most studies agreed that the cAMP-dependent release of fish hepatic glucose metabolism by CAs was the principal mechanism for the majority of fish species (for a review see Fabbri et al., 1998).

It is well established that cAMP formation is catalyzed from cellular ATP by the catalytic moiety of the enzyme adenylyl cyclase (AC; E.C. 4.6.1.1). This catalytic moiety is a component of a complex formed by a membrane receptor with a typical seven transmembrane-domain (7-TMD) structure, a heterotrimeric (αβγ) G-protein, and the AC catalytic moiety itself. Adrenergic receptors are members of this large 7-TMD family of receptors that are coupled to G-proteins (Wnorowski and Jozwiak, 2014). CA binding drives these receptors into a conformation that leads to the activation to a G-protein via protein–protein interactions. A GDP/GTP exchange on the guanine nucleotide binding site of the G-protein α-subunit occurs, leading to the dissociation of the heterotrimeric protein into two components, a GTP-bound α-subunit and the free βγ dimer. The α-subunit of the Gs-protein activates the downstream effector AC, whereas the βγ complex may positively or negatively affect the enzyme, depending on the particular AC isoform expressed in the tissue (Sunahara and Taussig, 2002). Signal termination occurs through the hydrolysis of GTP to GDP by the GTPase activity that is intrinsic to the α-subunit, allowing the α-subunit to re-associate with the βγ dimer. The α-subunits are divided into four classes, according to their amino acid sequence identities: Gs, Gi, Gq, and G12. Gs and Gi are directly involved in cAMP-dependent signaling, and all AC isoforms are stimulated by the activated Gs-α, whereas some are inhibited by Gi-α (Neves et al., 2002; Jalink and Moolenaar, 2010; Katada, 2012).

There are at least nine genes in mammals leading to multiple AC isoforms (Linder and Schultz, 2003). Each isoform is differentially expressed in tissues and activated or inhibited by specific G-proteins. Moreover the various AC isoforms are grouped into four subclasses according to their regulatory molecules (Sunahara and Taussig, 2002): AC I, III, and VIII are modulated by Ca^{2+}; AC II, IV, and VII are modulated by the βγ-dimer; AC V and VI are inhibited by Giα/Ca^{2+}; and, AC IX is insensitive to the prototypical AC activator forskolin (Fsk). In some cells an AC X is also present, not regulated by G-proteins, which is implicated in sperm motility, fertilization, and neurite outgrowth of neuronal cells (Sadana and Dessauer, 2009). Overall, the AC complex is regulated by agonists at the receptor level, by guanine nucleotides and sodium fluoride at the G-protein level (Bigay et al., 1987), and by the diterpene Fsk and a series of endogenous molecules on the catalytic moiety.

CA binding to β_1-, β_2- or β_3-AR elicits Gs activation, a rise in cellular cAMP levels and activation of the cAMP-dependent protein kinase (PKA). CA binding to the α_2-AR (typically present in fish integument and responsible for color changes; Svensson et al., 1997) elicits Gi activation, leading to decreased cAMP levels and reduced PKA activity. CA binding to α_1-AR elicits Gq activation, leading to increases in intracellular levels of inositol-trisphosphate (IP_3) and mobilization of Ca^{2+}. Both β- and α_1-ARs are expressed in mammalian liver, where β-AR blockers (e.g. propranolol, PROP) and α_1-AR blockers (e.g. prazosin, PRZ) counteract CA effects on glycogenolysis and glucose release (Ahles and Engelhardt, 2014).

4. The challenge of investigating fish liver adrenergic signaling

During the 1980s, and somewhat in parallel with the studies on mammals, interest regarding the regulation of fish liver metabolism was increasing. Sulakhe et al. (1988), using radioligand-binding experiments, examined the abundance of α_1- and β-ARs on hepatic membranes isolated from a selection of vertebrate species (but excluded fish). The conclusion was that β-ARs (using ^{3}H-dihydroalprenolol) were detected in the membranes of all animals studied, from mammals to birds, reptiles, and amphibians. Conversely, α_1-ARs (using ^{3}H-PRZ) were barely detectable in lizards and frogs, while present in liver membranes of birds and mammals at different concentrations depending on the species (Sulakhe et al., 1988). The authors suggested that β-AR is the phylogenetically more primitive AR, making an early appearance in