General and Comparative Endocrinology 240 (2017) 214–226

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Research paper

Claudin tight junction proteins in rainbow trout (*Oncorhynchus mykiss*) skin: Spatial response to elevated cortisol levels

Julia Gauberg, Dennis Kolosov, Scott P. Kelly *

Department of Biology, York University, 4700 Keele St, Toronto, ON M3J 1P3 Canada

ARTICLE INFO

Article history:
Received 23 March 2016
Revised 12 September 2016
Accepted 18 October 2016
Available online 19 October 2016

Keywords:
Skin
Epidermis
Cortisol
Tight junction
Claudin
Osmoregulation

ABSTRACT

This study examined regional distribution and corticosteroid-induced alterations of claudin (*cldn*) transcript abundance in teleost fish skin. Regional comparison of mRNA encoding 20 Cldns indicated that 12 exhibit differences in abundance along the dorsoventral axis of skin. However, relative abundance of *cldns* (i.e. most to least abundant) remained similar in different skin regions. Several *cldns* appear to be present in the epidermis and dermal vasculature whereas others are present only in the epidermis. Increased circulating cortisol levels significantly altered mRNA abundance of 10 *cldns* in a region specific manner, as well as corticosteroid receptors and 11β-hydroxysteroid dehydrogenase (type 2). Epidermis and epidermal mucous cell morphometrics also altered in response to cortisol, exhibiting changes that appear to enhance skin barrier properties. Taken together, data provide a first look at spatial variation in the molecular physiology of the teleost fish integument TJ complex and region-specific sensitivity to an endocrine factor.

1. Introduction

To maintain ionoregulatory homeostasis in freshwater (FW), teleost fishes must balance passive (obligatory) ion loss to the surrounding water by actively acquiring ions from the same medium (Marshall and Grosell, 2006). The acquisition of ions is accomplished by transcellular ion transport mechanisms, using channels and transporters to move salts across cell membranes. In contrast, passive ion loss to FW occurs primarily via the paracellular pathway across tissues that directly interface with the aquatic medium, i.e. the gill epithelium and the epidermis of the skin. In vertebrates, the paracellular movement of ions and other solutes is regulated by the epithelial tight junction (TJ) complex and the molecular components of the TJ complex include transmembrane proteins (e.g., occludin, claudins) as well as cytosolic scaffolding adaptor proteins (e.g., cingulin, ZO-1) (Günzel and Fromm, 2012). However, the permeability properties of an epithelium appear to be regulated predominantly by claudin (Cldn) composition (Günzel and Fromm, 2012). In teleost fishes studied to date, over 60 *cldns* have been described and their expression has been found to vary in different tissues and cell types (Kolosov et al., 2013).

The role of TJ proteins in the regulation of paracellular permeability across epithelia that directly interface with an aquatic environment is beginning to reveal itself primarily through studies conducted on the teleost gill (for review see Chasiotis et al., 2012a). The TJ complex and select TJ proteins of the gill epithelium are sensitive to environmental change and to ionoregulatory hormones that mediate the physiological response of fishes to environmental perturbation (for review see Chasiotis et al., 2012a). In the case of the latter, cortisol (the principal corticosteroid of teleost fishes), has a well documented tightening effect on the gill epithelium that is strongly associated with a number of TJ proteins (Bui et al., 2010; Chasiotis et al., 2010; Kelly and Chasiotis, 2011; Kelly and Wood, 2001, 2002; Kolosov and Kelly, 2013). However, in the skin of teleost fishes the contribution of epidermal TJ proteins to ionoregulatory homeostasis under conditions of environmental change or through the actions of ionoregulatory endocrine factors has been largely overlooked.

In general, the role of the adult teleost fish skin in ionoregulation has received much less attention than other ionoregulatory organs. This is most likely because a primary focus of work in this area has been mechanisms of transcellular solute movement, and the integument of most adult teleost fishes is classically regarded as a relatively static, passive barrier to solute movement (Marshall and Grosell, 2006). However, in all other aspects the adult teleost integument is a dynamic and complex tissue usually composed of dermis, scales and epidermis that functionally separates the external and internal milieu (Vernerey and Barthelat, 2014). Moreover, as we begin to understand the tremendous molecular plasticity of the vertebrate TJ complex, it is becoming

* Corresponding author.
E-mail address: spk@yorku.ca (S.P. Kelly).

http://dx.doi.org/10.1016/j.ygcen.2016.10.006
0016-6480/

more apparent that the maintenance of a seemingly static adult fish skin barrier may involve intricate and highly specific molecular adjustments of the TJ complex under different environmental and physiological conditions. For example, in the puffer fish *Fugu rubripes* it has been reported that over half of the 50+ *cldn* isoforms present in the organism can be found expressed in the skin (Loh et al., 2004). In addition, several recent studies have shown that *cldns* found in fish skin are responsive to altered environmental conditions or the actions of pathogens (Adamek et al., 2013; Bagherie-Lachidan et al., 2008, 2009; Bui and Kelly, 2014; Tacchi et al., 2015). Therefore, a closer look at the molecular physiology of the fish skin TJ complex and its contribution to the dynamics of fish skin as a barrier to solute movement now seems warranted. But in order to do this, it would be prudent for studies to take into consideration morphological and functional differences that are acknowledged to occur along the dorsoventral axis of fish skin (Zuasti, 2002; Varsamos et al., 2005; Park et al., 2006; Lazado and Caipang, 2014). Indeed, given these observations, it seems logical to hypothesize that spatial differences in the abundance and/or presence of *cldns* will occur in teleost fish skin, especially since Cldns are well known to exhibit differences in abundance within and between organs and tissues (for review see Günzel and Yu, 2013; Kolosov et al., 2013).

The primary objectives of this study were to: (1) investigate whether spatial differences in transcript encoding Cldn TJ proteins occurred in the skin of adult rainbow trout (*Oncorhyncus mykiss*) by examining *cldn* mRNA abundance along the dorsoventral axis (i.e. in dorsal, lateral and ventral skin regions) and (2) examine whether elevated systemic levels of cortisol alter region-specific molecular physiology of the adult fish skin TJ complex. With regard to the second objective it can be hypothesized that alterations in the molecular physiology of the adult fish epidermal TJ complex will occur in response to elevated circulating cortisol levels. This is because the epidermis, like the cortisol-responsive gill epithelium, directly interfaces with water, and both of these tissues must limit passive ion loss across the paracellular pathway. Furthermore, recent studies conducted on larval teleost fish provide some evidence to support this view (Kwong and Perry, 2013a). Therefore, it can be anticipated that cortisol may alter the molecular physiology of the adult fish epidermis in a manner that is consistent with its tightening effect on the gill epithelium (Kelly and Wood, 2001, 2002) and larval fish skin (Kwong and Perry, 2013a).

2. Materials and methods

2.1. Experimental animals

Rainbow trout (~4.5″ in length, ~18 g) (*Oncorhynchus mykiss*) were obtained from a local supplier (Humber Springs Trout Hatchery, Orangeville ON, Canada) and held in 200 L opaque polyethylene tanks supplied with flow-through dechlorinated freshwater (FW, ~composition in μM: $[Na^+]$: 590, $[Cl^-]$: 930, $[Ca^{2+}]$: 760, and $[K^+]$: 43). Fish were held under a constant photoperiod of 12:12-h light-dark and fed *ad libitum* once daily with commercial trout pellets (Martin Profishent, Elmira, ON, Canada). Fish were acclimated to laboratory conditions for at least two weeks prior to being used for experiments. All animal husbandry and experimental procedures were conducted in accordance with a York University Animal Care protocol that adhered to Canadian Council for Animal Care guidelines for the husbandry and care of experimental fishes.

2.2. Cortisol treatment

Size-matched rainbow trout housed in 200 L experimental tanks (20 fish/tank) were fed either a control or cortisol-supplemented diet for 17 days. Experimental diet was prepared according to a previously described protocol (Bernier et al., 2004) so as to produce a diet containing 150 μg cortisol/g food. Briefly, cortisol (hydrocortisone, Sigma-Aldrich, St. Louis, MO) was dissolved in ethanol and applied evenly on to trout food pellets by spraying. Ethanol alone was applied to control diet. Ethanol on sprayed food was allowed to evaporate and food was stored at −20 °C in pre-packaged daily rations for each tank. Control and experimental fish were fed a ration size of 2% body weight once daily.

2.3. Skin sampling

Fish were quickly net-captured and placed in an anaesthetic bath of buffered 0.5 g/L tricaine methanesulfonate TMS-222 (Syndel Laboratories, Vancouver, Canada). Blood was then collected from the caudal vessels prior to euthanizing by spinal transection. A dorso-ventrally spanning flank of skin was removed from the left side of each fish and separated into three equal sections: dorsal, lateral and ventral. Tissue designated "dorsal" spanned 1 cm ventrally from the mid-dorsal line. Tissue designated "ventral" extended dorsally 1 cm from the mid-ventral line. Tissue designated "lateral" spanned 0.5 cm on either side of the lateral line. Samples of each skin region (~80 mg) were placed into 1 mL of TRIzol® Reagent (Invitrogen Canada, Inc., Burlington, ON, Canada), flash frozen in liquid nitrogen and stored at −80 °C until further processing (see Section 2.5). For molecular analysis of TJ proteins in muscle tissue, a piece of epaxial white muscle was also placed into 1 mL of TRIzol® Reagent, flash frozen and stored at −80 °C.

For histological analysis, identical dorsal, lateral and ventral regions of skin were isolated and fixed in Bouin's fixative solution for 4 h at room temperature. Skin tissue samples were then washed in 70% ethanol to clear Bouin's and subsequently stored in 70% ethanol at 4 °C until further processing (see Section 2.7).

2.4. Serum cortisol levels, ion concentration, and muscle moisture content

A commercial cortisol enzyme immunoassay (EIA) kit (Oxford Biomedical Research, Rochester Hills, MI, USA) was used to measure serum cortisol levels according to the manufacturer's instructions. Parallelism curves generated from serially diluted rainbow trout serum were used to validate the cortisol EIA kit. In brief, a four-parameter logistic model was generated with the EIA standards plotted as a log(relative dilution factor) versus optical density. Parallelism between the serum and the standards was tested using R programming software (https://www.r-project.org/) and 'drc' package (www.bioassay.dk). The F-test value was 0.89. Intra- and inter-assay variation was 3% and 1–5% respectively. Values for inter-assay variation were supplied by the manufacturer. Serum $[Cl^-]$ was determined using colorimetric mercuric thiocyanate/ferric perchlorate chloride assay by diluting serum samples and comparing them to a standard curve of known chloride concentration (Zall et al., 1956). Serum $[Na^+]$ was measured using reference and ion-selective microelectrodes (ISMe). Electrodes were constructed according to Donini and O'Donnell (2005). A full flank of epaxial white muscle was collected from every fish and oven-dried to a constant weight at 60 °C for the determination of muscle moisture content.

2.5. RNA extraction and cDNA synthesis

Total RNA was extracted from skin or muscle tissue using TRIzol Reagent (Invitrogen Canada, Inc.) according to manufacturer's instructions. DNase I (Amplification Grade; Invitrogen Canada, Inc.) was used to treat extracted RNA, and first-strand cDNA was