Urea based osmoregulation and endocrine control in elasmobranch fish with special reference to euryhalinity

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Abstract

Since the landmark contributions of Homer Smith and co-workers in the 1930s there has been a considerable advance in our knowledge regarding the osmoregulatory strategy of elasmobranch fish. Smith recognised that urea was retained in the body fluids as part of the ‘osmoregulatory ballast’ of elasmobranch fish so that body fluid osmolality is raised to a level that is iso- or slightly hyper-osmotic to that of the surrounding medium. From studies at that time he also postulated that many marine dwelling elasmobranchs were not capable of adaptation to dilute environments. However, more recent investigations have demonstrated that, at least in some species, this may not be the case. Gradual acclimation of marine dwelling elasmobranchs to varying environmental salinities under laboratory conditions has demonstrated that these fish do have the capacity to acclimate to changes in salinity through independent regulation of Na\(^+\), Cl\(^-\) and urea levels. This suggests that many of the presumed stenohaline marine elasmobranchs could in fact be described as partially euryhaline. The contributions of Thomas Thorson in the 1970s demonstrated the osmoregulatory strategy of a fully euryhaline elasmobranch, the bull shark, Carcharhinus leucas, and more recent investigations have examined the mechanisms behind this strategy in the euryhaline elasmobranch, Dasyatis sabina. Both partially euryhaline and fully euryhaline species utilise the same physiological processes to control urea, Na\(^+\) and Cl\(^-\) levels within the body fluids. The role of the gills, kidney, liver, rectal gland and drinking process is discussed in relation to the endocrine control of urea, Na\(^+\) and Cl\(^-\) levels as elasmobranchs acclimate to different environmental salinities.

Keywords: Euryhaline; Elasmobranch; Osmoregulation; Gill; Kidney; Rectal gland; Drinking; urea; Na\(^+\); Cl\(^-\)

1. Introduction

Euryhalinity in fish involves regulation of renal and extra-renal processes in response to changes in the environmental salinity. It is well documented that in euryhaline teleost fish acclimating to FW and SW physiological processes such as drinking and gill function are of major importance with sodium (Na\(^+\)) and chloride (Cl\(^-\)) acting as the principal osmotic components. Plasma osmolality is maintained hyper-osmotic to the environment in FW and hypo-osmotic to the environment in SW (Evans, 1993; Karnaky, 1998).

Euryhaline elasmobranch fish, however, in addition to elevating plasma levels of Na\(^+\) and Cl\(^-\) in
comparison to teleost fish, also utilise organic osmolytes as part of their overall osmoregulatory strategy. With the exception of the FW elasmobranchs, Potamotrygonidae, all elasmobranchs are ureotelic. That is they synthesise and excrete urea as an end product of nitrogen metabolism. Urea also acts as the major organic osmolyte in marine and euryhaline elasmobranchs and is retained in the body fluids in large quantities. As a consequence body fluid osmolality is increased in comparison to teleosts and plasma is maintained hyper-osmotic to FW and iso-or slightly hyper-osmotic to SW. As a consequence osmolality from *C. leucas* and *S. canicula* in 50% SW and SW environments is to a large extent negated by the presence of additional organic osmolytes, namely, methylamines, and function of some proteins and enzymes in intracellular ratio of 2:1 urea:methylamines and postulated that methylamines evolved as osmolytes due to their stabilising effects on urea sensitive enzymes. Trimethylamine oxide (TMAO) constitutes approximately 90% of methylamine compounds in elasmobranch plasma (Vyncke, 1970) and is stored in large quantities in the muscle tissue (Goldstein et al., 1967). A further group of organic osmolytes in elasmobranchs is free amino acids. Free amino acids are concerned principally with the regulation of intracellular fluid volume and have been shown to represent approximately 1% of the total osmolyte concentration of the extracellular fluid compartment in the skate *Raja erinacea* (King and Goldstein, 1983). The focus of this review is the regulation of urea as an extracellular fluid osmolyte and as a consequence the role of free amino acids will not be discussed further. For a detailed review on the role of free amino acids in elasmobranch osmoregulation see Goldstein and Perlman (1995).

With the exception of recent reports on the euryhaline Atlantic stingray, *Dasyatis sabina* (De Vlaming and Sage, 1973; Piermarini and Evans, 1998, 2000, 2001; Janech and Piermarini, 2002) our knowledge on the osmoregulatory strategy of fully euryhaline elasmobranchs is based largely on work carried out on the bullshark, *Carcharhinus leucas* (Thorson, 1962, 1967; Thorson and Gerst, 1972; Thorson et al., 1973). Table 1 demonstrates the percentage contribution of urea, Na\(^+\) and Cl\(^-\) to the overall plasma osmolality of euryhaline *C. leucas* and *D. sabina* in the FW and SW environments. As a consequence of the paucity of information regarding the osmoregulatory strategy of fully euryhaline elasmobranchs our understanding of how elasmobranchs acclimate to varying environmental salinities has been based largely on studies utilising predominantly marine elasmobranchs. Laboratory acclimation of a variety marine species to varying environmental salinities has demonstrated that they can survive for extended periods of time in dilute saline environments. This suggests that these species are at least partially euryhaline even though they do not enter full FW environments as part of their natural life cycle. Comparison of partial euryhalinity in the marine elasmobranchs, *Scyliorhinus canicula*, and *R. erinacea* acclimated to 50 and 100% SW is presented in Table 1. However, it is evident that there is a marked shift in the percentage contribution that Na\(^+\), Cl\(^-\) and urea provide in the dilute and concentrated environments, particularly in the case of *S. canicula*. In the first instance this

<table>
<thead>
<tr>
<th>Species</th>
<th>Freshwater</th>
<th>Seawater</th>
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<tbody>
<tr>
<td></td>
<td>Na(^+)</td>
<td>Cl(^-)</td>
</tr>
<tr>
<td><em>C. leucas</em>(^a)</td>
<td>37.1</td>
<td>33.2</td>
</tr>
<tr>
<td><em>C. leucas</em>(^b)</td>
<td>32.4</td>
<td>31.6</td>
</tr>
<tr>
<td><em>D. sabina</em>(^c)</td>
<td>34.1</td>
<td>33.4</td>
</tr>
<tr>
<td><em>S. canicula</em>(^d)</td>
<td>36.6 (50% SW)</td>
<td>37.0 (50% SW)</td>
</tr>
<tr>
<td><em>R. erinacea</em>(^e)</td>
<td>Not measured</td>
<td>30.6 (50% SW)</td>
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**Table 1** Percentage contribution of Na\(^+\), Cl\(^-\) and urea to total plasma osmolality in *C. leucas* and *D. sabina* from FW and SW environments, and plasma osmolality from *S. canicula* in 50% SW and SW environments

*Source:* \(^a\)(Thorson et al., 1973); \(^b\)(Pillans and Franklin, 2003); \(^c\)(Piermarini and Evans, 1998); \(^d\)(Hazon and Henderson, 1984); \(^e\)(Goldstein and Forster, 1971b). All values are expressed as a percentage of the total plasma osmolality.
suggests independent regulation of $\text{Na}^+$, $\text{Cl}^-$ and urea in all three species acclimating to different environmental salinities. In addition, however, it appears that the fully euryhaline species, $D.\text{sabina}$ and $C.\text{leucas}$, and also the partially euryhaline $R.\text{erinacea}$ have a greater capacity to retain urea as they acclimate to reduced environmental salinities.

Organs and tissues involved in the regulation of plasma concentrations of $\text{Na}^+$, $\text{Cl}^-$ and urea will undoubtedly include the gills, kidney, rectal gland, liver and intestine with each tissue/organ influencing $\text{Na}^+$, $\text{Cl}^-$ and urea to a greater or lesser extent. Investigations reporting the function of these organs and tissues in fully euryhaline species are limited. However, laboratory based studies utilising at least partially euryhaline elasmobranchs such as, the spiny dogfish, $S.\text{canicula}$ and the lesser-spotted dogfish, $S.\text{canicula}$ at least provide an insight into the function of these organs/tissues during acclimation to changing environmental salinities.

Our knowledge of hormonal control of osmoregulation in fully euryhaline elasmobranchs is nonexistent. However, studies have demonstrated that numerous partially euryhaline elasmobranchs do possess homologues of major peptides and hormones that are involved in controlling osmoregulation in higher vertebrates. These include, the renin angiotensin system (RAS) (Takei et al., 1991), C-type natriuretic peptide (CNP) (Suzuki et al., 1991), neurohypophysial peptides including arginine vasotocin (AVT) (Acher, 1996) and a corticosteroid unique to elasmobranch fish, 1α-hydroxycholecalciferol (1α-OH-B) (Idler and Truscott, 1966) Peptidergic/hormonal control of osmoregulatory organs and the role they play in $\text{Na}^+$, $\text{Cl}^-$ and urea regulation in both partially euryhaline and where possible fully euryhaline species will be the focus of the present review.

2. Gills

2.1. $\text{Na}^+$ and $\text{Cl}^-$ regulation

Chloride cells, which are abundant in teleost gill epithelia, are characterised by a high concentration of mitochondria and a complex basolateral tubule system. They are known to be involved in the regulation of $\text{Na}^+$ and $\text{Cl}^-$ ions in teleost fish and their function and properties have been extensively reviewed by previous authors (Foskett, 1987; McCormick, 1995; Zadunaisky, 1997). The activity of the transport enzyme, $\text{Na}^+$, $\text{K}^+$-ATPase, is integral to the ion-regulatory function of chloride cells and is found primarily on the basolateral membrane. Although $\text{Na}^+$, $\text{K}^+$-ATPase is involved in the secretion of salt in a SW environment, the exact role of this enzyme during acclimation to changes in salinity has yet to be determined (McCormick, 1995).

In comparison to teleost fish, our understanding of gill function in elasmobranchs is very limited. Chloride cells have been identified, however, the basolateral membrane lacks the complex tubular network seen in teleost fish and is characterised more by a series of in-foldings (Wright, 1973). Despite the presence of chloride cells $\text{Na}^+$, $\text{K}^+$-ATPase activity has been shown to be 10–15 times below that reported for teleost fish (Jampol and Epstein, 1970). More recently investigation of $\text{Na}^+$, $\text{K}^+$-ATPase activity and abundance in $D.\text{sabina}$ was reported by Piermarini and Evans (2000). This study demonstrated that SW acclimation induced a decrease in the activity and relative abundance of $\text{Na}^+$, $\text{K}^+$-ATPase in the gills of $D.\text{sabina}$. Furthermore, FW $D.\text{sabina}$ were shown to have a higher number of $\text{Na}^+$, $\text{K}^+$-ATPase -rich cells localised primarily on the gill lamellae. The activity, location and abundance of $\text{Na}^+$, $\text{K}^+$-ATPase in FW acclimated $D.\text{sabina}$ seems to suggest a role in ion-uptake for $\text{Na}^+$, $\text{K}^+$-ATPase-rich cells (Piermarini and Evans, 2000). However, there is most probably a role in acid–base balance for $\text{Na}^+$, $\text{K}^+$-ATPase rich cells in the FW animal similar to that which has been demonstrated for FW teleosts and SW elasmobranchs (Bentley et al., 1976; Perry, 1997). Following the identification and localisation of the vacuolar proton-ATPase (V-H$^+$-ATPase) in the gills of $D.\text{sabina}$ a dual role was proposed for V-H$^+$-ATPase and $\text{Na}^+$, $\text{K}^+$-ATPase cell types in combined regulation of $\text{Na}^+$ and $\text{Cl}^-$ with acid base balance in both FW and SW acclimated $D.\text{sabina}$ (Piermarini and Evans, 2001). Whether the primary role of the $\text{Na}^+$, $\text{K}^+$-ATPase-rich cell type identified by Piermarini and Evans is that of salt regulation or acid–base balance remains to be determined.

It is apparent that the role of the gill in $\text{Na}^+$ and $\text{Cl}^-$ regulation in elasmobranch fish is uncertain. Indeed it has been suggested that the gill epithelia do not make a significant contribution to salt balance in marine elasmobranchs (Shuttle-
worth, 1988). This is in part because of the reported net influx of salt across the gill epithelia (Payan and Maetz, 1973) in marine elasmobranchs but primarily because the elasmobranchs are equipped with a specialised salt secreting gland, the rectal gland (Burger and Hess, 1960). The role of the rectal gland in response to changing environmental salinities will be discussed below (Section 5.1).

2.2. Urea regulation

It is well known that elasmobranch gill epithelia is particularly impermeable to urea (Boylan, 1967; Payan et al., 1973; Wood et al., 1995; Part et al., 1998; Walsh and Smith, 2001). However, the large surface area combined with a huge concentration gradient means that diffusional loss of urea is greatest across the gills, indeed this loss has been reported to be almost equivalent to the rate of urea synthesis across the gills. Initially, this conclusion was discounted. For example, a more recent examination of the process of urea transport in the elasmobranch gill epithelia revealed an extraordinarily high cholesterol content that was considered to significantly contribute towards the urea impermeability of the elasmobranch gill epithelia (Fines et al., 2001). Furthermore, Part et al. (1998) demonstrated that the basolateral membrane of the gill epithelia in S. acanthias was some 14 times more permeable to urea than the apical membrane. This selective permeability of urea thus supported Boylan’s conclusions and led these researchers to postulate that the gills in elasmobranchs form an intermediary layer between the internal and external environment with respect to urea (Wood et al., 1995; Part et al., 1998).

Using competitive urea transport inhibitors in vivo Wood et al. (1995) also demonstrated an increase in urea efflux from the gill of S. acanthias suggesting the presence of a urea ‘back-transporter’ on the basolateral membrane. This was later supported by a similar study using an in vitro perfused gill preparation Part et al. (1998). Further evidence for the presence of a urea transport system in the gills of elasmobranchs was provided by Fines et al. (2001) who demonstrated the presence of a phloretin sensitive sodium–urea counter-transporter directed to return urea to the blood stream. Finally, Wood et al. (1995) hypothesised that urea synthesis could also be occurring in the gills to support the ‘back transport’ of urea into the blood stream by creating a higher concentration of urea in the intermediary gill layer. It appears, therefore, that the observed low urea flux rate at the elasmobranch gills is a result of structural differences between the apical and basolateral membranes combined with possible urea synthesis in the epithelia and also specific urea transport mechanisms on the basolateral membrane.

In the context of the present review, how the physiological process of urea retention at the gills responds to alteration in environmental salinity is unknown. Manipulation of internal urea concentration only, using S. acanthias acclimated to 100% SW, did not yield any direct relationship between plasma urea concentration and urea efflux across the gills (Boylan, 1967; Wood et al., 1995; Part et al., 1998). Furthermore, reduced salinity acclimation of the partially euryhaline skates R. erinacea and Raja radiata and the lemon shark Negaprion brevirostris did not appear to affect gill permeability to urea (Goldstein et al., 1968; Goldstein and Forster, 1971a; Payan et al., 1973). These studies suggest that the observed decrease in plasma urea concentration in elasmobranchs acclimated to reduced environmental salinity is not a function of urea transport at the gills but rather some other physiological process involved in urea maintenance, such as synthesis at the liver or reabsorption by the kidney.

The evidence to date suggests that while the gill epithelia in elasmobranch fish appears to act as an intermediary barrier to perturbations in environmental salinity with respect to regulation of plasma urea, it does not behave as an active osmoregula-
tory tissue with respect to plasma Na\(^+\) and Cl\(^-\) levels as in teleost fish. As a consequence there is a greater emphasis on the role of the kidney and rectal gland in elasmobranch fish so as to maintain salt and urea balance within the body fluids.

2.3. Control of gill function

To date the only evidence for peptide control of gill function in elasmobranchs is indirect. Receptors for angiotensin II (Ang II), the principal bioactive component of the RAS, have been identified in membrane fractions prepared from gill cells of the Japanese dogfish, *Triakis scyllia* (Tierney et al., 1997). Although it is highly probable that Ang II would influence the perfusion of blood through the gills due to its vasopressor activity on the vasculature (Hazon et al., 1999) it is unknown if Ang II has a controlling influence on Na\(^+\), K\(^+\)-ATPase in the gills of elasmobranchs as has been demonstrated in the euryhaline eel, *Anguilla anguilla* (Marsigliante et al., 1997).

Evidence for the action of AVT or CNP in the elasmobranch gill is unavailable, however, as with Ang II both these peptides are known to have vasoactive properties in elasmobranchs with AVT exerting a vasopressor effect (Hazon, unpublished) and CNP a vasodilatory effect in isolated gill arteries (Bjenning et al., 1992; Evans et al., 1993) although the actions of these peptides on branchial capillaries remains unknown. It is likely, therefore, that these two peptides, as with Ang II, would at least influence gill function in elasmobranchs in terms of altering directional blood flow to the gill epithelia. However, clearly more work is required to establish the location of peptide receptors in the elasmobranch gill to gain a greater understanding of the actions of these peptide systems on elasmobranch gill function in terms of osmoregulation.

1α-OH-B binding activity has been demonstrated in the gills of the skate *Raja ocellata* (Moon and Idler, 1974) and subsequent identification of a cytosol receptor glycoprotein for 1α-OH-B was reported in the gills of *R. ocellata* (Idler and Kane, 1980). Furthermore, concentration of the gill-type glycoprotein and affinity for radio-labelled 1α-OH-B was much greater in the gill tissue than the blood, indicating that the gill epithelia would easily compete with the plasma for binding of the steroid (Idler and Kane, 1980). Although the exact role of 1α-OH-B has yet to be determined in the gills of elasmobranchs it is clear that the steroid may influence salt and/or urea flux across the gills, particularly when considering the effects of cortisol, the major teleost corticosteroid, on chloride cell morphology and function in teleost fish (Laurant and Perry, 1990).

3. Kidney

Elasmobranch kidneys are elongate, paired structures lying along the dorsal wall of the body cavity (Chester-Jones, 1957). Blood supply to the kidneys originates from both the arterial and renal portal systems, with portal blood mixing freely with blood from the glomerular efferent arteries before leaving the kidney via the renal veins. There is also evidence of a glomerular bypass shunt whereby blood may pass directly from the afferent arteriole to the post-glomerular circulation, therefore, avoiding filtration (Brown and Green, 1992).

The functional component of renal tissue, the nephron, is a long and intricate tubular structure and elasmobranch fish possess the most complex nephron of all vertebrates. Renal tissue is separated into two distinct zones: a sinus zone, where tubules are loosely packed and segregated from each other by large blood sinuses; and a zone of lateral bundles, where tubule segments are packed tightly into discrete bundles (Lacy and Reale, 1995). Each individual nephron spans both zones, forming two hairpin loops in the bundle zone and two extended convolutions in the sinus zone (Hentschel et al., 1998). In the sinus zone, proximal and distal tubules belonging to different nephrons extend in a common bed of sinusoidal capillaries belonging to the venous renal portal system (Hentschel, 1988). In contrast, the portions of each nephron that penetrates into the bundle zone are separated from each other, as each bundle is enclosed in a sheath of squamous epithelial cells (Hentschel, 1987). Ultrastructural analysis of the nephron in the little skate, *R. erinacea* (Lacy and Reale, 1991a,b) and *S. canicula* (Hentschel et al., 1993, 1998) provides strong evidence for a countercurrent system involving highly specialised and diverse epithelial cell types. A full account of elasmobranch renal anatomy has been described in detail by Lacy and Reale (1995).

3.1. Na\(^+\), Cl\(^-\) and urea regulation

Elasmobranchs produce a urine that is hypotonic to blood plasma. The heterogeneity of tubular epithelial cells may be an adaptation for
the retention of urea with approximately 70–99% of filtered urea being reabsorbed in the elasmobranch kidney (Kempson, 1953; Boylan, 1967). It has long been speculated that a carrier-mediated process is involved in elasmobranch renal urea reabsorption. Urea reabsorption has been shown to be specific, with only 35% of the urea analogue, thiourea, being reabsorbed in the elasmobranch nephron (Boylan, 1967). The fact that urea is reabsorbed against a sizeable concentration gradient has led to the proposal that the reabsorption mechanism is active, possibly coupled to the movement of sodium (Schmidt-Nielsen and Rabnowitz, 1964; Schmidt-Nielsen et al., 1972 Hays et al., 1977; Stolte et al., 1977). The kidney of S. acanthisias contains at least one protein representing a facilitated urea transporter (shUT) belonging to the UT-A family of urea facilitated transporters in mammals, and there is preliminary evidence for a similar urea transport protein in R. erinacea (Smith and Wright, 1999). Recently, a second facilitated urea transporter was sequenced and identified in D. sabina (Jancec et al., 2003). A 71% sequence identity was reported between the shUT transporter and the stingray (strUT) urea transporter. This evidence undoubtedly adds support for facilitated urea transport but it remains to be determined if facilitated urea transport is solely responsible for urea reabsorption in the elasmobranch kidney.

A passive model of urea reabsorption has been proposed (Friedman and Herbert, 1990) which relies on the presence of a countercurrent multiplication system and differential permeabilities of tubular segments to urea, water and Na⁺, as occurs in mammals. The renal countercurrent bundles and the microvasculature of the elasmobranch nephron were recently examined in detail in S. canicula and R. erinacea (Hentschel et al., 1998). This study identified a single lymph capillary-like vessel that runs in close contact with the collecting tubule along the entire bundle, before merging with the venous sinusoidal capillaries of the peritubular blood circulation. It was suggested that this central vessel provides a channel for the convective flow of sodium chloride rich-fluid to the portal system, and may, therefore, be involved in countercurrent exchange of urea, from the collecting tubule urine to the fluid in the central vessel (Hentschel et al., 1998). This could account for the low concentration of urea in the fluid of the collecting duct as observed by micropuncture (Stolte et al., 1977). A more detailed description of urea transport is provided in a recent review by Walsh and Smith (2001), however, it is clear that detailed analysis of urea permeabilities, and sites of transport along the elasmobranch nephron are required to determine whether urea reabsorption is active, passive or both.

The kidney also plays a role in the regulation of Na⁺ and Cl⁻ plasma concentration. In addition to sodium-linked urea transport (see above), ultrastructure studies have demonstrated that tubular cells in the early distal tubule have characteristics very similar to cells that are known to actively transport sodium (Friedman and Herbert, 1990; Lacy and Reale, 1991a). Histochemical studies have only isolated Na⁺, K⁺-ATPase activity in the early and late distal tubules and the collecting duct (Endo, 1984; Hebert and Friedman, 1990).

In addition, isolated perfused proximal tubules from S. acanthisias have been shown to actively secrete sodium and chloride ions (Beyenbach and Fromter, 1985) and this appears to drive net fluid secretion (Sawyer et al., 1985). However, the ultrastructural studies used as evidence for active tubular reabsorption; (Lacy et al., 1975; Endo, 1984) could just as easily be applied in support of active tubular secretion (Henderson et al., 1988) as no directional element to this tubular movement was established. It is therefore clear that further study is required to determine whether sodium is reabsorbed or secreted across the tubular epithelium.

In the context of euryhalinity interpretation of data regarding the transport of ions and urea in the elasmobranch kidney in response to salinity change is undoubtedly complicated by the intricacy of the nephron. However, basic renal parameters such as urine flow rate (UFR), glomerular filtration rate (GFR) and ion clearance still provide an opportunity to examine the effects of environmental acclimation on kidney function and its relationship to urea and salt excretion/reabsorption. Integral to the process of acclimation to varying environmental salinity is the necessity to vary urine output in order to control extracellular fluid volume (Brown and Green, 1987). Values for urine flow rate in FW acclimated euryhaline elasmobranchs have been shown to greatly exceed those of FW teleosts (Smith, 1931a; Jancec and Piermarini, 2002) demonstrating the potential for a large reabsorptive capacity for urea in these fish. Acclimation of S. canicula to varying dilute environmental salinities induces a progressive increase...
in renal clearance of Na\(^+\), Cl\(^-\) and urea in vivo (Wells et al., 2002). As with previous reports utilising partially euryhaline elasmobranchs, urine flow and renal clearance of Na\(^+\), Cl\(^-\) and urea all increase upon acclimation to dilute environmental salinity (Goldstein et al., 1968; Goldstein and Forster, 1971b; Wong and Chan, 1977; Sulikowski and Maginniss, 2001). It is apparent that both partially euryhaline and fully euryhaline elasmobranchs have the capacity to alter kidney function in response to environmental salinity change. During acclimation to dilute environments reduction in plasma levels of Na\(^+\), Cl\(^-\) and urea (see Table 1) may simply be achieved through an increase in urine flow (Smith, 1931a). Although it is unlikely that there is no alteration in urea and/or Na\(^+\) and Cl\(^-\) reabsorptive mechanisms at a tubular level, particularly in the case of FW acclimated euryhaline elasmobranchs.

3.2. Control of renal function

Early studies, using primarily pharmacological doses of catecholamines, presented conflicting results in terms of the control of elasmobranch GFR. However, a clear glomerular diuresis caused by adrenaline was demonstrated in S. canicula (Brown and Green, 1987). GFR has also been modulated by other heterologous peptide hormones including vasoactive intestinal peptide (VIP) and prolactin (Yokota and Benyajati, 1988) which both appeared to significantly increase GFR. In addition, homologous AVT, Ang II and natriuretic peptides (NP) have all been implicated in the control of renal function in teleost fish species (Brown et al., 1980; Amer and Brown, 1995; Takei and Kaiya, 1998) although their role in elasmobranch fish has only recently been investigated. One of the major reasons for a lack of more conclusive data is that renal studies in at least partially euryhaline marine elasmobranch fish are technically difficult to perform. Some species, including S. canicula, possess very long and convoluted urinary sinuses and urine output in vivo occurs intermittently. Consequently, establishing basal control urine flow rates, a prerequisite for in vivo renal studies, is almost impossible unless extremely long clearance intervals are used. In addition, whole animal data can be difficult to interpret because of the potential effects of a range of factors (e.g. paracrine and endocrine) that may increase or decrease blood pressure and thereby change GFR and renal function.

In order to avoid at least some of these complications and to determine the actions of individual peptides on kidney function, a perfused trunk preparation with the kidney in situ was developed for elasmobranch fish by Wells et al. (2002) based on a technique previously described in teleosts (Dunne and Rankin, 1992; Amer and Brown, 1995). Addition of 10\(^{-9}\) and 10\(^{-10}\) M homologous AVT to the in situ perfused trunk preparation caused a decrease in urine flow rate and GFR in SW acclimated female dogfish (Wells et al., 2002). Renal clearances of ions and urea were also reduced in line with reductions in GFR and there were no indications of specific tubular actions of AVT. These data suggest that AVT induced a glomerular antidiuresis in S. canicula as previously reported for the trout (Amer and Brown, 1995). Similar results to those of AVT were obtained when 10\(^{-9}\) M Ang II and 10\(^{-10}\) M ANG II were added to the perfusate (Wells et al., 2003). As both AVT and Ang II are profound vasoconstrictor hormones in elasmobranch fish, it is possible that this effect is mediated by a process of glomerular de-recruitment, through vasoconstriction, in which the total number of filtering nephrons is decreased, therefore, reducing the total GFR. Wells et al. (2002) assessed filtering populations of nephrons indirectly by measurement of the transport maximum for glucose (TmG). Addition of 10\(^{-9}\) M and 10\(^{-10}\) M AVT and Ang II resulted in significant decreases in TmG indicative of reductions in the population of functional glomeruli (Wells et al., 2002, 2003).

CNP is the only circulating NP reported in elasmobranch fish (Suzuki et al., 1994; Kawakoshi et al., 2001) and perfusion of the dogfish trunk preparation with 10\(^{-9}\) M CNP resulted in an increase in urine flow rate, GFR and TmG, coupled with an increase in the clearance and excretion of urea, Na\(^+\) and Cl\(^-\) (Wells et al., 2003). In S. acanthias, synthetic mammalian ANP (atriopeptin II) was reported to be antidiuretic and anti-natriuretic in vivo (Benyajati and Yokota, 1990). This renal anti-diuresis and anti-natriuresis was associated with a drop in mean aortic pressure and GFR. The renal effect of the heterologous NP was temporally dissociated from the vasodepressor effect by at least an hour. However, it is possible that the renal effects were secondary rather than a direct effect. The drop in blood pressure may in
fact have caused a release of vasopressor factors such as Ang II that would then induce the observed antidiuresis. However, following volume-expansion in 90% SW, the renal effect of atriopeptin II is reversed to diuresis and natriuresis (Benyajati and Yokota, 1987). The authors suggested that the actions of atriopeptin II depend upon the hydration status of the animal and that this effect may be modulated by catecholamines. It would appear, therefore, that CNP has the opposite renal action to both AVT and Ang II although the precise location of receptors for these peptide hormones needs to be established. Together these peptides may play an important role in the renal function of euryhaline elasmobranchs and indirectly control clearance of ions and urea via their effects on the renal vasculature in the control of GFR and urine flow rate during acclimation to FW or SW environments.

4. Liver

Elasmobranch fish are ureotelic and urea production in the liver is primarily carried out by the ornithine urea cycle, which has been detailed in many reviews (Goldstein, 1967; Anderson, 1995, 2001; Walsh and Mommsen, 2001). Marine elasmobranchs retain 300–500 mmol/l of urea in the blood plasma and excrete relatively large amounts of urea and small amounts of ammonia almost completely via the gills (Wood et al., 1995). The ornithine urea cycle has been demonstrated in the FW rays, Potamotrygonidae. However, these fish do not retain urea in the body fluids and excrete large amounts of ammonia and small amounts of urea (Goldstein and Forster, 1971b). Furthermore, activities of ornithine urea cycle related enzymes are much lower in Potamotrygon compared to marine species (Goldstein and Forster, 1971b). Indeed, it is considered that Potamotrygonidae lack a functional ornithine urea cycle as described in other elasmobranchs. For a detailed review on the role of the various ornithine urea cycle enzymes and urea synthesis in elasmobranchs see Anderson (2001). In the context of the present review we will briefly investigate urea metabolism in the elasmobranch in relation to changes in environmental salinity.

Following transfer to 50% SW there was shown to be a reduction in urea plasma levels in the partially euryhaline lemon shark, Negaprion brevirostris (Goldstein et al., 1968). This was attributed to be due entirely to an increase in urea loss at the kidney with no change in urea biosynthesis. Similarly transfer to 50% SW for the partially euryhaline R. erinacea and S. canicula reduced plasma urea concentration, However, in these species the reduction was due to the combined increase in plasma clearance and decrease in production (Goldstein and Forster, 1971a; Hazon and Henderson, 1984). Therefore, current evidence suggests that plasma urea reduction in elasmobranchs acclimating to reduced environmental salinity is a result primarily of increased urine flow and, therefore, increased renal clearance of urea. However, in addition there is the possibility of a reduced urea biosynthesis at the liver, which appears to be species-specific. The contribution of urea to total plasma osmolality in the partially euryhaline elasmobranch S. canicula acclimated to 50% SW is only 16%, whereas urea contribution in FW in both C. leucas and D. sabina is between 25 and 31% of total plasma osmolality (Table 1). It appears, therefore, that the ability to retain urea in dilute media in fully euryhaline elasmobranchs is greater. Surprisingly we must go back to Homer Smiths’ original articles for further information where he postulates that urea reduction in FW elasmobranchs is simply a function of increased urinary output and reduced tubular reabsorption in the kidney. It is unfortunate that there is still no definitive evidence to support the ideas put forward by Smith over 70 years ago (Smith, 1931a,b).

5. Rectal gland

5.1. Na+ and Cl− regulation

The capacity of the rectal gland to secrete a fluid that is iso-osmotic to blood plasma, which is almost entirely composed of Na+ and Cl− (Burger and Hess, 1960) makes it perhaps the most studied elasmobranch tissue. Given the composition of rectal gland fluid the contribution of the rectal gland to urea balance in elasmobranchs is almost negligible so this section will report only the function of the rectal gland in terms of the Na+ and Cl− component of osmoregulation.

The rectal gland is known to secrete on an intermittent basis (Burger, 1962), which has led to the suggestion that its activity is stimulated by acute salt/volume loading perhaps related to feeding. Structurally the rectal gland consists of a series of interconnected tubules that are lined with
salt secreting epithelia and conjugate to form a single duct that empties into the posterior end of the intestine close to the rectum. It is supplied by a single arterial branch from the dorsal aorta, the rectal gland or posterior mesenteric artery. The artery enters a complex arrangement of arterioles and capillaries that surround the tubular/secretory network. A recent investigation has demonstrated that blood flow through the capillary network flows in a countercurrent direction to tubular flow, suggesting modification of rectal gland fluid within the tubular network (Newbound and O’Shea, 2001). However, it is also recognised that secretion rate from the gland is highly dependent on blood flow to the secretory epithelia (Shuttleworth and Thompson, 1986; Anderson et al., 2002a).

The rectal gland has contributed greatly to our understanding of salt secretion on a cellular level (Silva et al., 1990; Riordan et al., 1994) and a key enzyme involved in this process is Na⁺, K⁺-ATPase. Na⁺, K⁺-ATPase activity in the rectal gland of marine elasmobranchs is extremely high and has been shown to increase both in terms of activity and expression in response to feeding in the partially euryhaline elasmobranch S. canicula (MacKenzie et al., 2002). Furthermore, Na⁺, K⁺-ATPase activity and abundance was shown to be significantly greater in the rectal gland of SW acclimated D. sabina compared to FW acclimated D. sabina (Piermarini and Evans, 2000). These reports suggest that a stimulus for secretion from the rectal gland is salt or osmotic load associated with the SW environment or dietary intake of sodium. However, an increase in blood volume has been shown to act as a major stimulus for secretion in the rectal gland of both S. acanthis and S. canicula (Solomon et al., 1984a,b; Anderson et al., 2002a). Recently, Anderson et al. (2002a) demonstrated that transfer from 100 to 70% SW induced an increase in secretion rate and blood perfusion to the secretory epithelia in the rectal gland of S. canicula. Therefore, as S. canicula acclimates to a reduced environmental salinity there would be an initial increase in blood volume (Hazon and Henderson, 1984). This would, in turn, stimulate rectal gland secretion (Solomon et al., 1984a,b) thereby reducing plasma osmolality in line with the reduced environmental salinity of 70% SW.

In a FW environment euryhaline elasmobranchs are hyperosmotic to their environment and must retain sodium and chloride. Secretion of Na⁺ and Cl⁻ would, therefore, be an inappropriate response and indeed Na⁺, K⁺-ATPase activity in the rectal gland of FW D. sabina was shown to be considerably lower than that of SW acclimated D. sabina (Piermarini and Evans, 2000).

Rectal gland size and morphology have been shown to be significantly different between FW and SW elasmobranchs (Oguri, 1964) with FW species generally having smaller rectal glands and a reduced secretory tubule network. Furthermore, although Na⁺, K⁺-ATPase activity in SW wild caught and SW-acclimated (8 days in 1005 SW aquaria) was similar (Piermarini and Evans, 2000) the rectal gland in SW acclimated D. sabina was some 50–70% smaller than wild caught SW conspecifics (Piermarini and Evans, 1998). This suggested that the total NaCl secretory capacity of the rectal glands (Na⁺, K⁺-ATPase activity X rectal gland mass) of wild SW caught animals would be greater (Piermarini and Evans, 2000). Indeed, Burger (1972) demonstrated that chloride secretion from the rectal gland of SW wild caught D. sabina was comparable to that of rectal glands from SW S. acanthis. However, Piermarini and Evans (2000) did demonstrate that although Na⁺, K⁺-ATPase activity in the rectal gland of FW acclimated D. sabina was about half that from rectal glands of SW acclimated D. sabina it was seven times greater than Na⁺, K⁺-ATPase activity in the gills of FW acclimated D. sabina. This suggests that rectal glands from FW acclimated D. sabina have at least the potential to secrete Na⁺ and Cl⁻. Recently, Anderson et al. (2003) reported an allometric scaling relationship between rectal gland mass and total body mass in SW acclimated S. canicula. Although functional aspects of rectal gland activity such as secretion rate or Na⁺, K⁺-ATPase activity were not reported in this study it was shown that smaller dogfish had proportionally larger rectal glands. It would be interesting to apply similar scaling relationships to rectal gland mass in addition to functional aspects such as secretion rate and Na⁺, K⁺-ATPase activity to the body mass of both FW and SW acclimated euryhaline elasmobranchs. In this way the true contribution of the rectal gland to Na⁺ and Cl⁻ balance in these different environments could be determined.

5.2. Control of rectal gland function

In addition to the potential morphological and functional changes in the rectal gland of FW and
SW acclimated elasmobranchs, alteration in peptide/diegetic and or hormonal control must also be considered. As has been stated, perhaps the major stimulus for rectal gland secretion is blood volume expansion. For vertebrates in general, release of natriuretic peptides from the heart is directly associated with blood volume expansion. C-type natriuretic peptide (CNP) is the principal, and perhaps the only (Suzuki et al. 1994; Kawakoshi et al., 2001) circulating natriuretic peptide in elasmobranchs. Natriuretic peptide like-binding sites have previously been demonstrated in the capsular and sub-capsular regions of the rectal gland of S. canicula (Masini et al., 1994) and more recently a NPR-B type natriuretic peptide receptor was cloned from the rectal gland of S. acanthias (Aller et al., 1999). CNP has been shown to stimulate salt secretion from cultured rectal gland cells (Karnaky et al., 1992) in addition to in vitro (Solomon et al., 1992; Anderson et al., 2002a) and in vivo (Anderson and Hazon, unpublished) preparations. As such CNP is believed to be the causative factor for rectal gland stimulation in response to volume load (Solomon et al., 1985; Loretz and Pollina, 2000). It is reasonable to assume that FW euryhaline elasmobranchs would be volume loaded in comparison to their SW counterparts, however, CNP stimulation of rectal gland secretion in this situation would be an entirely inappropriate response. Whether circulating levels of CNP are greater in FW or SW remains to be determined. Furthermore, identification and quantification of CNP receptors in the rectal gland of FW and SW euryhaline elasmobranchs has yet to be reported. Additional complications must also be considered given the vasodilatory action of CNP in elasmobranchs (Bjenning et al., 1992; Evans et al., 1992, 1993) and the close correlation between secretory activity and vascular perfusion of the rectal gland secretory epithelia in S. canicula (Anderson et al., 2002a).

In addition to stimulation (or lack of it) the possibility of direct inhibition of rectal gland secretion in FW acclimated euryhaline elasmobranchs must also be considered. Anderson et al. (2002a) reported a significant decrease in rectal gland secretion rate in S. canicula acclimated to 120% SW when compared to those acclimated to 100% SW presumably in response to a requirement to retain salt in the hyper-osmotic salinity of 120% SW. Recently, an increase in circulating levels of Ang II was demonstrated in S. canicula following transfer from 80 to 100% SW and this increase was the result of hypo-volaemia (Anderson et al., 2002c). Ang II like binding sites and angiotensin converting enzyme like activity have been reported in the rectal gland of S. canicula (Masini et al., 1994; Hazon et al., 1997a). However, 10−9 M Ang II perfusion of the isolated rectal gland of S. canicula did not affect either secretion rate or vascular perfusion of the secretory epithelia in the isolated rectal gland of S. canicula (Anderson et al., 2002a). It is likely, therefore, that the reported inhibition in 120% SW from the rectal gland of S. canicula was the result of direct inhibition by NPY or somatostatin as suggested for the rectal gland in S. acanthias (Silva et al., 1985, 1993). Whether such inhibition occurs in the rectal gland of FW acclimated euryhaline elasmobranchs remains to be determined.

Additional reports regarding control of rectal gland function include investigations into the actions of 1α OH-B, vasoactive intestinal peptide (VIP) and scyliorhinin II. Interestingly, VIP has been reported to act as a potent secretagogue in S. acanthias (Stoff et al., 1979) but induced no response in S. canicula or Raja clavata (Shuttleworth and Thorndyke, 1984; Thorndyke and Shuttleworth, 1986; Anderson et al., 1995). To date this discrepancy has yet to be fully explained and at best can be described as a species difference. Attempts to elucidate an intestinal peptide that did stimulate rectal gland secretion in S. canicula were initially made by Shuttleworth and Thorndyke (1984) and an endogenous stimulatory peptide from the gut of S. canicula was isolated but not sequenced and named `rectin’. Later Anderson et al. (1995) purified and sequenced a stimulatory peptide (most likely `rectin’) from the intestine of S. canicula that was found to be a previously identified intestinal peptide, syclorhinin II. Although it is unlikely that VIP or scyliorhinin II are released in response to changes in environmental salinity, their release may well be influenced by changes in feeding activity and/or dietary intake.

As has been described 1α-OH-B is the principal corticosteroid secreted from the interrenal gland in elasmobranch fish (Truscott and Idler, 1968) and 1α-OH-B receptors have been identified in the rectal gland of the ray, R. ocellata (Idler et al., 1967; Moon and Idler, 1974). The steroid has been implicated in a variety of osmoregulatory processes including rectal gland secretion (Holt and Idler,
1975) and may have a potential mineralocorticoid role in the retention of sodium from renal and extra-renal sites in elasmobranch fish (Armour et al., 1993). Removal of interrenal tissue in R. ocellata caused a decrease in secretion by the rectal gland, which was reversed following injection of 1α-OH-B (Holt and Idler, 1975). However, injections of heterologous corticosteroids in intact Hemiscyllium plagiogum inhibited rectal gland secretion (Chan et al., 1967). The role of 1α-OH-B in the control of rectal gland secretion is therefore not clear. Whether the action of 1α-OH-B is at a molecular, cellular or morphological level remains to be determined.

6. Drinking

Historically it was thought that elasmobranch fish did not have the physiological requirement to drink (Smith, 1931b). However, this assumption was based largely on marine elasmobranchs that maintain their plasma osmolality iso- or slightly hyper-osmotic to the surrounding SW and are, therefore, faced with a small but constant influx of water across the gills in an environment that has little to no fluctuations in salinity.

Euryhaline elasmobranchs are faced with a very different problem as they migrate from FW to SW. They must rapidly raise their plasma osmolality to that of SW as they enter the marine environment so that plasma osmolality is maintained iso- or slightly hyper-osmotic to the surrounding SW and are, therefore, faced with a small but constant influx of water across the gills in an environment that has little to no fluctuations in salinity.

Euryhaline elasmobranchs face the problem of rapidly increasing plasma osmolality when entering a hyper-osmotic medium. Clearly euryhaline elasmobranchs would face such an acute change in environmental osmolality as they migrate from FW to SW and it is probable that these animals would utilise a drinking response as part of their overall osmoregulatory strategy as they enter the marine environment.

6.1. Control of drinking

To date Ang II is recognised as the most potent dipsogenic peptide in higher vertebrates and is released in response to cellular dehydration in mammals (Fitzsimons, 1979, 1998). It is known to have similar dipsogenic properties in both teleost (Takei et al., 1979) and elasmobranch fish (Anderson et al., 2001a) but is released in response to extra-cellular dehydration in both vertebrate groups (Takei et al., 1988; Anderson et al., 2002c). Interestingly a peak in circulating levels of Ang II in S. canicula transferred from 80 to 100% SW was observed to coincide with the peak in drinking in these animals (Anderson et al., 2002c). These data strongly implicate the involvement of Ang II in the control of drinking in at least partially euryhaline elasmobranch fish. It is not known, however, if Ang II is involved in the control of an as yet undetermined drinking response in fully euryhaline elasmobranchs.

The only other peptide to be investigated to date with regard to the control of drinking in elasmobranchs is CNP. Natriuretic peptides in general are known to be antagonistic to Ang II in the physiological mechanisms they control. Indeed, in the euryhaline teleost, Anguilla japonica, atrial natriuretic peptide (ANP), the principal circulating natriuretic peptide, is 100 times more potent in its inhibition of drinking than Ang II is in stimulating drinking (Takei, 2000). A similar role in the inhibition of drinking in the elasmobranch S. canicula was recently reported, where CNP was 50 times more potent in its inhibition than Ang II was in its stimulation (Anderson et al., 2001b). How CNP interacts with Ang II in fully euryhaline elasmobranchs has yet to be examined although it would be reasonable to assume that these peptides would influence a drinking response in these fish similar to that described for the partially euryhaline elasmobranch S. canicula. Other hormones may well influence the drinking response in elasmobranch fish and further work is required to elucidate fully the control of the drinking response.
In summary, it would appear that the fundamental physiological mechanisms that mediate acclimation to different environmental salinities in partial and fully euryhaline elasmobranch fish are similar. Undoubtedly expression of key transporters, and action of osmoregulatory peptides such as Ang II, AVT and CNP are markedly different between partially and fully euryhaline elasmobranchs. Nevertheless, we are only just beginning to elucidate the mechanisms that control such an extraordinary osmoregulatory strategy. Future research involving an integrated approach from molecular biology to whole animal physiology will help to determine the processes that allow euryhaline elasmobranchs to survive and flourish in both FW and SW environments.

References


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