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During the last decades the complicated behaviour of three-spined sticklebacks has not only attracted the attention of ethologists, but also of many students of osmoregulation. The migratory behaviour, as shown especially by the \textit{trachurus} form of the three-spined stickleback, makes high demands upon the osmoregulatory capacities of the fish during its journey from freshwater to seawater and vice versa. Many investigators have studied the physiological mechanisms of adaptation that enable these fish to live in environments that are totally different in osmolality and ion composition (Koch \& Heuts, 1943; Lam, 1968; Wendelaar Bonga, 1973, 1976; Wendelaar Bonga \& Veenhuis, 1974a, b; see also Wootton, 1976).

The nestbuilding behaviour displayed by male sticklebacks during the reproductive period requires osmoregulatory adaptations as drastic as seaward migration. However, whereas migration is shown by many other fish, the nestbuilding behaviour of sticklebacks has consequences for osmoregulation that are quite unique among the more than 20,000 species of teleosts. The materials selected for nestbuilding by male sticklebacks are glued together with a mucous substance secreted by the kidneys. To this end the kidneys are transformed, structurally and functionally, from an important osmoregulatory organ into a mucus secreting gland. This phenomenon offers the opportunity of studying the osmotic and ionic homeostasis in a freshwater fish that lacks normal kidney functions. Our studies have been concerned with the glandular transformation of the kidneys, the endocrine control of this process, and its consequences for hydromineral control.

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Some principles of hydromineral regulation in freshwater fish

Ion concentrations in the blood and other body fluids are maintained at rather constant levels, which results in an osmolality of about 330 mOsmol/l in freshwater sticklebacks. Freshwater is strongly hypotonic compared to the body fluids (less than 5 mOsmol/l), and this results in osmotic water influx and loss of ions via the gills (Conte, 1969). The kidneys normally play an important role in the elimination of the water surplus, via excretion of large volumes of urine (Hickman & Trump, 1969). Due to the high ion-reabsorptive capacity of the kidney tubules, the elimination of the water can take place without substantial loss of ions (Hickman & Trump, 1969). Any loss of ions via urine or via diffusion through the gills are compensated by active uptake of ions from the food in the intestine and from the ambient water by the chloride cells in the gills (Conte, 1969; Bierther, 1970). Thus, in normal freshwater fish the kidneys, intestine and gills are vital organs for maintaining water and ion homeostasis. It is therefore surprising that male sticklebacks can live in freshwater with their kidneys transformed into mucus glands.

Structure and function of the normal stickleback kidney

The kidneys are located in the body cavity, basolaterally to the spinal column, and consist of numerous nephrons—coiled tubule-like structures—embedded in haematopoietic tissue. The nephrons are connected by short branches to the two ureters, which run laterally of the kidneys and terminate in the urinary bladder. A nephron consists of a renal corpuscle, and the attached nephronic tubule which consists of four segments.

The renal corpuscle consists of a Bowman’s capsule with inside a tuft of bloodcapillaries, the glomerulus. In the three-spined stickleback the renal corpuscles are situated in small groups, mostly at the periphery of the kidney.

The nephronic tubules consist of a short neck-segment, which connects the Bowman’s capsule to the remaining part of the tubule, and three main segments, namely the first and second proximal segments and the remaining collecting tubule, which opens into the ureters (Figs 1 and 2).

A glomerular filtrate is formed by means of ultrafiltration of the blood. This is processed into urine in the nephronic tubule segments, via reabsorption and secretion of substances such as ions, small proteins, sugars and amino acids. Reabsorption of ions, water and macromolecules takes place in the first proximal segment, while in the other segments mainly ions and water are reabsorbed.
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Fig. 1. Photomicrograph of a part of an immature freshwater male stickleback renal section showing a group of renal corpuscles. One of them is showing the attachment of the neck segment (ns) and the first proximal segment ($P_1$) of the nephronic tubule with Bowman’s capsule ($Bc$), (glomerulus; ($cl$) capillary lumen. Silvermethenamine staining. $\times 475$.

A so called basal labyrinth is present in the basal parts of the epithelial cells of both the proximal segments and the collecting tubule (Fig. 4A). The basal labyrinth is an intracellular membrane system, consisting of membrane folds connected with the basal and lateral cell membranes (Wendelaar Bonga & Veenhuis, 1974a, b; Figs 2 and 4A). The presence
FRESHWATER SEXUALLY IMMATURE

Fig. 2
FRESHWATER SEXUALLY MATURE

LOW GFR →

MUCUS SECRETION
of a basal labyrinth is characteristic for ion and water transporting cells (Berridge & Oschman, 1972). It is generally accepted that ion transporting enzyme systems, e.g. Na+/K+ activated ATPase, are located in the lateral cell membranes and especially in the membranes of the basal labyrinth (Dibona & Mills, 1979; Flik et al., 1980). These enzyme systems utilize ATP produced by mitochondria, which are closely associated with the membranes of the basal labyrinth (Figs 2 and 4A). Thus in summary, the kidneys of freshwater fish are characterised by a high glomerular filtration rate and a high ion-reabsorptive activity in comparison to seawater fish.

Effects of testosterone on structure and function of the stickleback kidney

In spring, rising water temperature and increasing daylength stimulate the production of testosterone in the gonads of the males (Baggerman, 1957, 1966, 1968; Gottfried & van Mullem, 1967). This androgen plays an important role in spermatogenesis and is essential for the development of the secondary sex characteristics, such as nuptial coloration of the skin, as well as reproductive and nestbuilding behaviour (Hoar, 1962; Wai & Hoar, 1963; Baggerman, 1966, 1968; see also Wootton, 1976). As soon as the nuptial coloration of the skin becomes noticeable, the first signs of glandular activity appear in the kidneys. The most important structural changes in the kidneys concern the transformation of the second proximal tubule and the collecting tubule, which comprise about 90% of the nephronic tubule cells (Fig. 2). The basal labyrinth almost completely disappears in these segments, probably as a

Fig. 2. Diagram of a nephron of a sexually immature and a sexually mature male stickleback. In the glomeruli of mature males the capillary lumina (cl) and number of fenestrations (f) in the endothelium (end) are smaller. In these males the filtration barrier (fb; = capillary wall) has thickened, while the number of filtration-slit-membranes between the pedicels (ped) of the podocytes (pod) also has decreased. These phenomena point to a reduced glomerular filtration rate. In contrast to immature males in the mature male nephronic tubule cells of the second proximal segment (P2) and the collecting tubule (CT) the basal labyrinth (b) and closely associated mitochondria (mit), characteristic structures for ion reabsorptive cells, almost have completely disappeared. The cytoplasm of these cells is almost completely occupied by stored secretory granules (sg) and granular endoplasmic reticulum (ger; mainly in the P2), which is very characteristic for mucus secreting cells. The single epithelial cells of the three main segments are drawn to scale to demonstrate the considerable differences in cell size between immature and mature males. (nu) nucleus; (Go) Golgi apparatus; (mu) microvilli.
Fig. 3
result of autophagous digestion. This implies that the ion reabsorbing capacity of the kidneys, and thus the capacity to produce hypotonic urine, is greatly reduced. Simultaneously these cells have grown considerably and an extensive granular endoplasmic reticulum develops, especially in the second proximal segment. The Golgi apparatus also proliferates and starts forming secretory granules. After this transformation, the cells of the second proximal tubule show all the structural characteristics of serous gland cells and those of the collecting tubule attain the appearance typical of mucous cells (de Ruiter & Mein, 1982; Fig. 2). The transformed kidney of a sexually mature stickleback resembles a salivary gland more than an excretory organ and has increased up to sevenfold in size.

This glandular transformation of the kidney can also be induced experimentally by administration of methyltestosterone to castrated males or (intact) females (Wai & Hoar, 1963; Mourier, 1972; de Ruiter & Mein, 1982). Furthermore, our in vitro studies have shown that the effect of testosterone on the kidneys is direct, and not mediated by other hormones, since the glandular transformation can also be induced in small kidney pieces cultured for 10 days in the presence of testosterone (de Ruiter & Mein, 1982).

The changes in the nephronic tubules are accompanied by changes in the glomeruli. At light microscopic level, measurements have shown that in sexually mature males the diameter of the renal corpuscles and of glomerular bloodcapillaries decreases markedly, which points to a reduced glomerular bloodflow (de Ruiter, 1980a; Fig. 3). In addition, electron microscopical observations have clearly shown that thickening and partial obstruction of the filtration barrier takes place. This barrier consists

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Fig. 3A, B, C. Photomicrograph of renal corpuscles as they are present in: A) immature freshwater males, B) mature freshwater males and C) immature seawater males. The diameters of both glomeruli and capillary lumina of the mature freshwater (B) and immature seawater (C) males are smaller than those of immature freshwater males (A). (P2) second proximal segment. See Fig. 1 for other abbreviations. Silvermethenamine staining. × 875.

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Fig. 4A, B. Electron micrograph of the basal part of a second proximal tubule cell of an immature (A) and of a mature (B) male. The basal labyrinth (b) in mature males, in contrast to that in immatures, has almost completely disappeared and is replaced by dilated cisterns of the granular endoplasmic reticulum (ger) and numerous secretory granules (sg). This together with a well developed nucleolus (nucl) in the nucleus (nu) points to a high mucus secretory activity. (bcm) basal cell membrane. 4A × 34500; 4B × 13000.
of capillary endothelium, mesangium, basal lamina and podocytes (Fig. 2). *In vivo* and *in vitro* experiments have shown that these glomerular structural changes also are induced directly by testosterone (de Ruiter, 1981). The changes that occur in the glomeruli of sexually mature males are similar to those that take place in the glomeruli of freshwater fish that migrate to seawater. Since it is known that in the latter these changes are connected with a firm reduction of the glomerular filtration rate (GFR) and low urine production (Hickman & Trump, 1969), we have concluded that the GFR and urine production in sexually mature males also is reduced (de Ruiter, 1980a, 1981). This conclusion has been confirmed experimentally for urine production (de Ruiter, 1980b). Thus the effects of testosterone on the kidneys of male sticklebacks lead to reduction of glomerular filtration (de Ruiter, 1981), the loss of most of the ion-reabsorptive capacity of the nephronic tubules, and a firm reduction of urine production. The reduction of urine flow is understandable, since the loss of ion reabsorptive capacity of the nephronic tubules would lead to substantial loss of ions and other valuable substances via the urine. On the other hand, the loss of the kidneys as the main pathway for the elimination of osmotically accumulated water will greatly disturb water and ion balance unless the loss of normal kidney function is compensated elsewhere in the body.

Compensatory mechanisms for water elimination

In sexually mature male sticklebacks living in freshwater there are two alternative ways to drain off the surplus of water; via the secretion of mucus by the kidneys, and the excretion of intestinal fluid. The loss of water via renal mucus secretion may partially compensate the reduction of urine excretion. However, we have demonstrated that the main compensatory pathway for the elimination of water in mature males is the intestine (de Ruiter, 1980b, de Ruiter *et al.*, 1985). As has been observed for some other teleost species, sticklebacks normally excrete through the anal opening some fluid, produced in the intestine. This fluid is more or less iso-osmotic with the body fluids. The collection of this intestinal fluid is complicated by the circumstance that, in sticklebacks, the urinary bladder opens into the posterior part of the intestine, and thus urine is mixed with intestinal fluid. Using micro-surgery and cannulation procedures, we were able to collect separately the fluids produced in the intestine and the kidneys (Fig. 5). We found that the rate of intestinal fluid production in mature males increased up to five-fold after glandular transformation.
of the kidneys. Furthermore, the osmolality of the fluid is reduced significantly, possibly by increased reabsorption of ions from this fluid in the posterior intestine (de Ruiter, 1980b, de Ruiter et al., 1985).

Electron microscopical observations on intestinal and gall-bladder showed that the epithelial cells lining these organs, are provided with a well developed basal labyrinth, similar to that of the nephronic tubule cells (de Ruiter et al., 1985; Fig. 6). The presence of such a labyrinth provides structural evidence that these epithelia are capable of transcellular transport of water and ions (Diamond & Bossert, 1967, 1968; Berridge & Oschman, 1972).

By means of morphometrical techniques we established that the extent of basal labyrinth in the epithelium of the posterior intestine increases markedly in sexually mature males and in testosterone-treated castrates or female fish (de Ruiter et al., 1985). Thus in mature males the testosterone induced disappearance of most of the basal labyrinth in the nephronic tubule cells is accompanied by a considerable increase in extent of this membrane system in the intestine.

**Concluding remarks**

Nestbuilding behaviour in male sticklebacks strongly affects hydromineral regulation. The transformation of the kidneys into mucus
glands leads to complete loss of normal kidney functions. The loss of the water excreting capacity of the kidneys is partially counterbalanced by the secretion of mucus and, more importantly, by enhanced release of intestinal fluid. Other kidney functions, such as formation and removal of nitrogenous waste products, have not yet been studied, but may be taken over by the gills.
All the complicated structural and functional changes that follow sexual maturation in males can be induced by administration of testosterone in castrated males or even females. Our in vitro studies have shown that the glandular transformation of the nephronic tubules is a direct effect of testosterone on the tubular cells. The processes accompanying this transformation, such as the reduction of both the extent of renal tubule basal labyrinth and glomerular filtration rate, the increase of intestinal water permeability, growth of the basal labyrinth in the intestinal cells, and enhanced secretion of intestinal fluid, are also induced by testosterone, either directly or mediated by other hormones. Thus, in sticklebacks testosterone is a genuine multifunctional hormone: in addition to the well known control of spermatogenesis, nuptial coloration and reproductive behaviour it has pronounced effects on hydromineral regulation.

Summary

Nestbuilding behaviour, which takes place in sexually mature males as a consequence of an enhanced level testosterone in the blood, considerably influences hydromineral balance. As shown by in vivo and in vitro experiments testosterone is directly involved in the glandular transformation of the nephronic tubule cells into gland cells, that secrete mucus for nestbuilding. Light and electron microscopical observations as well as physiological data provide strong evidence that, in sexually mature males both ion reabsorptive capacity of most of the nephronic tubule cells and glomerular filtration rate c.q. urine production has decreased considerably. As a consequence of this the water balance of these fish, living in freshwater, would be greatly disturbed. However in these males the intestine and apparently also the gall-bladder, compensate for the loss in renal function in maintaining hydromineral balance. Again, this is influenced by testosterone.

References

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