Polar Biol (1999) 21: 385–390

ORIGINAL PAPER

M.A. Masini · M. Sturla · P. Prato · P. Romano
B. Uva

Regulatory peptides in the urinary bladder of two genera of Antarctic teleosts

Accepted: 1 November 1998

Abstract Somatostatin 14, prolactin, atrial natriuretic peptide, galanin and urotensin II were found using immunohistochemistry in the urinary bladders of the Antarctic fishes *Trematomus bernacchii* (Nototheniidae) and *Chionodraco hamatus* (Channichthyidae) caught in the Ross Sea. The urinary bladders of the two species showed a different histology in the epithelial layer. In *T. bernacchii* the epithelium comprises a single type of columnar cells, while in *C. hamatus* the columnar cells are restricted to the ventral portion of the bladder, and the dorso-lateral region is lined by cuboidal cells. No difference in the intensity of the immunostaining was observed in the two cell types; the only variation was a different distribution of the immunoreactions, which were present in the whole cytoplasm in the cuboidal cells and restricted to the apical and/or basal portion of the columnar cells.

Introduction

The urinary bladder in marine fishes is known to be involved in the control of water and salt homeostasis. Teleosts can modify the urine composition in order to survive in hyperosmotic environments as a result of the activity of the epithelial cells (Loretz and Bern 1980; Fossat and Lahlou 1982). The transport properties vary with the challenge offered by the environment: in euryhaline teleosts, water reabsorption increases in fishes adapted to seawater as compared to freshwater fishes (Johnson et al. 1974; Foster 1975).

M.A. Masini (✉) · M. Sturla · P. Prato · B. Uva
Dipartimento di Biologia Sperimentale, Ambientale ed Applicata, Università di Genova, viale Benedetto XV 5, I-16132 Genoa, Italy
e-mail: uvab@unige.it, Tel.: +39-10-3538049,
Fax: +39-10-3538047

P. Romano
Dipartimento di Medicina Sperimentale, Università di Genova, via De Toni 14, I-16132 Genoa, Italy

Neurohypophysial and adrenal hormones influence the movement of water and ions across the urinary bladder membranes of mammalian (Lewis and Diamond 1976) and non-mammalian vertebrates, but a number of other regulatory peptides may also be involved in transport at this site.

Somatostatin (SST) is a neuropeptide that inhibits the release of many hormones and other proteins (Patel et al. 1990); however, an osmoregulatory function has also been postulated for somatostatin as, in mammals, it inhibits renin secretion following infusion into the renal artery (Gomez-Pan et al. 1976) and in the toad urinary bladder it inhibits the hydro-osmotic response to antidiuretic hormones (Forrest et al. 1980). An SST immunofluorescence was identified in the urinary bladder epithelial cells of the toad *Bufo marinus* (Bolaffi et al. 1980). A form of somatostatin, somatostatin-14 (SST-14), is synthesised in the lungfish kidney (Masini et al. 1998a) and the sequence of SST-14, even if part of a large variant of somatostatin, is well conserved from fish to mammals (Andrews et al. 1988; Sower et al. 1994; Conlon et al. 1995a,b). Prolactin (PRL) is a well-known hormone involved in milk production in female mammals but its function also involves water and sodium metabolism. In teleosts, prolactin regulates water and ion movements across the urinary bladder (Doneen 1976). Injections of prolactin into freshwater-adapted fish have been shown to reduce permeability to water reabsorption (Johnson et al. 1974; Hirano 1975). Specific binding sites for prolactin have been identified in the urinary bladder cells of the toad (Dunand et al. 1985). Prolactin occurs in multiple forms in vertebrates, and teleostean prolactin shares 30% sequence homology with mammalian prolactin (Henderson 1997); nevertheless, it has been successfully employed in immunohistochemical investigations in the skin and gills of Antarctic teleosts (Uva et al. 1994; Sturla et al. 1996).

The primary effects of atrial natriuretic peptide (ANP) in mammals are natriuretic, diuretic and vasorelaxant (Cantin and Genest 1985). ANP has been observed in lungfishes (Masini et al. 1996) and freshwater

teleosts (Kim et al. 1991), as well as in the heart, kidney, gills and skin of Antarctic teleost fishes (Masini et al. 1998b). ANP isolated from teleosts is structurally similar to mammalian ANP (Takei et al. 1989).

Galanin (GAL), a 29 amino acid peptide, was first isolated from porcine small intestine (Tatemoto et al. 1983). In the rat stomach it may inhibit the release of somatostatin (Madaus et al. 1988). An involvement of galanin in the regulation of fluid homeostasis has been postulated in mammals (Koenig et al. 1989). The N-terminal part of the GAL molecule is well conserved, whereas the C-terminal part shows different amino acids in several positions (Rökaeus 1994).

Urotensin I and urotensin II are peptides produced by the caudal neurosecretory system (the urophysis) of teleostean fishes. Urotensin II influences ion transport in teleosts' gill and urinary bladder (Loretz and Bern 1981).

The presence of the above-mentioned peptides in the urinary bladder is, therefore, worthy of investigation.

Compared with temperate-zone fishes, Antarctic teleosts have an elevated serum osmolarity (Dobbs and De Vries 1975) due, apart from the antifreezing peptides, to a high serum ion concentration, which is, however, still below seawater values. Antarctic fish are therefore, as temperate-water marine teleosts, hypoionic regulators which need to promote salt excretion and water reabsorption. The urinary bladder is very likely involved in these processes, as in many other vertebrates. The aim of the present study was to investigate the occurrence of SST-14, PRL, ANP, GAL, and urotensin II immunoreactivity in the epithelial lining of the urinary bladders of the red-blooded *Trematomus bernacchii* and the icefish, *Chionodraco hamatus*.

Materials and methods

Animal and tissue preparation

Three specimens of *C. hamatus* Lönnberg (Channichthyidae), about 35 cm long, and three *T. bernacchii* Boulanger (Nototheniidae), about 25 cm long, were caught using nets at depths of 80 and 150 m during the Italian expeditions 1997–1998 at Terra Nova Bay in the Ross Sea. The fishes were housed in a recirculated-seawater aquarium at 0°C, and within a few days were killed by a blow to the head and spinal cord transection. Urinary bladders were immediately removed at 4°C, fixed in Bouin's fluid and then processed for embedding in paraffin wax, or for scanning electron microscopy.

Animal manipulations were performed according to Ethical Committee recommendations and under the supervision of authorised investigators.

Bouin-fixed urinary bladders were dehydrated in ethanol, cleared in xylene and embedded in paraffin wax. Sections (5 μm thick) were cut on a microtome and mounted on glass slides coated with 0.5% gelatin containing 0.05% chrome alum.

Histological and histochemical procedures

Sections were processed for histological analysis using the hematoxylin and eosin (H&E) technique; parallel sections were treated with alcian blue periodic acid-Schiff (PAS) for the detection of mucus material.

Immunohistochemical procedures

Tissue sections were processed for immunohistochemistry following the indirect immunofluorescence method and the peroxidase-antiperoxidase (PAP) method using the following rabbit antisera: Ab-SST-14, Ab-hANP (1–28), Ab-ovine PRL (diluted 1:200, UCB, Braine-l'Alleud, Belgium), Ab-porcine GAL (diluted 1:2000, Biogenesis, Poole, UK) and Ab-Dogfish urotensin II (diluted 1:200, Chartrel et al. 1996). The PAP reaction was developed with 0.025% 3,3′-diaminobenzidine-HCl and 0.1% H_2O_2 in PBS. The specificity of the immunoreaction was verified by preabsorbing the primary antisera with the homologous antigens (10^{-6} M), by omitting one of the steps of the immunohistochemical procedure, or by replacing the primary antiserum with nonimmune rabbit serum or PBS.

Scanning electron microscopy

The specimens were fixed in Bouin's fluid and identified by means of a Wild M3 C stereo-microscope and a Leitz Diaplan microscope. For the electronic microscopy, samples were dehydrated in ethanol of increasing concentration up to 100% and critical point dried, with liquid CO_2 as transition fluid (Cohen 1979). Specimens were mounted using small pieces of double-sided adhesive tape on aluminium stubs and coated with a 20-nm gold layer in an argon atmosphere flow discharge sputter-coating-unit (Polaron E 5100). Samples were examined in an ISI SS-40 scanning electron microscope operated at an accelerating voltage of 10–20 kW.

Results

Histomorphology and histochemistry

The urinary bladders in both the species examined are elongated sacs (about 1–1.5 cm long) protruding from the conjoined ends of the urinary ducts. The bladder is internally lined by a simple epithelium on top of a connectival layer, which is arranged in several folds in the empty bladder. Epithelium and connectival layers are surrounded by a smooth-muscle layer.

The epithelium shows a different aspect in the two species examined. In *Trematomus* the epithelium is composed in every region of columnar cells (Fig. 1a), while in *Chionodraco* the bladder is divided in two very distinct regions: a ventral region, close to the gonads, is covered by columnar epithelial cells, while the dorso-lateral region is covered by cuboidal cells (Fig. 1b).

The alcian-PAS histochemical method shows an intense reaction on the surface of the columnar epithelium both in *Trematomus* (Fig. 2) and *Chionodraco*, while a less intense staining is visible on the cuboidal cells (Fig. 3a–c). Alcian blue-positive goblet cells were extremely rare in both species; the PAS reaction was completely absent.

In the scanning electron microscopy, both the columnar and the cuboidal epithelial cells showed short microvilli in the apical region (Fig. 4). In the columnar epithelium, the cells possess basal nuclei, oval in shape, while in the cuboidal cells the nuclei are centrally located.

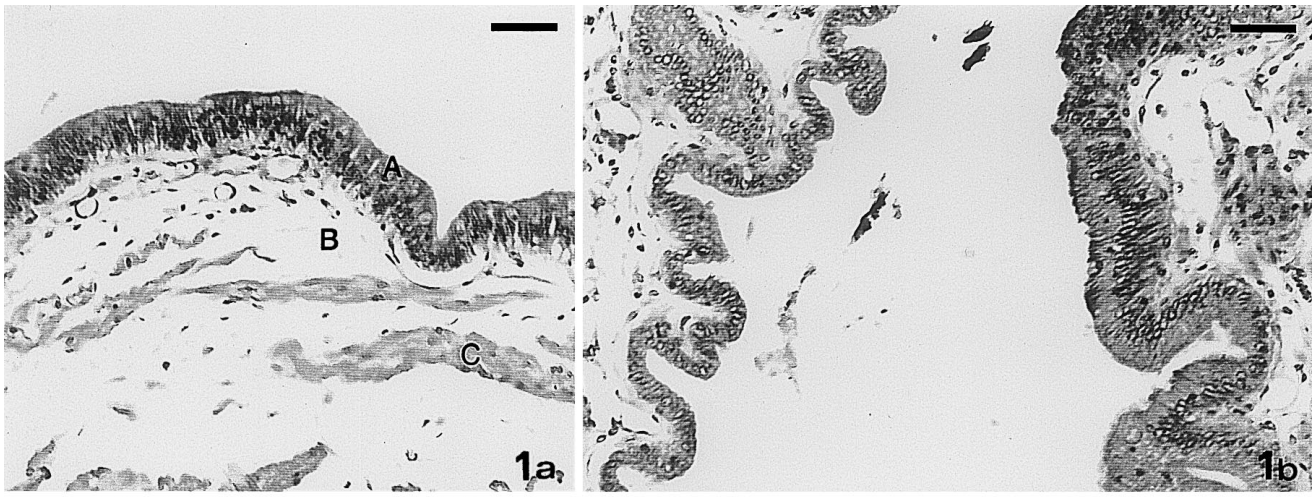

Fig. 1 a Urinary bladder of *Trematomus bernacchii* showing the general architecture of the structure: *A* columnar epithelium, *B* connectival layers, *C* smooth muscle layers. **b** The micrograph shows the different cells (columnar and cuboidal) in the urinary bladder epithelium of *Chionodraco hamatus*, H&E. The *bar* indicates 20 μm

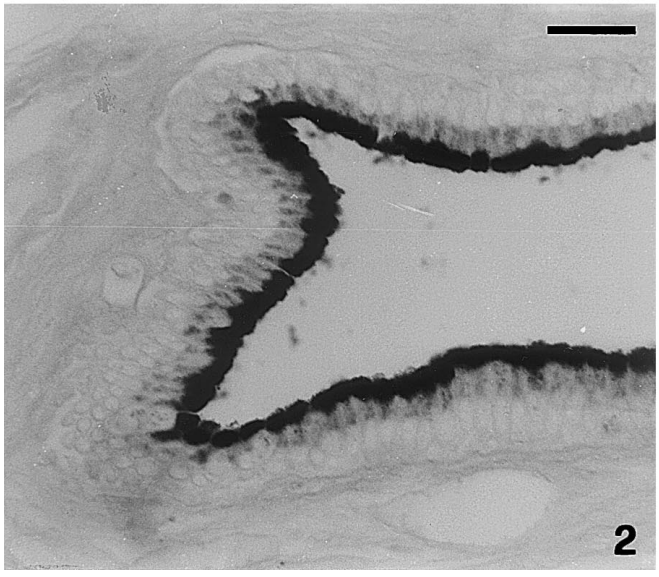

Fig. 2 Alcian-blue-positive reaction in the epithelium of *T. bernacchii*'s urinary bladder. The *bar* indicates 15 μm

Immunohistochemistry

Immunoreactivity for SST-14, PRL, ANP, GAL and urotensin II is present in the urinary bladder epithelium of both the species examined.

In the cuboidal cells of *Chionodraco,* the immunoreactivity is localised in the whole cytoplasm (Fig. 5). The immunoreaction in the columnar epithelium shows a different localisation.

Immunoreactivity for SST-14 seems to be localised in the apical region of the columnar cells both in *Chionodraco* and *Trematomus* (Fig. 6), while GAL immunoreactivity is present in the whole cytoplasm (Fig. 7). ANP is localised in the columnar cells both in the apical and basal regions (Fig. 8). Prolactin and urotensin II show the same localisation as SST-14 in both species. No immunoreaction is present in the control sections (Fig. 9).

Discussion

There is a most striking histological difference between the urinary bladders of the two species examined. The epithelial layer comprises a single type of cells, columnar in shape, in the whole urinary bladder of *Trematomus* while in *Chionodraco* the columnar cells are restricted to the ventral region and the dorso-lateral region is lined by cuboidal epithelial cells. Topographically separated regions lined by two cell types, mitochondria-rich columnar cells and cuboidal cells, have been observed in the gobiid teleost *Gillichthys mirabilis*. Different involvement of the two region in the activity of ions-water transport has also been established (Loretz and Bern 1980). An explanation of the structural differences in the urinary bladder of the two species of Antarctic teleosts examined and in the gobiid, may, at this stage, only be hypothesised as a result of the double embryological origin of the urinary bladder in *C. hamatus* and in *G. mirabilis*. The urinary bladder in fish is, in the majority of cases, of mesodermal origin, unlike the urinary bladder of tetrapods, which derives from the endoderm. In fact, the

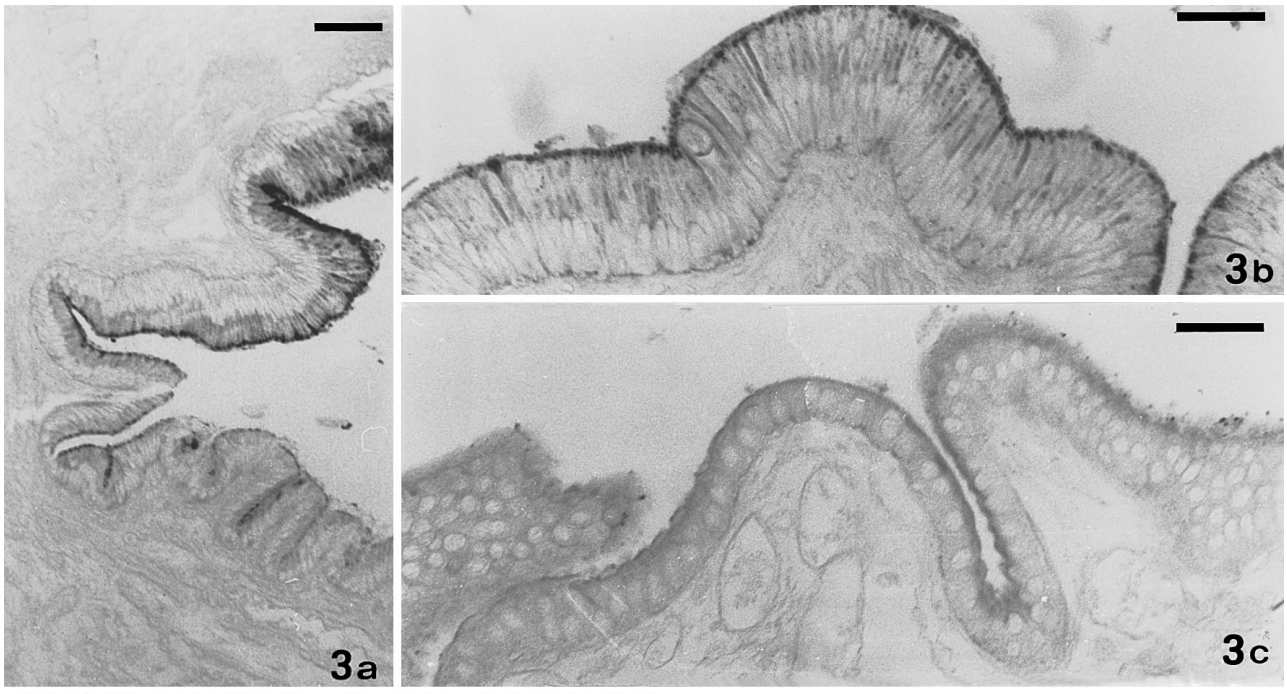

Fig. 3 **a** Micrograph showing in *C. hamatus* the different response to the Alcian blue-PAS technique in the columnar and cuboidal epithelium. **b** Higher magnification of the columnar epithelium. **c** Higher magnification of cuboidal epithelium. The *bars* indicate: **a** 20 μm, **b,c** 15 μm

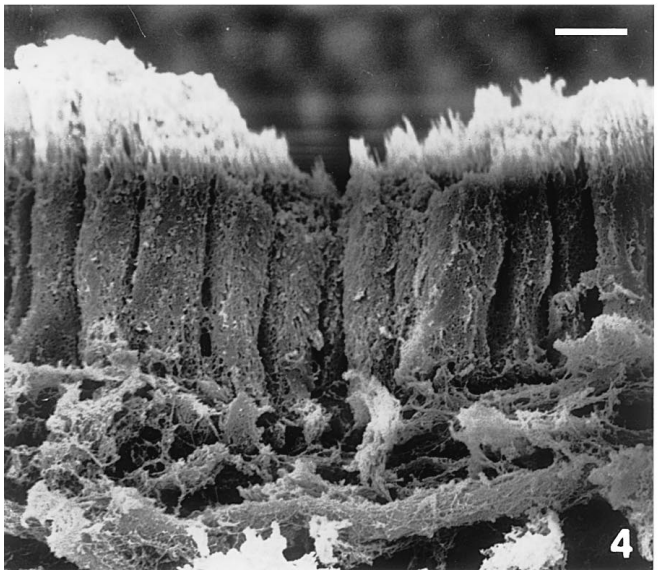

Fig. 4 Scanning electromicrograph of the columnar epithelium in *C. hamatus*. Note the apical microvilli. The *bar* indicates 5 μm

urinary bladder of lower actinopterigian teleosts develops as an enlarged sac from the urinary ducts at their conjoined ends, where urine may be stored. In other teleosts the urinary bladder has a twofold origin, since a portion of the cloaca, of endodermal source, takes part in the formation of the urinary bladder wall (Romer 1968).

The hypothesised different embryological origin may suggest a different involvement of hormones regulating ion transport and water reabsorption in the two regions. It has been demonstrated that urotensin II has a direct effect on ion transport in the columnar region of the *Gillichthys* urinary bladder (Loretz and Bern 1980) but, from our data, all the hormonal peptides are present in both the columnar and cuboidal epithelium of the bladder in the two species of Antarctic teleosts and different functions of the two cell types are not, therefore, easy to determine. No difference has been observed in the two cell types, columnar and cuboidal, in terms of presence or intensity of the immunoreactions; the only variation noticed was the localisation of the immunostaining, distributed in the whole cytoplasm in the cuboidal cells and localised in the basal and/or apical regions in the columnar cells. The thickness in the mucus coating may indicate a different function of the simple columnar and cuboidal epithelia.

It is very likely that the peptides investigated take part, along with other hormones, in the regulation of movements of ions and water across the urinary bladder membranes of these Antarctic teleosts; however, a pos-

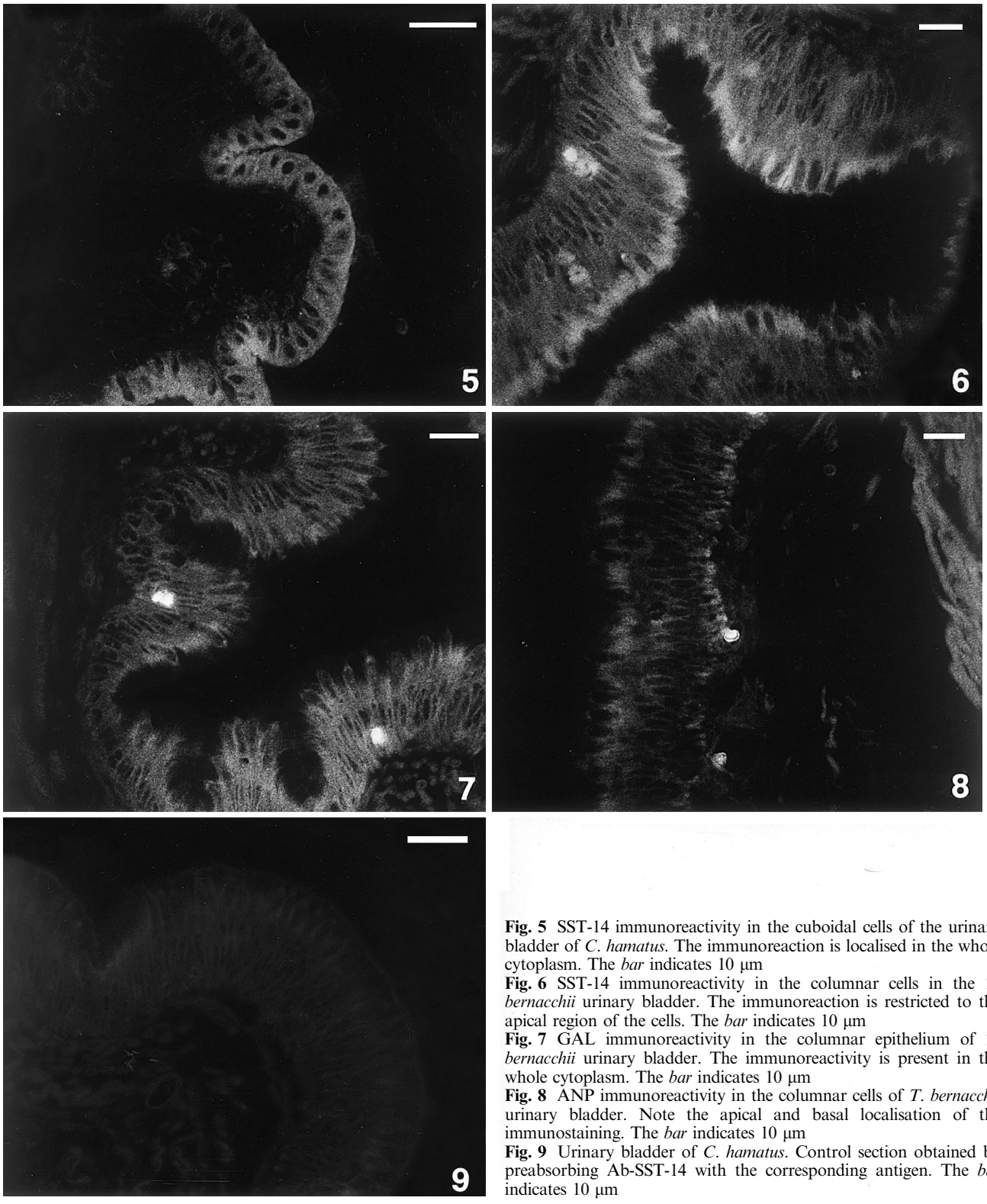

Fig. 5 SST-14 immunoreactivity in the cuboidal cells of the urinary bladder of *C. hamatus*. The immunoreaction is localised in the whole cytoplasm. The *bar* indicates 10 μm

Fig. 6 SST-14 immunoreactivity in the columnar cells in the *T. bernacchii* urinary bladder. The immunoreaction is restricted to the apical region of the cells. The *bar* indicates 10 μm

Fig. 7 GAL immunoreactivity in the columnar epithelium of *T. bernacchii* urinary bladder. The immunoreactivity is present in the whole cytoplasm. The *bar* indicates 10 μm

Fig. 8 ANP immunoreactivity in the columnar cells of *T. bernacchii* urinary bladder. Note the apical and basal localisation of the immunostaining. The *bar* indicates 10 μm

Fig. 9 Urinary bladder of *C. hamatus*. Control section obtained by preabsorbing Ab-SST-14 with the corresponding antigen. The *bar* indicates 10 μm

sible difference in the influence of the hormones studied on the water-ion transport in the two regions remains to be established.

Acknowledgements This study was supported by a grant from the Italian PNRA.

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