Na-pump sites in the oviduct of *Salamandra salamandra* (L.) (Amphibia, Urodela)

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Summary. Using ^{3}H-ouabain autoradiography, Na^+-K^+-ATPase has been localized on the basolateral membranes of ciliated and nonciliated cells in the oviduct (pars recta, p.convoluta I, II, III) of the European fire salamander, *Salamandra salamandra*. The mucous and seromucous gland cells of the p.convoluta I, II, III, however, do not show any significant labelling. An asymmetrical distribution of ouabain binding sites is a main feature of transporting epithelia.

The oviduct of the ovoviviparous European fire salamander, *Salamandra salamandra*, lined by a single-layered epithelium, is differentiated into 2 main portions, the pars recta and the p.convoluta. The pars convoluta consists of glandular parts (p.convoluta I, II, III) with gland cells of different sizes and chemical composition, and the uterus (p.convoluta IV). Both main portions - with the exception of the uterus - exhibit ciliated and nonciliated, rather undifferentiated cells[1]. In the uterus epithelium, which consists only of a single cell type[2,3] a Na^+-K^+-ATPase has recently been demonstrated on the basolateral plasmalemma by ultrahistochemistry and autoradiography. Preliminary electrophysiological experiments suggest a transport of solute to the underlying connective tissue and blood vessels[4].

When the ultrastructure of the epithelial lining, made up of ciliated and nonciliated cells, is considered, it also seems to fulfill the criteria suggested for transporting epithelia[5,6].

An attempt was made to localize ouabain binding sites in the oviduct by ^{3}H-ouabain autoradiography which indirectly gives evidence for the presence of a Na^+-K^+-ATPase. This enzyme is regarded as serving as a Na^+-pump and as being characteristic for vertebrate transporting epithelia[7-9].

Material and methods. Na^+-K^+-ATPase was demonstrated by autoradiography[4,10]. Small pieces of the pars recta and the glandular parts of the oviduct of a pregnant female of *S. salamandra* were incubated for 50 min in aerated Ringer's solution for salamanders[11] containing 2.2×10^{-6} M (25 µmCi/ml) ^{3}H-ouabain (Amersham, Buchler). Then, the specimens were washed in ouabain-free Ringer's solution 5 times to remove unbound ouabain. After freeze-drying, the tissue was fixed under vacuum with osmium tetroxide and vacuum-embedded in styrene-methacrylate[12]. 1-µm sections were mounted on glass slides, coated with the Kodak stripping film AR 10, exposed for 21 and 30 days at 4 °C, developed and fixed. Appropriate controls were done by incubating a) in saline only and b) in labelled solution, which contained additional unlabelled ouabain in a concentration of about 10^{-3} M.

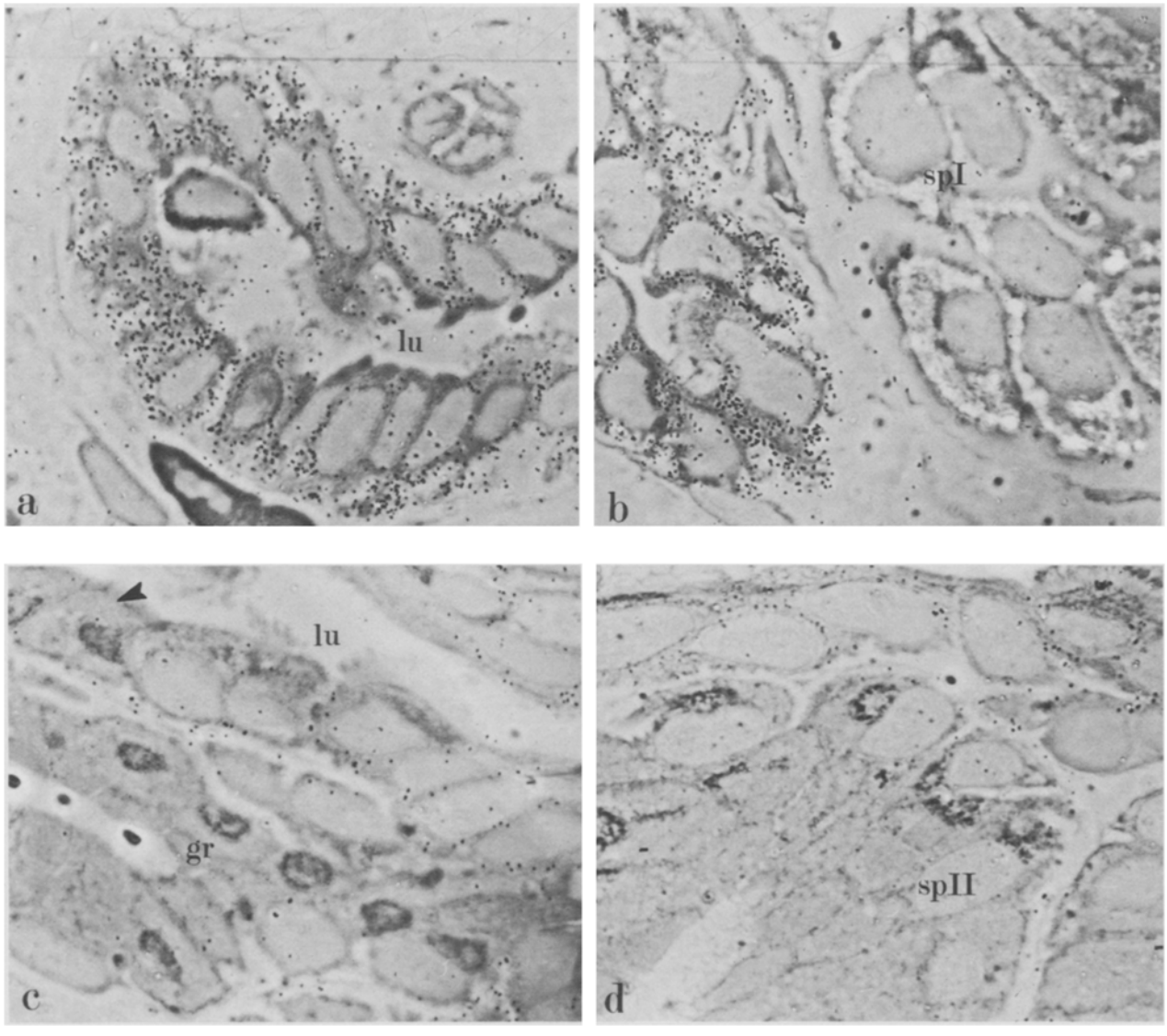
Results. Pars recta: The autoradiographs show a clear distribution of silver grains along the basolateral border of the epithelial cells. Only a few granules were seen apically; these were not above background. Both the connective tissue and the blood vessels were unlabelled. Although exact grain counts were not done the ciliated and nonciliated cells showed no apparent differences in grain location or density (fig., a, b).

Pars convoluta: In principle the epithelium of the glandular parts of the oviduct is single-layered, a pattern which is somewhat obscured by the different types of mucous and seromucous gland cells partly forming deep crypts, in particular the spongiocytes (p.convoluta I), the granulocytes (p.convoluta II) and the spongiocytes II (p.convoluta III). Smaller gland cells (microgranulocytes, microspongiocytes), cilated and nonciliated cells make up the lumenal epithelium[1]. The gland cells show, if anything at all, an irregular distribution of a few silver grains, not above background, whereas in this region of the oviduct the ciliated and nonciliated cells also exhibit a distinct but somewhat less intensive labelling (fig., b, c, d). This fact may be the result of the poor penetration of the labelled solution into the sites within the epithelium of the collapsed oviduct.

No silver grains were found in either of the controls. Using Ringer's solution without tritiated ouabain, chemography can be excluded; using labelled inhibitor together with high amounts of unlabelled ouabain, the latter occupies the ouabain binding sites which results in a reduction of silver grains in the autoradiographs.

Discussion. It has been shown in the present study that Na^+-K^+-ATPase can be localized along the entire extension of the oviducal epithelium in *S. salamandra* on the basal and lateral cell membranes of the ciliated and nonciliated cells, but not in the gland cells. Ciliated and nonciliated cells exhibit rather long intercellular spaces and projections of the basolateral plasmalemma enlarging their surface[1], a feature which is also known from the uterine epithelium[3]. The clear asymmetrical, i.e. basolateral distribution of ouabain binding sites - a main characteristic of most of the transporting epithelia in vertebrates so far investigated - has been found in absorbing as well as in secreting epithelia[8,9]. The uterine epithelium in *S. salamandra* obviously represents an absorbing epithelium ('forward' transporting epithelium according to the terminology of Diamond[5]), which transports solute out of the uterus to the connective tissue layer and to the blood vessels[4]. As further data are not available at present the direction of transport in the pars recta and the glandular parts of the oviduct is still an open question especially as an uneven distribution of the transport enzyme as well as a possible structural polarity (not present in the oviducal epithelium) are not necessarily indicative of reabsorption or secretion[8,9]. Nevertheless, the localization of the Na^+-K^+-ATPase on the basolateral plasmalemma is in agreement with the model for transepithelial transport of sodium suggested by Koefoed-Johnsen and Ussing[13]. This fact together with the general 'design'[6,8] of the epithelium may suggest a 'forward', i.e. absorbing epithelium. Assuming that the Na^+-K^+-ATPase plays a direct role in a significant active transport rather than being responsible only for cellular homeostasis, and assuming further that the oviducal epithelium (with the exception of the gland cells) is reabsorptive along its whole extent, then it follows that the oviduct of *S. salamandra* has available a considerable bulk of cells adapted to absorb ions (and solute) from the oviducal lumen. The origin of the reabsorbed material which contains electrolytes and possibly substances from the glands and/or from the coelomic cavity, into which the p.recta opens, is, however, still under discussion[4].

The absence of ^{3}H-ouabain binding sites on the membranes of the larger gland cells (spongiocytes, granulocytes) is interpreted as the absence of Na^+-K^+-ATPase activity, or at least as a low level of activity not demonstrable by the technique used. This is in agreement with similar observations on the central acinar cells of cat submandibular



Radioautographs of different portions of the oviduct of *S. salamandra* exposed to ^{3}H-ouabain. *a* Pars recta epithelium. Grains are associated with the basolateral cell membranes of all cells. *b* Transition from the p. recta (left side) to the p. convoluta I (right side). Note the absence of grains in the spongiocytes I (sp I). *c* P. convoluta II. The granulocytes (gr) and the smaller gland cells (arrow) in the lumenal epithelium are unlabelled. *d* P. convoluta III with sparsely labelled ciliated and nonciliated cells (above) and unlabelled spongiocytes II (sp II). lu, lumen of the oviduct. × 1000.

glands[14] and may indicate that these cells do not contribute significantly to transport of ions. The ultrastructure of the spongiocytes and granulocytes reveals an almost straight lateral border not typical of epithelia involved in solute transport. The same applies to the smaller glands between the ciliated and nonciliated cells. Here the resolution of the technique applied does not in all cases allow one to decide whether the ouabain binding sites are localized on the lateral plasmalemma of a small gland cell or on the plasmalemma of the adjacent ciliated or nonciliated cell.

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