Glutaraldehyde Fixation Preserves the Permeability Properties of the ADH-Induced Water Channels

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Summary. Unidirectional and net water movements were determined, in frog urinary bladders, before and after glutaraldehyde fixation. Experiments were performed in three experimental conditions: 1) in nonstimulated preparations, 2) after the action of antidiuretic hormone (ADH) and 3) in nonstimulated preparations to which amphotericin B was incorporated from the luminal bath. As previously observed for net water fluxes, the increase in the unidirectional water movement induced by ADH was well preserved by glutaraldehyde fixation. After correction for the effects of unstirred layers and nonosmotic pathways, the observed correlation between the ADH-induced increases in the osmotic $(P_f)$ and diffusional $(P_d)$ permeability coefficients was not modified by the fixative action (before glutaraldehyde: slope $11.19, r: 0.87 \pm 0.07; n = 12$; after glutaraldehyde: slope $10.67, r: 0.86 \pm 0.04, n = 39$). In the case of amphotericin B, $\Delta P_f/\Delta P_d = 3.08 (r: 0.83 \pm 0.08)$, a value similar to that observed in lipid bilayers or in nonfixed toad urinary bladders. It is concluded that 1) The experimental approach previously employed to study water channels in artificial lipid membranes and in amphibian urinary bladders, can be applied to the glutaraldehyde-fixed frog urinary bladder. 2) Glutaraldehyde fixation does not modify the permeability properties of the ADH-induced water channels. 3) Any contribution of exo-endocytic processes or cell regulatory mechanisms to the observed permeability parameters can probably be excluded. 4) Glutaraldehyde-fixed preparations are a good model to characterize these water pathways.

Key Words  water channels · glutaraldehyde fixation · frog urinary bladder · unstirred layers · osmotic and diffusional permeabilities · Rana esculenta

Introduction

Antidiuretic hormone (ADH) increases the water permeability of target epithelial barriers and strong evidence indicates that the hormonal action is due to the transfer of water channels [3, 16] from cytoplasmic vesicles into the apical plasma membrane of granular cells [21]. This membrane traffic, induced by ADH, can be at the origin of another experimental observation: glutaraldehyde fixation can “freeze” the epithelial barrier in a “high permeability state” (after ADH) [4, 14]. Nevertheless, while glutaraldehyde-fixed epithelial tissues have been frequently employed [5, 6, 7, 11, 15], it is until now unknown if the fixative modifies the intrinsic permeability of the water pathway. ADH-induced water channels have been recently characterized in frog [22] and toad [18] urinary bladders, by a careful estimation of the osmotic $(P_f)$ and diffusional $(P_d)$ water permeability coefficients. We have now measured these parameters in frog urinary bladders, before and after glutaraldehyde fixation.

As previously made [17, 18, 22], the experimental approach developed to study water permeability in artificial membranes [9, 29, 30] was applied to frog urinary bladders. In pure lipid bilayers $P_f/P_d = 1$, after appropriate correction of the unstirred layer effects. The incorporation of polyene antibiotics into the membrane increases both $P_f$ and $P_d$ but now $P_f/P_d = N$, $N$ being a number characteristic of the inserted channel. Amphotericin incorporation ($N = 3$) has been previously employed to test water permeability measurements in toad urinary bladder [18]. We have now also incorporated amphotericin B to frog urinary bladders previously fixed by glutaraldehyde.

The obtained results show that the permeability properties of the ADH-induced channels are not modified by glutaraldehyde and indicate that fixed preparations are a good model for the biophysical characterization of these water pathways.

Materials and Methods

Frog (Rana esculenta) urinary bladders were (vertically) mounted as a flat sheet between two twin-barrel Lucite® cells (Fig. 1). A 10-cm hydrostatic pressure applied on the mucosal bath maintained the tissue against a nylon mesh placed on its serosal surface. The exposed tissue area was twice 1.77 cm². The four chambers (mucosal and serosal bath, control and experimental channels) were filled with 5 ml buffer solution (R) con-
Table 1. Effect of glutaraldehyde fixation on the observed net water fluxes

<table>
<thead>
<tr>
<th>Net water fluxes</th>
<th>Before fixation</th>
<th>After fixation</th>
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<tbody>
<tr>
<td>(µl/min/cm², R/Rhypo)</td>
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<tr>
<td>Nonstimulated (n = 8)</td>
<td>0.12 ± 0.01</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>Oxytocin, 2.2 × 10⁻⁸ M (n = 8)</td>
<td>2.39 ± 0.15</td>
<td>1.87 ± 0.12</td>
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Fig. 1. Schematic representation of the experimental device.

Fig. 2. Effect of glutaraldehyde (GLUTA) fixation on the net water flux, in control conditions and after ADH (oxytocin 2.2 × 10⁻⁸ M).

Net Water Measurements

The net water transfer was measured with the technique of Bourguet and Jard [1]: Water was injected into the mucosal chamber to maintain a constant volume. The amount injected every minute, and equivalent to the net flux, was automatically recorded. At the end of a measurement period performed in the presence of an osmotic gradient, the net water flux was again estimated with both the mucosal and serosal chambers filled with isosmotic solutions. The osmotic permeability coefficient (Pf) was estimated by subtracting the permeability corresponding to the "nonosmotic net flux" (driven by the applied hydrostatic pressure), from the osmotic permeability coefficient observed in the presence of an osmotic gradient (Pfobs).

Unidirectional Water Fluxes

The unidirectional water transfer was estimated from ³HHO fluxes in the absence of an osmotic gradient, as previously described [24]. The radiotracer (2 µC/ml) was added to the mucosal chamber at the beginning of the experiment. All the serosal volume was aspirated every two minutes and replaced by cold buffer. The ³HHO transfer was measured in at least 15 consecutive periods, by scintillation counting, and the water permeability coefficient (Pdobs) determined.

The thickness of the unstirred layers (d) in series with the tested membrane was estimated from ¹⁴C-butanol permeability experiments [9, 13]:

\[ d = \frac{D}{P_{but}} \]  

(1)

where \( D \) is the diffusion coefficient for butanol in water and \( P_{but} \) the observed butanol permeability. In 10 different experiments performed in our experimental conditions we obtained for the unstirred layer thickness a value of 442 ± 29 µm. This figure was used to correct the observed diffusion permeability coefficient (Pdobs) according to [10, 12, 30]:

\[ \frac{1}{P_{dobs}} = \frac{1}{P_d'} + \frac{d}{D_D} \]  

(2)

where \( P_d' \) is the water diffusional permeability coefficient after correction for the unstirred layer effects and \( D_D \) the diffusion coefficient for water in water.

The existence, in our experimental conditions, of "nonspecific leaks" was estimated from ¹⁴C-sucrose permeability experiments [28]. This radiotracer was added (1 µC/ml) to the mucosal bath, together with ³HHO, in most unidirectional flux experiments. Appropriated corrections for double marking were performed to calculate sucrose and water fluxes. Assuming that water moves together with sucrose across this "nonosmotic pathway" and that the ³HHO/¹⁴C-sucrose specific activity ratio remains constant all the way, the amount of water moving through this route can be easily calculated, as well as its water diffusional permeability (Pd). Finally, the water diffusional permeability (Pd) can be obtained from: