Correlation between Net Water Flux, Osmotic Concentration of the Interstitial Fluid, and Osmotic Water Permeability of the Isolated Skin of Bufo bufo bufo (L.)

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Summary. The present study was carried out in order to test the hypothesis that an increase in net water flux through the isolated toad skin causes a decrease in osmolarity of the interstitial fluid resulting in a decreased osmotic water permeability of the epithelium. A high net water flux is associated with a high water content and low sodium and potassium contents (Fig. 1). The calculated concentration of sodium plus potassium was about 120 mM in skins with a water flux below 0.5 μl cm⁻² min⁻¹ and decreased to about 70 mM in skins with a water flux of 2–3 μl cm⁻² min⁻¹ (Fig. 2). During perfusion of the vascular system of isolated pelvic skins with hypotonic media, the osmotic water permeability was reduced. Perfusion with 50% Ringer’s solution reduced the permeability by 33% in February and by 60% in June (Fig. 3).

Introduction

When neurohypophyseal hormone is added to the inside medium bathing the isolated pelvic toad skin exposed to an osmotic gradient, the net water flux is increased (Bentley and Main, 1972; Baldwin, 1974).

If the skin is perfused through the vascular system with a perfusion medium to which AVT (arginine vasotocin) has been added, the water flux is several times higher than that of unperfused skins containing AVT in the inside medium (Christensen, 1974). The corium probably represents an unstirred layer, and the relatively low water flux through unperfused skins may be due to a decreasing osmotic concentration of the interstitial fluid, resulting in a reduced gradient across the epithelium. In amphibian bladders, cellular or interstitial hypotonicity causes a decrease in osmotic water permeability (Bourguet et al., 1971; Eggena, 1972), and a similar situation may be found in the toad skin.

The present study aims:
1) To investigate whether the osmolarity of the interstitial fluid in the unperfused skin is correlated with the magnitude of the transepithelial water flux.
2) To measure the osmotic water permeability of the epithelium while the osmotic concentration of the interstitial fluid is decreased by perfusion of the vascular system with hypotonic media.

Methods and Materials

Experimental Animals

The experiments were performed on female toads, Bufo bufo bufo (L.). One batch of animals was collected in April and the experiments were carried out in June. Another batch of
animals was collected in September, and the experiments were carried out during the months of October to February. All animals were kept in shallow tap water at 4 °C until the start of the adaptation period. Some were adapted to “aquatic” conditions: shallow tap water at 20 °C, 1–2 weeks prior to the experiment. These animals weighed 30–60 g and were unfed. Others were adapted to “terrestrial” conditions: animals on dry filter paper, access to water, 20 °C, 4–8 weeks prior to the experiment. These animals weighed 70–120 g and were fed meal worms ad libitum.

Water Flux

The transepithelial water flux through the isolated skin was measured volumetrically, and perfusion of the pelvic skin was performed as described by Christensen (1974). These experiments were carried out at 20 ± 0.5 °C.

Total Content of H₂O, Na, and K

A piece of skin was taken from the flux chamber, and the water content was determined by dehydrating the skin for 24 hours over silica gel. Subsequently, the skin was boiled in water for 15 minutes, and after one hour of equilibration, the concentrations of sodium and potassium in the extract were determined by means of an Eppendorf flame photometer.

Media

The inside medium was frog’s Ringer containing 112.2 mM NaCl, 2.4 mM NaHCO₃, 1.9 mM KCl, and 1.0 mM CaCl₂ (220 mOsm). The outside medium was “artificial tap water” containing 2.4 mM NaHCO₃, 1.9 mM KCl, and 1.0 mM CaCl₂ (10 mOsm). The perfusion medium was always the same as the inside medium. In some of the experiments, the perfusion medium and the inside medium were diluted with distilled water. The osmotic concentration of the media was measured on an Advanced Osmometer Model 3L.

The water flux was stimulated with synthetic AVT [arginine vasotocin, Schwartz/Mann, 135 ± 20 U/mg (rat pressor)].

Results

Correlation between Net Water Flux and Osmotic Concentration of the Interstitial Fluid in Unperfused Skins

In order to obtain a wide range of water fluxes, both pelvic and pectoral skins isolated from animals adapted to “aquatic” as well as to “terrestrial” conditions were used. Some of the skins were unstimulated, and in other cases 5 × 10⁻⁶ M AVT was added to the inside medium from the start of the experiment.

When the water flux was constant, after 80 min, the skins were removed from the flux chamber and analyzed for content of water, sodium, and potassium.

The results given in Fig. 1 indicate that the total water content increased and the total content of sodium and potassium decreased with increasing water flux. The sodium and water contents were generally higher in pelvic skins than in pectoral skins, possibly due to a smaller extracellular space.

The total amount of sodium and potassium divided by the total amount of water has been used as an estimate of the osmotic concentration in the skin (Fig. 2). This concentration was about 120 mM in slowly transporting skins and about 70 mM in maximally transporting skins. The mean concentration in stimulated pelvic skins from “terrestrial” toads was 79 ± 3 mM (SEM, 12 skins). In all types of skins the concentration seems to depend on the magnitude of water flux rather than on skin area, preadaption, or presence of AVT.

Table 1 shows the mean water fluxes through the different types of skins.