

WATER TRANSFER BY THE TOAD BLADDER

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ABSTRACT

Studies have been made on the isolated urinary bladder of the toad, *Bufo marinus*, in an attempt to investigate the effect of vasopressin on the permeability of water from mucosal surface to serosal surface of the toad bladder. The method adapted was that described by Bentley (1). The bilobed bladder of the toad is divided into two separate sacs. Each of the sacs is filled with a dilute Ringers solution and then immersed in aerated isotonic Ringers solution. The rate of water loss along the imposed osmotic gradient is estimated by weighing the sacs in air at 30 minutes intervals and noting the weight loss in that time period. In most studies one bladder sac serves as a control for the contralateral experimental obtained from the same animal. Osmotic flow of water is negligible in both sacs during the initial control periods. However, the addition of vasopressin to the solution bathing the serosal surface of the membrane results in a marked increase in net water movement. The effect is readily reversed by rinsing the bladder and adding hormone free Ringers solution to the serosal surface. Characteristically no response is elicited by addition of hormone to the mucosal bathing solution.

INTRODUCTION

One of the biological membranes which has been given much attention by physiologists in the past few years is the amphibian urinary bladder which have long been useful preparations for studying membrane transport process.

Morphologically, the urinary bladder of a toad is much simpler than the abdominal skin of a frog. Toad bladder consists of but a single layer of mucosal cell plus a small amount of connective tissue and non cellular serosal membrane. Toads conserve water by reabsorbing it from urine in its bladder to replenish body water as the animal becomes dehydrated. Water flows osmotically the active transport of salt by this single layer of cell. This water, which may amount to 30-40% of the body weight in a day, is excreted by the kidneys as a hypoosmotic urine. The urine contains solutes such as sodium that are physiologically significant. This sodium is pumped from the inside of the bladder to the outside or into the body of toad. The net flux

of sodium equals the short circuit current (10), which means that sodium is basically the only ion transported. The potential difference across the bladder averages near 50 mV; the inside (mucosal) surface is negative with the respect to the outside (serosal) surface. Both water movement and sodium transport are under hormonal control, such as vasopressin. The bladder is under resting conditions, relatively impermeable to water and urea, but becomes more permeable to these simple substances upon being treated with vasopressin (14, 15). This neurohypophysial hormone stimulates the net active transport of sodium ions and increases the permeability of the amphibian urinary bladder to water and a number of other small molecules from the mucosal to the serosal surface of the bladder (1, 2, 10).

The object of the work to be presented here was to see if the urinary bladder of the toad showed such directional in the presence and absence of vasopressin on net water movement.

MATERIALS AND METHODS

Chemicalia. The experiments were carried out by using of the following chemicalia: Ringers solution (which consisting of the following composition (g/l): NaCl 6.5, KCl 0.14, CaCl₂ 0.12 and NaHCO₃ 0.2), Dilutes ringers solution (1:10, 1:25 and 1:35 respectively), Vasopressin (10 milliunits/ml, which then was added to 1:10 dilute Ringers).

Living material. Studies were done using the urinary bladder of the toad, *Bufo marinus*, in vitro.

Equipment. The equipment used in the experiment were: 2 glass tubing (mounting tube, 12 cm long and 0,5 cm diameter), 2 test tubes (150 mm long and 25 mm diameter), 2 rubber stoppers # 4, 2 stands for clamping the test tubes, 1 petri dish, Strong suture thread, 1 set dissection kit, Pipet, Syringe with needle, Plastic tubing, Compressed air or aquarium aerator, Analytical balance.

PROCEDURES

Preparing the isolated toad bladder

For the in vitro studies, bladder halves were removed from pithed animals and mounted according to the method of Rentley (1).

Mounting the bladders

Each bladder lobe then was attached to the mounting tube assembly and tied by using strong suture thread and transferred to the test tube containing enough Ringers solution to just cover the bladder by using a syringe with needle and plastic tubing bladders were filled with 1-1.5 ml of dilute Ringers solution (1:10). The two preparations were left out of the test tube in order to examine weather there were any leaks. After checking them up, the two bladders were replaced into the test tubes. Then, the level of dilute Ringers solution within the bladders and the test tubes were adjusted, so that it just covered the bladders. The air flow in the two test tubes were adjusted, so that it delivered a stream of fine air bubbles.

EXPERIMENTAL

A. Permeability of bladder to water

In this procedure water transfer along an osmotic gradient was measured. After

mounting the bladders and adjusting fluid volumes as described above the bladders were removed carefully from the test tube where the stoppers and glass tubes were dried with kimwipes or other absorbent tissue. Then, the mounting assemblies were hung from the upper hook of an analytical balance and weighed to measure their weight. After weighing, the bladders were replaced in aerated Ringers for 30 minutes, then they were removed and dried as before, and weighed again. The bladders should lose 10-25 mg of water in 30 minutes, depending upon size and condition of the bladder tissue. If it has lost more than 25 mg weight it usually means that the bladders have a hole in it. The first of the two bladders served as a control. Then the experiment was continued by using dilute Ringers 1:25, 1:35, and dilute Ringers 1:10 served as a control. After completing this measurement the investigation was continued on the following part.

B. Effect of neurohypophysial hormones on bladder permeability to water

In this experiment both bladder were run in parallel. By using the same preparations in part A the experiment was continued on the following steps:

1. The old 1:10 Ringers was aspirated from the two bladders until they were empty.
2. One empty bladder was refilled with ordinary 1:10 Ringers to which had been added 10 milliunits/ml of vasopressin and the other with 1:10 Ringers solution.
3. Two baths of ringers solution was prepared, one containing 10 mU/ml of vasopressin and the other containing no hormone.
4. Each bladder was weighed and then it filled with ordinary dilute Ringers was placed into the bath containing the hormone and the bladder containing the hormone was placed into the bath lacking hormone, so that, one bladder had hormone at mucosal surface and the other at the serosal surface.
5. After 30 minutes the bladders were weighed to measure water transfer. The bladder with hormone at the serosal (outside) surface should lose 100-400 mg of water, while the other bladder with hormone at mucosal (inside) surface should lose water at about the rate of the control.



bladder. This results is consistent to the previous investigations.

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