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Thirst and sodium appetite in rats with experimental nephrotic syndrome

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HIGHLIGHTS

- Injections of Adriamycin were used to create a model of nephrosis in rats.
- Adriamycin treatment attenuated water drinking to renin-dependent challenges.
- Adriamycin treatment reduced sodium intake after acute hypovolemic challenge.
- Adriamycin treatment attenuated release of renin and aldosterone to isoproterenol.
- Water drinking to osmotic challenge and angiotensin II were not affected.

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ABSTRACT

Nephrotic syndrome is a renal disease accompanied by abnormal body fluid balance. The present experiments investigated the role of behavioral mechanisms in contributing to disordered fluid homeostasis in rats with experimentally-induced nephrotic syndrome. The studies examined water and sodium ingestion under ad libitum conditions and in response to dehydration-related challenges in rats made nephrotic by treatment with the antibiotic, adriamycin. Rats with nephrotic syndrome had greater ad libitum water intakes beginning 3 weeks after treatment, but daily sodium (0.3 M NaCl) intakes were not affected. Nephrotic rats showed attenuated water and sodium intakes after combined treatment with furosemide (10 mg/kg) and captopril (2 mg/kg), reduced water intakes after 20 h of water deprivation, and diminished water intakes, plasma renin activity and aldosterone secretion after subcutaneous isoproterenol (30 μg/kg). However, the adriamycin-treated animals had normal water intakes in response to subcutaneous hypertonic saline (4% at 0.75 ml/100 g) and central injections of angiotensin II (10, 20, and 50 ng). The results suggest that water and sodium ingestion in response to hypovolemic/hypotensive stimuli are disturbed in nephrotic rats, and provide evidence that the disordered behaviors reflect disturbances of the peripheral renin–angiotensin–aldosterone system.

1. Introduction

Nephrotic syndrome is a clinical disorder of body fluid regulation characterized by edema, hypoalbuminemia and proteinuria [4]. Much of the pathology of nephrotic syndrome can be modeled in animals by inducing renal damage with systemic injections of adriamycin (doxorubicin), an anthracycline antibiotic. In the rat, a single intravenous (iv) injection of this drug induces severe proteinuria that appears within 2 weeks, plateaus around 4 weeks and remains elevated for several months [2]. Rats made nephrotic by adriamycin treatment also exhibit sodium retention, polyuria, and increased plasma urea nitrogen and plasma creatinine concentrations [2,7,8,16]. Histological studies show that adriamycin-injected rats have severe generalized renal lesions characterized by tubular dilation and atrophy, interstitial fibrosis and focal, global glomerulosclerosis [7].

Renal damage associated with nephrotic syndrome alters fluid balance by affecting not only excretory mechanisms, but also other renal functions important for maintaining body fluid homeostasis. There are data indicating alterations in renin–angiotensin mechanisms in nephrotic syndrome and that such changes may contribute to various aspects of the pathology of the nephrotic syndrome. For example, infusions of renin or angiotensin increase the urinary excretion of

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protein in nephrotic animals [3] and proteinuria can be reduced in nephrotic humans and some experimental animal models by administering angiotensin converting enzyme inhibitors, such as captopril [9].

There is evidence that drinking mechanisms are altered in nephrotic syndrome. Hall et al. [7] reported elevated water intake and urine output in adriamycin-treated rats suggesting that drinking is altered in nephrotic syndrome. However, it is not clear whether the change in drinking is primary or secondary to increased urine output. Additionally, a complete assessment of changes in stimulated drinking has not yet been done. Therefore, the goal of the present studies was to investigate the effects of adriamycin treatment on 1) ad libitum water and sodium ingestion; 2) water and sodium ingestion in response to acute intracellular and extracellular thirst challenges; 3) short-term water and sodium ingestion mediated by peripheral and/or brain renin–angiotensin systems; and 4) blood pressure and hormonal responses to activation of the peripheral renin–angiotensin system.

2. General methods

2.1. Animals

Male Sprague–Dawley rats weighing 370–430 g at the beginning of the experiment were purchased from Harlan Laboratories (Indianapolis, IN). They were housed individually in hanging wire-mesh cages for at least 5 days before being introduced into experimental protocols. Standard diet (Purina Rat Chow 5012), tap water and 0.3 M NaCl solution were available ad libitum except as indicated. Water and 0.3 M NaCl were provided from 100 ml graduated cylinders with 1 ml divisions that were fitted with metal drinking spouts. A 12-h light–dark cycle (0600–1800, light) was maintained in the colony. Room temperature was controlled at approximately 22 °C. All work was conducted according to procedures approved by the University of Iowa Institutional Animal Care and Use Committee and conformed to the guidelines of the American Physiological Society.

For all surgeries, rats were anesthetized with an Equithesin-like anesthetic cocktail (composed of 0.97 g of sodium pentobarbital and 4.25 g of chloral hydrate/100 ml distilled water and prepared by the University of Iowa Hospitals and Clinics Pharmacy; 0.33 ml/100 g body-weight) [6].

2.2. Materials and procedures

2.2.1. Adriamycin treatment

Rats were randomly assigned to experimental and control groups and anesthetized. Adriamycin (3.5 mg/kg) or an equal volume of isotonic saline vehicle (1 ml/kg) was injected through the sublingual vein, and the rats were returned to their home cages.

2.2.2. Catheter surgery

Rats received carotid catheters under anesthesia. The catheters were made from 25 cm pieces of polyethylene tubing (PE-50) that were heat-welded to 4-cm pieces of PE-10. The PE-10 end was inserted into the carotid artery and the other end was tunneled under the skin to exit at the base of the neck. Catheters were filled with heparinized saline (50 U/ml) and plugged with 23-gauge obturators, and the rats were allowed to recover from surgery for at least 2 days before testing.

2.2.3. Cannula surgery

Anesthetized rats were placed in a Kopf stereotaxic instrument. The scalp was incised and the periosteum reflected. The skull was then leveled between bregma and lambda. A 23-gauge stainless steel cannula was implanted to terminate in the lateral ventricle using the coordinates 1.2 mm caudal to bregma, 1.5 mm left of the midline and 4.0 mm below the dura mater. The cannula was fixed to the cranium using dental acrylic resin and jeweler's screws and a 30-gauge metal obturator was placed in the cannula between tests.

2.2.4. Blood pressure measurements

Arterial catheters were connected to transducers and recorders by l m of PE-50 tubing. Arterial blood pressure was recorded on a polygraph (Dynograph Recorder, Model R611, Sensormedic, Anaheim, CA) using Cobe transducers. Mean arterial blood pressure (MAP) was obtained by reducing the gain on the electronic arterial signal.

2.2.5. Drugs

Adriamycin (Sigma, St. Louis, MO) was dissolved in sterile isotonic saline and administered at a dose of 3.5 mg/kg in 1 ml/kg volume through the sublingual vein. Furosemide (Furo; 10 mg/kg body-weight; Elkins-Sinn Inc., Cherry Hill, NJ) was administered subcutaneously. Captopril (Cap; 2 mg/kg body-weight; 1 mg/ml volume; SQ-14, 225; Bristol-Myers-Squibb, Princeton, NJ), was dissolved in sterile isotonic saline immediately before each experiment and was also administered subcutaneously. Isoproterenol (HCl, Elkins-Sinn Inc., Cherry Hill, NJ) was diluted with isotonic saline to 30 μg/ml before each test and administered (30 μg/kg body-weight) subcutaneously. Angiotensin II (ANG II; Sigma, St. Louis, MO) was dissolved in isotonic saline and stored frozen in aliquots. Aliquots were thawed, and diluted to the appropriate concentration with isotonic saline, immediately before each test.

2.2.6. Statistical analysis

The results are reported as means ± SEM. The data were analyzed using one-way or two-way ANOVA. Urinary protein and acute (i.e., 90–120 min) water and 0.3 M NaCl intakes were analyzed with treatment (control vs adriamycin) as the between-subjects factor. Daily water and 0.3 M NaCl intakes were analyzed with treatment as the between-subjects factor and time (i.e., days, weeks) as the within-subjects repeated factor. Mean arterial pressure and plasma hormones were analyzed with treatment and drug condition (vehicle vs isoproterenol) as between-subjects factors. Water drinking to icv ANG II was analyzed with treatment and dose as factors. Differences were considered significant at $p < 0.05$.

3. Experiment 1: the effects of adriamycin treatment on ad libitum and experimentally-induced thirst and sodium appetite

3.1. Experimental groups

Experiment 1 determined the effects of nephrotic syndrome on daily, ad libitum intakes of water and saline, and on water and saline drinking responses to acute dipsogenic and natriorexigenic challenges. Twenty-two rats weighing 370–430 g were randomly assigned to receive adriamycin ($n = 12$) or vehicle ($n = 10$) treatment. Ad libitum water and 0.3 M NaCl intakes were recorded for the next 23 days. Then rats received a series of drinking tests with a few days between testing. Urine was also collected on one day to evaluate effectiveness of adriamycin treatment by measuring urinary protein excretion.

3.2. Experimental protocols

3.2.1. Quantitative total urine protein test

Twenty four-hour urine samples were collected on day 28 after adriamycin treatment to verify the presence of proteinuria. Urine samples were centrifuged to remove suspended matter. Total urinary protein was measured with a turbidimetric test kit (Stanbio Laboratory, Inc., San Antonio, TX). Absorbance reflecting the turbidity of the solution was determined spectrophotometrically at 420 nm (Spectronic 601, Milton Roy, Riviera Beach, FL) and compared to protein standards.

3.2.2. Furo/Cap test

The combined administration of Furo and Cap [5,13] was used to induce a rapid-onset sodium appetite and thirst. The Furo/Cap test was conducted on day 23 after adriamycin treatment. Rats were placed in