

An in-depth study on the importance of neurohormonal control on osmoregulatory function of *Dipodomys spectabilis*; how arginine vasopressin causes an increase in urine concentration.

Project Summary

Organisms that live in desert regions have evolved unique adaptations to live in these harsh environments. Some organisms, like *Dipodomys spectabilis* the kangaroo rat, have developed extremely efficient ways of conserving water. This adaptation is the product of other factors, such as behavior, microhabitats, and physiology. However, given that the kangaroo rat's physiology seems to be the largest contributor, the main question is this: what is the importance of neurohormonal control on the osmoregulatory system of the kangaroo rat?

The study put forth by this proposal will take an in-depth look at the ability of kangaroo rats to concentrate urine with respect to the anti-diuretic hormone, arginine vasopressin. This physiological question is interesting because it highlights exactly how important hormones such as ADH and aldosterone are to the process of urine concentration. All of these hormones play an important part, but this study will focus only on arginine vasopressin for the sake of brevity. This study hypothesizes that vasopressin itself can cause an increase in urine concentration – even if no environmental stressors are present – thereby underscoring the degree to which neurohormonal control affects osmoregulation. Kangaroo rats will be subjected to exogenous vasopressin as well as adrenomedullin, a vasopressin inhibitor. By showing that vasopressin causes increase in urine concentration, this will answer an important physiological question that as of yet has not been studied this specifically.

Introduction and Background

Desert ecology is very unique, as is the physiology of the organisms that inhabit such environments. Many species of plants, vertebrates, and invertebrates alike have developed extreme adaptations for coping with the characteristics of desert environments; specifically high temperatures and water scarcity. One such example is the Heteromyd family. This family includes small rodents living in the southwest region of North America. The two most studied species in this family are kangaroo rats (*Dipodomys*) and pocket mice (*Perognathus*). Both of these species have shown the ability to conserve water by producing highly concentrated urine.

For many vertebrates that live in desert environments, certain adaptations are the product of other factors, such as behavior, microhabitats, and physiology. The kangaroo rat is certainly no exception, yet these three factors raise interesting questions. Is the kangaroo rat's ability to concentrate its urine the result of all of these factors working in concert, or is there a single factor that contributes more to the osmoregulatory ability of this organism? The kangaroo rat is a small, nocturnal mammal that spends most of the day in a burrow humidified by its own respiration, which helps the rat to not lose additional water by respiring hot and dry air. Other desert organisms are nocturnal, but do not share the kangaroo rat's remarkable urine concentrating abilities. Additionally, other organisms make use of microhabitats, like burrows and dens to stay out of high desert temperatures, yet these organisms also cannot produce highly concentrated urine. It seems most likely then, that the kangaroo rat's physiology is the largest contributor. This study proposes that within the kangaroo rat's physiology, neurohormonal control is essential. Therefore, the main question is this: how important is neurohormonal control on the osmoregulatory system of the kangaroo rat? If neurohormonal control was not present, would the

kangaroo rat still be able to produce urine that was as highly concentrated as the scientific community knows it to be?

Rodents of the family *Heteromyidae* are all known for their remarkable ability to concentrate their urine. Extensive studies have shown that this is largely due to the physiological structure of the kidney itself. For vertebrates, the kidney plays a very important homeostatic role when it comes to the fluids within the organism. The kidney is even more important for mammals, since it is the only osmoregulatory organ present in the organism, unlike birds and reptiles that have additional osmoregulatory organs, like salt glands. This is an extremely important function for an organism's survival because the plasma, interstitial fluid, and intracellular environments all need to be maintained at a careful balance and osmoregulation describes how the organism will correct any deviance from the optimal set-point that occurs when the organism eats, drinks, or breathes in materials from the external environment (Hill et al., 2004). Therefore, terrestrial vertebrates have the ability to produce hyperosmotic urine, or urine that is more osmotically concentrated than the blood plasma (Hill et al., 2004).

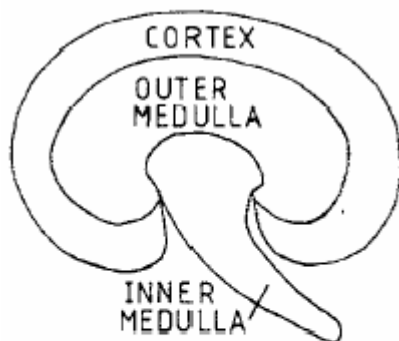


Fig. 1. Diagram of hemisected kidney of pocket mouse (*Perognathus parvus*).

(Yancey, 1988) This figure details the three main sections of the kidney commonly used for osmotic analysis. For the purpose of this proposal, this figure shows the outer medulla which is where the loops of henle are found.

Vertebrate kidneys are made up of many tubular structures called nephrons and birds and mammals both possess loops of Henle within their nephrons. The loops of Henle are primarily found in the outer zone of the medulla and structurally resemble a hairpin with two segments running parallel to each other. A mammalian nephron starts with the glomerulus and the Bowman's Capsule. The nephron then continues into the proximal convoluted tubule, the loop of Henle (descending limb, thin segment, and ascending limb), the distal convoluted tubule, and ends at the collecting duct (Hill et al., 2004). Much research has been done that shows that loops of Henle play a crucial role in urine concentration.

Hill et al. (2004) explain that the physiology of the loops of Henle differ greatly in mammals that live in aquatic environments as compared to mammals that live in arid environments. There is, in fact, a strong correlation between the length of the loop of Henle and the thickness of the medulla, where the longest loop of Henle determines the overall thickness of the medulla (Hill et al., 2004). A study done by Schmidt-Nielsen and O'Dell (1960) showed that there is a very strong correlation between the ability to maximally concentrate urine and the relative medullary thickness of the kidney. The kangaroo rat is a prime animal model to study because of its relatively thick medulla and longer loops of Henle.

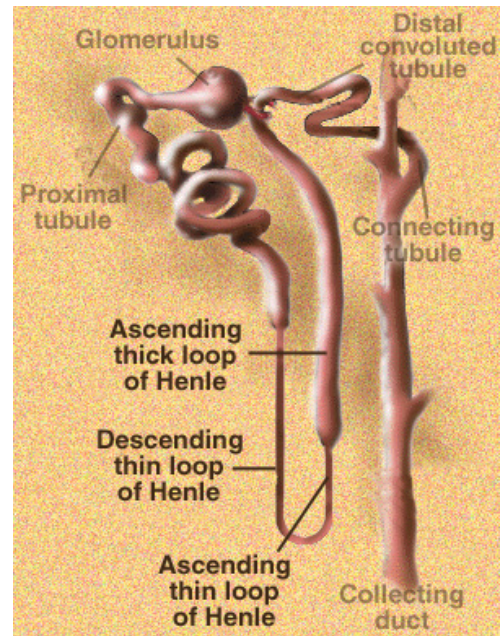


Fig. 2 (Renal Physiology) This figure shows the structure of the nephron, the functional unit of the kidney. Additionally, both birds and mammals have an additional segment, known as the loop of Henle (in bold type), where some of the urine concentration takes place

Concentration within the nephron can be said to happen in spatially distinct steps. The first concentration event occurs in the loop of Henle, where water freely diffuses out of the thick descending limb and highly concentrates the urine. Then, when the primary urine flows through the thick ascending limb, salts are actively pumped out of the loop and into the interstitial space. The primary urine that leaves the ascending limb is at a relative low concentration, and is usually hypotonic compared to the plasma. This arrangement where the flow of water and osmolytes goes in opposite directions in the ascending and descending limbs effectively produces a concentration gradient from one limb to the adjacent limb. The fact that metabolic energy is required to pump salts out of the ascending limb shows that the loop of Henle is a countercurrent multiplier, as opposed to a passive countercurrent exchanger (Hill et al., 2004).

Additional concentration takes place in both the distal convoluted tubule and the collecting duct. In these segments of the nephron neurohormonal control is exceedingly important. Aldosterone is the hormone responsible for sodium reabsorption in the distal tubule and ADH is the hormone responsible for water permeability in the collecting duct. There are many other hormones that have a large role in osmoregulation, however for this study the primary focus will be on ADH for the sake of brevity. The collecting duct is where the organism can “decide” if it wants to produce hypoosmotic or hyperosmotic urine. These alternatives are determined by the relative permeability of the collecting duct to water. The hormone that is responsible for this permeability is actually a family of very closely related hormones known as antidiuretic hormones (ADH). The antidiuretic hormone of humans is simply known as ADH, while the related hormone for amphibians, reptiles, and birds is arginine vasotocin, and the antidiuretic hormone for many other mammals (including kangaroo rats) is arginine vasopressin

(Hill et al., 2004). ADH – as well as its related forms – is a peptide hormone that is secreted by the posterior pituitary.

When the organism has no need to retain or conserve water, ADH levels in the body are low. These levels mean that the wall of the collecting duct will have low permeability to water. When salts are reabsorbed, water is unable to follow and thus will be excreted in a dilute urine. When an organism needs to retain or conserve water – such as organisms living in arid environments – the pituitary will increase production of ADH. Increased levels of ADH cause the walls of the collecting duct to become more permeable to water. In this case, as NaCl is actively reabsorbed back into the kidney, the water is better able to follow the salt as compared to when ADH levels are low. The result of increased ADH means that the urine passing through the collecting duct is hyperosmotic compared to the blood plasma. This study proposes that neurohormonal control on osmoregulation is extremely important on the ability of the kangaroo rat to concentrate urine. If neurohormones such as ADH and aldosterone were not produced and regulated in the kidney, the urine concentrating ability of the kangaroo rat would be greatly diminished.

A study that was done by Stallone and Brown (1988) showed that when kangaroo rats are subjected to drought-like conditions and become dehydrated, the pituitary releases elevated levels of plasma arginine vasopressin (Pavp). However, this study only showed correlation, not causation. It merely suggests that vasopressin is involved in a larger response of the organism to a lack of water. The majority of current literature also reiterates this view on the role of vasopressin. The purpose of this study will be to determine if vasopressin *itself* can cause an increase in urine concentration, even if no drought-like conditions are present. This increase in concentration will show the importance of vasopressin, and neurohormonal regulation in general,

to desert organisms with respect to water conservation. If this study shows that vasopressin causes an increase in urine concentration, the results could additionally underscore the importance of the conservation and evolution of the ADH family across taxa, especially in organisms that are highly adapted for life in arid environments.

Hypothesis:

(H) Hypothesis: Increasing plasma vasopressin causes an increase in urine concentration in kangaroo rats, even when no other environmental stressors consistent with drought are present.

(H₀) Null Hypothesis: Increasing vasopressin in kangaroo rats does not cause a change in urine concentration.

(H₁) Alternative Hypothesis: Vasopressin does not directly cause an increase in urine concentration in kangaroo rats, and rather is a result of the organism's cumulative response to dehydration.

The logic behind this hypothesis is based largely on the study that was done by Stallone and Brown (1988). By subjecting kangaroo rats to drought-like conditions, and causing them to become dehydrated, they were able to measure plasma arginine vasopressin (Pavp). This study showed a correlation between dehydration in kangaroo rats and an increase in vasopressin. However, I propose to show in this study that even if a kangaroo rat is not subjected to drought-like conditions such as high heat and extreme lack of water, introducing vasopressin into its system will cause an increase in urine concentration.

Proposed Study:

The organism that will be used in this study will be the kangaroo rat (*Dipodomys spectabilis* [*Heteromyidae*]) for two primary reasons. Firstly, as many previous studies have shown, all organisms in the genus *Dipodomys* not only display increased relative medullary thickness – which correlates with increased urine concentration – but they have also been shown to have increased levels of plasma vasopressin when dehydrated. Secondly, this species has been used in studies before, like the Stallone and Brown study (1988), and so more is known about this organism’s physiology. Since this experiment will attempt to determine if an increase in vasopressin directly causes an increase in urine concentration, the procedure will have to be controlled to show that other confounding factors are not contributing to urine concentration.

The first part of this study will involve acclimating *Dipodomys spectabilis* to standardized conditions within the lab as far as diet, temperature, access to water, etc. After a period of 30 days for acclimatizing the kangaroo rats, they will then be divided, at random, into six different groups based on two different sets of conditions. The first set of conditions will be whether the kangaroo rats are subjected to normal conditions or to drought conditions. The second set of conditions will involve vasopressin; the kangaroo rats will be placed in a control group, an experimental group that will be injected with exogenous vasopressin, and a second experimental group that will be injected with a compound to block the function of vasopressin. Adrenomedullin is a peptide isolated from human pheochromocytoma, and was shown in a study done by Yokoi et al. (1996) to inhibit vasopressin release in conscious rats.

Experimental Groups	Control	Exogenous Vasopressin	Adrenomedullin
Drought Conditions	Group 1	Group 2	Group 3
Non-Drought Conditions	Group 4	Group 5	Group 6

In a study done by Schmidt-Nielsen (2005), the researchers determined the relative humidity at which kangaroo rats will thrive and the relative humidity at which kangaroo rats begin to suffer detrimental effects. Based on this prior study, the groups being observed in the normal conditions will be in environments where the atmospheric humidity is 30%. The groups being observed under drought conditions will be in an environment where the atmospheric humidity is 15%. Both groups will be fed a similar diet of pearled barley, which the Schmidt-Nielsen (2005) study showed will absorb water from the environment such that the grain and the air will have an equal amount of moisture. The amount of grain given to each group will not be altered. In this way, experimental conditions can be reasonably controlled.

In respect to vasopressin, there will be a control group that will be injected with a placebo, most likely a saline solution. The first experimental group will be injected with a fixed amount of exogenous vasopressin to increase the total amount of P_{avp}. The second experimental group will be injected with adrenomedullin.

Once the experimental conditions are in place, measurements will be taken at four specific time intervals; 48, 96, 144, and 192 hours. At each time interval, one individual will be chosen at random from each of the six groups. Urine samples will be collected and analyzed to determine osmolarity by the urine collection and analysis method detailed in Yancey (1988). Finally, blood will be drawn and then analyzed using the radioimmunoassay method detailed in Stallone and Brown (1988) to determine precise levels of plasma vasopressin. This procedure will be repeated with as many individuals as is necessary to statistically show that vasopressin causes the increase in urine concentration.

Analysis of the results will be determined by looking at the concentration of urine and the amount of vasopressin in the plasma. The three groups that are under non-drought conditions will be especially important in determining the role of vasopressin in urine concentration.

Looking at the experimental group where vasopressin is inhibited, if the urine collected is less concentrated than the urine of the control group this supports the hypothesis that vasopressin causes an increase in urine concentration. Concerning the experimental group that is given exogenous vasopressin, if the urine collected is more concentrated than the urine of the control group this also supports the hypothesis. This will be further supported by the fact that these kangaroo rats will not have been under conditions that normally would cause a release in vasopressin in order to conserve water. Additionally, if vasopressin does indeed cause an increase in urine concentration, then inhibiting it should mean that for both experimental groups with inhibited vasopressin (in both normal and drought conditions) should have more dilute urine than their respective control counterparts.

These changes in urine concentration will show the importance of vasopressin, and neurohormonal regulation in general, to desert organisms with respect to water conservation. To show that vasopressin causes an increase in urine concentration will help to explain the importance of the conservation and evolution of the ADH family across taxa, especially in organisms that are highly adapted for life in arid environments.

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Budget Justification

For the purpose of this budget, I am going to assume that I already have access to laboratory facilities at the University of Arizona.

I will also assume that in my laboratory I will have access to all major equipment needed such as: equipment to perform a radioimmunoassay of arginine vasopressin in the plasma, and equipment to perform flame photometry to measure sodium and potassium in the urine.

I will also assume that as an in-state researcher, I will not have to pay for a collecting permit to go out into the field and collect *Dipodomys spectabilis*.

Estimated Duration of the Study: 14 months.

Salary for four undergraduate workers: pay at \$10 per hour plus 10% benefits.

Four undergraduate workers will be sufficient to help me in this study. Their responsibilities would include helping me in trapping and collecting the kangaroo rats, feeding and caring for the kangaroo rats during the duration of the experiment, performing the various analyses and collecting data.

Estimated total: \$16,000

Equipment:

computer for statistical analysis (This will be needed not only to keep collected data organized but also to perform statistical analyses)

humidifiers

dehumidifiers (Both of these will be needed to maintain the different experimental groups at the appropriate conditions; the humidifier for the non-drought conditions, and the dehumidifier for drought conditions).

standard plastic laboratory rodent cages

desert sand bedding

pearled barley

(The above three items will be needed to keep and maintain the kangaroo rats once they are in the laboratory)

All chemicals for the various assays will be obtained from Sigma Chemical Company

Estimated total: \$10,000

Travel: \$3,000

Since *Dipodomys spectabilis*, banner-tailed kangaroo rats live in a range that includes southeastern Arizona, travel costs will include the vehicles needed for transportation, gas required to travel between the trapping location and the University of Arizona, and any extra needed for maintenance of vehicles.

Publishing Costs: \$1,000

This is the estimated cost that will be needed to publish this study once the study is concluded and the report has been written.

Estimated Total Cost: \$30,000