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Seasonal acclimatization in water flux rate, urine osmolality and kidney water channels in free-living degus: molecular mechanisms, physiological processes and ecological implications

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Summary

The environmental modification of an organism's physiology in the field is often hypothesized to be responsible for allowing an organism to adjust to changing biotic and abiotic environmental conditions through increases in biological performance. Here, we examine the phenotypic flexibility of water flux rate, urine osmolality and the expression of kidney aquaporins (AQP; or water channels) in free-ranging *Octodon degus*, a South American desert-dwelling rodent, through an integrative study at cellular, systemic and organismal levels. Water flux rates varied seasonally and were significantly lower in austral summer than in winter, while urine osmolality was higher in summer than during winter. The observed water influx rate during summer was 10.3±2.3 ml day^{-1} and during winter was 40.4±9.1 ml day^{-1}. Mean urine osmolality was 3137±472 mosmol kg^{-1} during summer and 1123±472 mosmol kg^{-1} during winter. AQP-2 medullary immunolabeling was more abundant in the kidneys of degus captured during summer than those captured during winter. This immunoreactivity was higher in apical cell membranes of medullary collecting ducts of degus in summer. AQP-1 immunostaining did not differ between seasons. Consistently, AQP-2 protein levels were increased in medulla from the summer individuals, as judged by the size of the 29 kDa band in the immunoblot. Here, we reveal how the integration of flexible mechanisms acting at cellular, systemic and organismal levels allows a small desert-dwelling mammal to cope with seasonal water scarcity in its semi-arid habitat.

Key words: acclimatization, arid environment, water economy, rodent, aquaporin, degu, *Octodon degus*.

Introduction

Studies of phenotypic flexibility are central to the understanding of contemporary evolutionary biologists. Flexibility is heritable and appears to evolve through natural selection (e.g. Schlichting and Pigliucci, 1998). A working example is reversible phenotypic flexibility (*sensu* Stearns, 1989), which is the ability to modulate organismal traits in response to environmental conditions (Piersma and Drent, 2003). Ecological and evolutionary physiologists have studied reversible phenotypic flexibility under the paradigm of environmental acclimation and/or acclimatization (Willmer et al., 2000). The environmental modifications of an organism's physiology in the field, or acclimatization, are often hypothesized to be the process responsible for allowing organisms to adjust to changing biotic and abiotic environmental conditions through increases in biological performance (Garland and Carter, 1994; Huey and Berrigan, 1996).

Maintaining a positive water economy is a difficult task for desert-dwelling animals (Walsberg, 2000). Rodents from arid and semi-arid habitats live under conditions where the spatial and temporal availability of free water is limited, or scarce, so these rodents are faced with the problem of water conservation (Degen, 1997). Nevertheless, the physiology of water regulation among desert rodents appears to show remarkable flexibility in both time and space. The response of small mammals to unproductive desert environments and water deficits has been intensively investigated (e.g. Schmidt-Nielsen, 1964). However, current understanding of the cellular, systemic and organismal physiology of water economy relies heavily on short-term, laboratory-oriented experiments, which usually focus on responses at isolated levels of biological organization.

Here, we examined the phenotypic flexibility of water flux

rate, urine osmolality and expression of kidney aquaporins (AQP), or water channels, in a South American desert-dwelling rodent (see below) and, as far as we know, for the first time through a field study integrating cellular, systemic and organismal levels. Rates of water flux in the field represent the loss of water, *via* excretion and evaporative water loss, and the simultaneous input of water, *via* metabolic water production, and pre-formed water, *via* food and drink (Nagy and Costa, 1980; Nagy and Peterson, 1988; Speakman, 1997). In this context, labeled water could be used to estimate water flux (Speakman, 1997). In addition, for small mammals, urine osmolality represents the capacity of the kidneys to efficiently conserve water (e.g. Schmidt-Nielsen, 1964; Prosser, 1991; Willmer et al., 2000; McNab, 2002; Seldin and Giebisch, 2000). Finally, studies at cellular levels of biological organization have now shown that the urine-concentrating mechanism associated with water reabsorption relies on the activities of a series of NaCl co-transporters, ion channels, Na^+/K^+-ATPase and AQP water channels (Seldin and Giebisch, 2000). AQP water channels are probably central structures leading to the mechanisms responsible for the changes in water balance. Here, we studied AQP-1 and AQP-2 water channels. In the kidneys, AQP-1 channels are constitutively active water channels that allow rapid transmembrane osmotic water flux and are involved in the reabsorption of filtered water in proximal tubules and thin descending limbs (Nielsen et al., 2002). On the other hand, AQP-2 is the vasopressin-regulated water channel expressed in apical membranes of connecting and principal cells of the distal nephron. AQP-2 is localized in the apical cell membrane but it also resides in a pool of membrane vesicles within the cytoplasm and can be regulated by the animal's state of water balance. Through apical AQP-2 and basolateral AQP-3, principal cells and inner medullary collecting duct cells are able to efficiently reabsorb free water (Nielsen et al., 2002).

Since small desert mammals regularly encounter seasonal variation in both their physical and biotic habitats, we hypothesize that they should change their physiology through phenotypic flexibility to cope with desert conditions. We studied seasonal acclimatization of *Octodon degus* (degu; see below) to its habitats. We determined, under field conditions, the effect of seasonal changes in environmental water availability on the distribution and regulation of AQP-1 and AQP-2, on urine osmolality and on total water flux.

Because during the dry summer months the plants consumed by degus (*Vulpea* sp. and *Erodium* sp.) contained nearly 5.7% and 2.8% of water, respectively, while during the wet winter these plant species contained 70.6% and 76.9% of water, respectively (C. Veloso, personal communication), we predict that rates of water flux will be higher during winter than during summer; thus, urine osmolality should be significantly higher during the dry season, and, consequently, we also predict a constitutive activity of AQP-1 and an increase in the insertion of AQP-2 into apical kidney cell membrane during the dry summer period.

We used the degu, *Octodon degus* (Molina) (Rodentia: Octodontidae), as the study organism. The degu is endemic to Chile and is a good model because it is diurnal, allowing for field observations. Furthermore, the degu inhabits the seasonal, semi-arid and Mediterranean environments of northern and central Chile, where summers are hot and dry and winters are cold and rainy. Degus are efficient at water conservation (Cortés et al., 1990) but have a low capacity for evaporative thermolysis (Bozinovic et al., 1995, 2000).

Materials and methods

Study site and field water flux measurements

This field study was conducted between austral winter 2001 and autumn 2002 in San Carlos de Apoquindo (33°23′ S, 70°31′ W), a rugged area of 8.35 km^2 located approximately 20 km east of Santiago in the Andean foothills, with elevations ranging from 1050 m to 1915 m above sea level. San Carlos de Apoquindo is covered by sclerophyllous vegetation, which, physiognomically, may be described as an evergreen scrub, locally know as matorral, and is best characterized as shrubland. We used the facilities of the P. Universidad Católica de Chile field station [Estación de Investigaciones Ecológicas Mediterráneas (EDIEM); see http://www.bio.puc.cl/ediem/]. Details of the climate and habitat conditions at our study site can be found at http://www.bio.puc.cl/sca/. Briefly, San Carlos de Apoquindo has a Mediterranean climate, with an annual mean rainfall of 376.4 mm, which is concentrated (65%) during the austral winter months, from June to August. On average, it rains every month of the year, but precipitation is scant from December to March (3% of the yearly total). Mean temperature is highest from December to March (austral summer) and lowest from June to August (austral winter). The combination of high temperature and low precipitation from December to March results in a summer drought.

We selected a representative area within the semi-arid matorral of San Carlos de Apoquindo that encompassed an extensive open space with sparse shrub and tree cover. In this area, we constructed an enclosure measuring 70 m×50 m, with a 1.8 m high fence that was buried 40 cm into the substrate and covered with chicken wire. Conditions within the enclosure can be considered natural. In the enclosure, we released four families of adult degus that were previously captured from another population. A total of 32 degus were released in the enclosure.

We measured the mean rate of water flux and field metabolic rate during winter and summer using double-labeled water (DLW) mixture provided by The Center for Isotope Research in The Netherlands (CIO) (http://www.cio.phys.rug.nl). The water was enriched in both ^2H and ^{18}O (34.4% and 64.1%, respectively). Following the protocol established by CIO and Kenagy et al. (1989), we injected the DLW quantitatively (0.8 ml in a 1 ml insulin syringe). We always noted if some DLW was spilled. For equilibration times of the DLW, we also followed the protocol outlined by Kenagy et al. (1989). That is, after 1 h at equilibrium, a first blood sample was taken from the orbital sinus using 80 µl heparinized microhematocrit

capillary tubes; the tubes were subsequently flame-sealed and stored at 5°C until analysis. Animals were always anaesthetized prior to measurements. Each capillary tube contained approximately 15 μl of blood. We used a propane torch with a very fine pointed flame for flame sealing. Degus were weighed with a portable electronic balance (±0.01 g; Sartorius, Gottingen, Germany). The ease of trapping degus in the enclosure allowed us to resample individuals after 48 h and to collect a second series of blood samples. Samples were mailed to CIO for further triplicate analysis. Background samples were obtained from degus inhabiting the same enclosure. Water fluxes were calculated for each individual on the basis of the individual-specific size of the body water pool (based on the plateau value of the ^{2}H dilution; Visser et al., 2000) and the individual-specific fractional ^{2}H turnover rate. We took isotope fractionation effects into account, assuming that 50% of the water efflux was lost through evaporative pathways, following Kenagy et al. (1989). In three animals, the sizes of their body water pools could not be assessed due to minor leakage of the DLW dose. In these cases, we used the mean percentage of body water to estimate the individual-specific sizes of the body water pool. Observed water flux rates were compared against the standard water flux rates (in ml day^{-1}) predicted for small herbivorous mammals (Nagy and Peterson, 1988) using the following equation: water flux rate=$0.708\times M_b^{0.795}$, where M_b is body mass in g.

Field urine osmolality

We conducted field measurements of urine osmolality for individuals captured in Quebrada de las Vacas, Fray Jorge National Park, Northern Chile (30°38′ S, 71°40′ W) during winter and summer sampling occasions. These measurements were made during the winter and summer of 1994. Briefly, the biotic and abiotic habitat in Fray Jorge is semi-arid-Mediterranean, similar to our enclosure at San Carlos de Apoquindo. Rodents were captured using Sherman live traps during a period of five consecutive days. A total of 30 adult animals was captured for this study. To avoid resampling, all rodents were ear-tagged. Urine samples were obtained each morning, at the same hour, using microhematocrit capillary tubes, which were subsequently sealed with parafilm. Immediately after collection, we measured urine total solids (S; in g per 100 g), following the method of Cortés and Rosenmann (1988), using a field refractometer (AO TS Meter; Scientific Instruments, Lakewood, NJ, USA). Our refractometer measurements (urine samples) were continuously calibrated against a freezing-point osmometer (Advanced Instruments, Norwood, MA, USA). Finally, we estimated urine osmotic concentration (U; in mosmol kg^{-1}) using the following equation: $U=140\times S^{0.984}$ (Cortés and Rosenmann, 1988).

Seasonal changes in AQP distribution and regulation

To test for the effect of seasonal changes in water availability on the distribution and regulation of AQP-1 and AQP-2, three adult male degus were live-caught with Sherman traps at San Carlos de Apoquindo during winter and summer collecting trips and transferred to the laboratory. Immediately following capture, animals were sacrificed *via* an overdose of pentobarbital sodium (60 mg kg^{-1}; i.p.). In the laboratory, both kidneys were removed, placed in ice-cold phosphate-buffered saline (PBS; pH 7.4) and processed for immunocytochemistry and immunoblot.

Localization of AQPs was carried out *via* immunocytochemistry. Immunocytochemical studies were carried out in paraplast-embedded tissue sections (6 μm thick), previously fixed in Bouin solution. The steps for immunocytochemistry are described in Gallardo et al. (2002). The primary antibodies used were anti-AQP-1 and anti-AQP-2 (diluted 1:200; kindly provided by M. Knepper, NIH, USA). Immunoreactive sites were revealed using biotinylated swine anti-rabbit IgG (Dako, Carpinteria, CA, USA) followed by streptavidin-conjugated horseradish peroxidase; the chromogens used were 3,3′-diaminobenzidine (Dako Liquid DAB plus kit) or Vector SG substrate (Vector, Burlingame, CA, USA), in the presence of hydrogen peroxide. Tissue sections were observed and photographed on a Nikon Optiphot microscope.

Variations in AQP-2 protein abundance were studied through immunoblot. Membranes were prepared from renal medulla of both groups by differential centrifugation. The tissue was homogenized in buffer containing 250 mmol l^{-1} sucrose, 10 mmol l^{-1} triethanolamine, 21 μmol l^{-1} leupeptin, 57.4 μmol l^{-1} phenylmethyl sulfoxide (PMSF), 0.1 mg ml^{-1} aprotinin, pH 7.5 and centrifuged at 3000 ***g*** for 10 min at 4°C; the supernatant was centrifuged at 100 000 ***g*** for 1 h at 4°C. The final pellet was resuspended in the same buffer, and protein concentration was determined spectrophotometrically with Bradford reagent (BioRad, Hercules, CA, USA). For immunoblotting, 15 μg of protein was solubilized in Laemmli buffer and heated at 65°C for 15 min. Immunoblotting was performed in 12% SDS–PAGE mini-gels and run on a mini-gel system (BioRad Mini-Protean III). Proteins were blotted onto nitrocellulose membranes, blocked for 1 h, washed with TBS-T pH 7.4 and incubated for 18 h at 4°C with the primary antibody. The secondary antibody was goat anti-rabbit IgG coupled to horseradish peroxidase. Immunoreactivity in the membrane was detected by enhanced chemiluminescence. In addition, we measured plasma and urine osmolality in animals used for AQP determinations, using a freezing-point osmometer (Advanced Instruments). Plasma was obtained by centrifugation of a venous sample collected from the inferior cava vein. Urine samples were obtained from a single bladder puncture.

Statistics

Statistical analyses were performed using the STATISTICA® for Windows (2001; version 6.0) statistical package. Data were analyzed using analysis of covariance (ANCOVA), with body mass (M_b) as a covariable. Data fulfilled the assumptions of the tests. Results are reported as means ± 1 S.D.

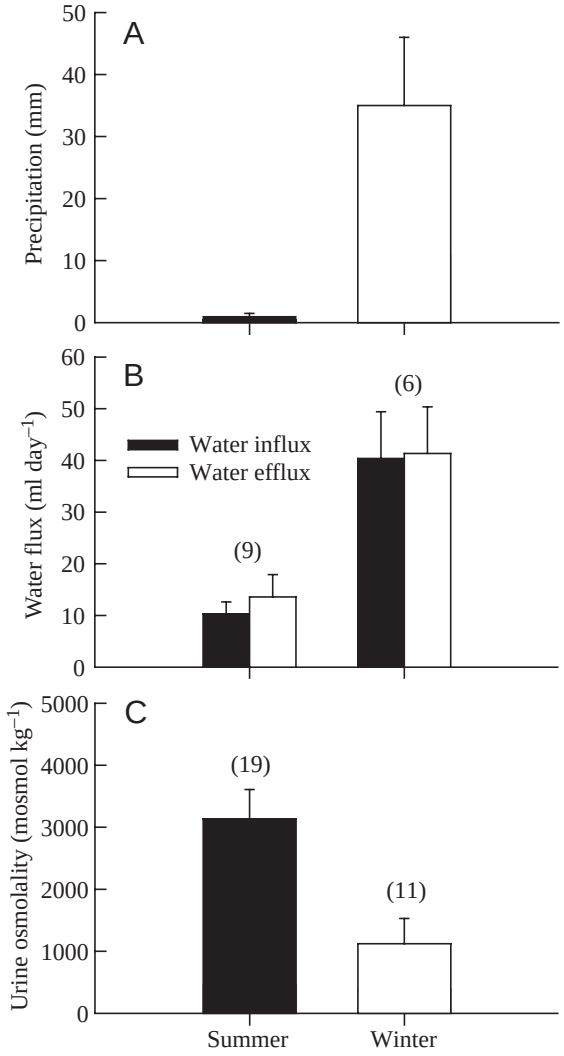

Fig. 1. Seasonal pattern of variation in (A) precipitation in the semi-arid habitats of north-central Chile, (B) water influx and efflux in free-living *Octodon degus* and (C) field urine osmolality in free-living degus. Values are means ± 1 S.D. Sample size (*N*) is shown in parentheses, and further statistical details are found in Tables 1 and 2.

Table 1. *ANCOVA test for seasonal water flux (ml day^{-1}) in* Octodon degus

Effect	SS	d.f.	MS	*F*	*P*
Body mass	57.158	1	57.158	1.1652	0.293
H_2O influx/H_2O efflux (1)	7.873	1	7.873	0.1605	0.693
Season (2)	5918.759	1	5918.759	120.6613	**<0.0001**
Sex (3)	22.570	1	22.570	0.4601	0.505
1×2	0.169	1	0.169	0.003	0.954
1×3	0.022	1	0.022	0.0005	0.983
2×3	156.669	1	156.669	3.1939	0.088
1×2×3	0	1	0	0	1.000
Error	1030.106	21	49.053		

Body mass (g) was used as a covariate.

Results

Climate

Climatic conditions at the study site were obtained from the Meteorological Service at the study site and in San Carlos de Apoquindo. Mean summer and winter rainfall at San Carlos de Apoquindo and Fray Jorge is plotted in Fig. 1A.

Field water fluxes and urine osmolality

There was no significant difference in mean body mass (M_b) between summer degus used for water flux measurements (186.2±14.39 g; six females and three males) and winter degus (187.8±11.75 g; four females and two males) (Table 1).

The mean rates of water flux in free-living degus for each season are shown in Fig. 1B. ANCOVA revealed that season was the only factor that had a significant effect on water flux rate. As predicted, rates of water flux were higher during winter than during summer (Fig. 1B; Table 1). Observed water influx rate during summer was 10.3±2.3 ml day^{-1}, which was 22.8% of the expected value (45.2 ml day^{-1}) based on M_b,. In winter, the rate of water influx (40.4±9.1 ml day^{-1}) was similar to the expected value, making up 88.8% of the value expected from M_b (45.5 ml day^{-1}). No significant effects of sex or interactions among factors were observed on the rate of water flux (Table 1). In addition, rates of water influx and efflux were not significantly different (Table 1).

In a second group of individuals, we seasonally measured field urine osmolality, *U* (Fig. 1C). The mean M_b of degus used for field *U* were not significantly different, being 124.79±6.99 g during summer (nine females and 10 males) and 119.72±9.19 g during winter (three females and eight males) (see Table 2). Interestingly, the same statistical pattern and results observed for the mean rates of water flux were also obtained for field *U* (Fig. 1C; Table 2). As expected, *U* was significantly higher during summer than during winter (Fig. 1C). The observed mean *U* during summer was 3137±472 mosmol kg^{-1}, which is 27.7% lower than the maximum *U* ever recorded in degus [Cortés et al. (1988) registered a mean *U* of 4338 mosmol kg^{-1}]. Our winter *U* (1123±472 mosmol kg^{-1}) was 74.1% lower than the maximum recorded *U* for degus.

Seasonal changes in AQPs and urine:plasma concentration ratio

The study of seasonal changes in AQP distribution and regulation was carried out using three degus (mean M_b=164.07±24.25 g) captured during summer and three degus (mean M_b=170.9±12.9 g) captured during winter. Body mass was not significantly different between seasons ($F_{1,3}$=1.202, P=0.353).

AQP-2 immunolabeling was observed in connecting tubules and cortical and medullary segments of collecting ducts. At the subcellular level, AQP-2 immunostaining was evident in apical plasma membrane as well as subapical cytosol. AQP-2 medullary immunolabeling was more abundant in kidneys of degus captured during the dry season (summer; Fig. 2A) than

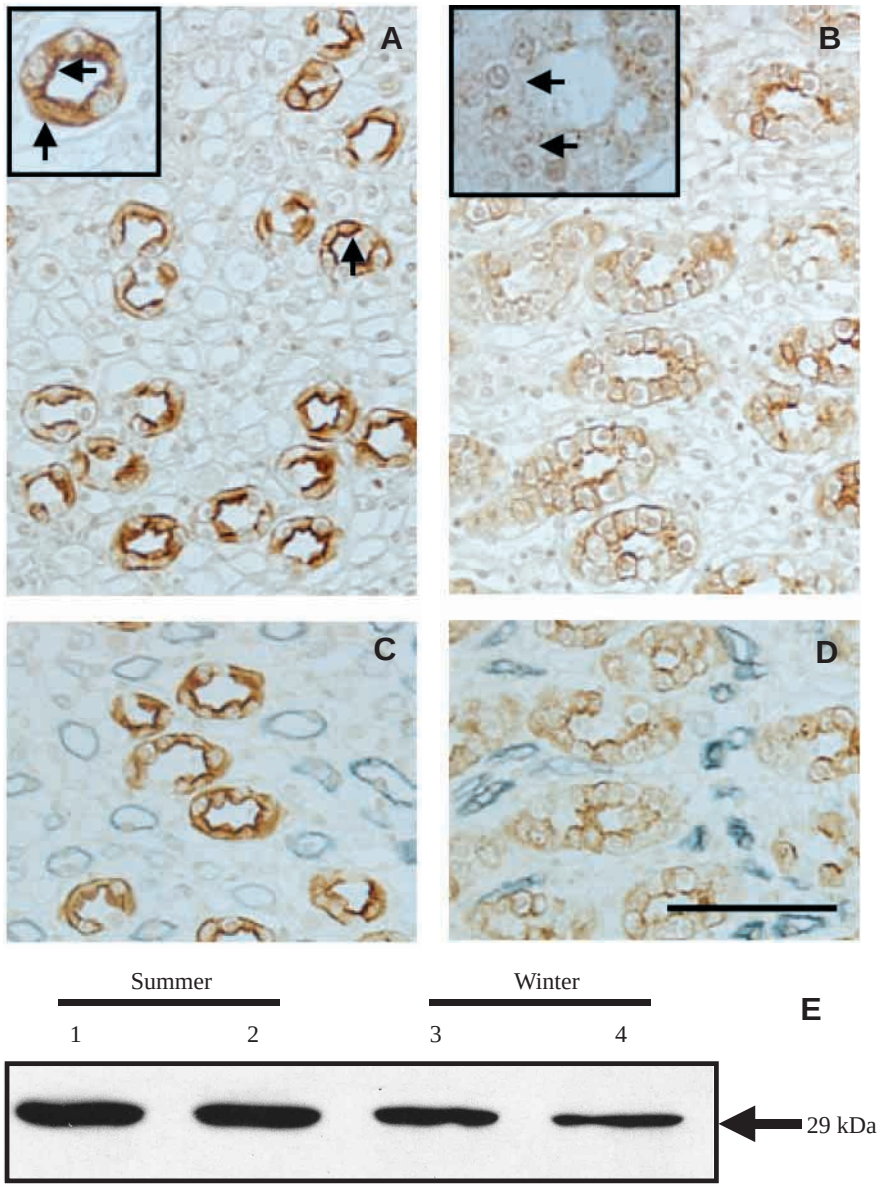

Fig. 2. Seasonal pattern of variation in aquaporin-2 (AQP-2) distribution and regulation in cells of the renal medulla. (A) Immunolabeling for AQP-2 in apical membrane (vertical arrow) and cytosol of inner medullary collecting duct cells during summer. (B) Scarce immunolabeling for AQP-2 can be observed in the same cells during winter. Unlabeled cells in A and B may correspond to intercalated cells. (C,D) Double immunolabeling for AQP-2 (inner medullary collecting duct cells; brown) and AQP-1 (thin descending cells of Henle's loop; blue) during summer and winter, respectively. Note the change in AQP-2 but not in AQP-1 immunoreactivity. (E) Immunoblot of proteins from renal medulla for AQP-2 during summer (lanes 1 and 2) and winter (lanes 3 and 4). Scale bar represents 100 μm in A–D and 50 μm in inserts of A and B.

Table 2. *ANCOVA test for seasonal urine osmolality (mosmol kg^{-1}) in* Octodon degus

Effect	SS	d.f.	MS	*F*	*P*
Body mass	141266	1	141266	0.6469	0.429
Season (1)	24985437	1	24985437	114.4153	**<0.0001**
Sex (2)	51947	1	51947	0.2379	0.629
1×2	1652	1	1652	0.0076	0.931
Error	5459373	25	218375		

Body mass (g) was used as a covariate.

the rainy season (winter; Fig. 2B). This immunoreactivity was higher in apical cell membranes of medullary collecting ducts of summer degus in comparison with those of winter degus. AQP-1 immunostaining did not differ between groups (Fig. 2C,D). Consistently, AQP-2 protein levels were higher in the medulla of the summer individuals compared with the winter individuals, as judged by the density of the 29 kDa band on the immunoblot (Fig. 2E).

Degus used for AQP determinations exhibited significantly higher *U* values during summer (2223.0±117.6 mosmol kg^{-1}) than in winter (794.0±166.06 mosmol kg^{-1}) ($F_{1,3}$=36.623, P=0.009). As expected, plasma osmolality (*p*) was constant throughout seasons, being 310.0±14.5 mosmol kg^{-1} in summer and 293.3±3.71 mosmol kg^{-1} in winter ($F_{1,3}$=1.202, P=0.353). Consequently, and as expected, the *U*/*p* ratio was significantly higher during summer (summer=7.58±0.31; winter=2.52±0.4; $F_{1,3}$=71.913, P=0.003).

Discussion

The environmental modification of an organism's physiology in the field is often hypothesized to be responsible for allowing organisms to adjust to changing spatial, as well as temporal, environmental conditions through increases in biological performance. Here, we reveal how the integration of flexible mechanisms acting at cellular, systemic and organismal levels allows a small desert-dwelling mammal to cope with seasonal water scarcity in its semi-arid habitat.

Degus live in open-scrub habitats subject to summer drought. Geographical and seasonal dietary differences in the relative proportions of shrub and grass foliage occur between localities. At the most arid and semi-arid localities, degus feed primarily on shrub foliage, seeds and conductive tissue. In Mediterranean environments, they feed primarily on foliage of forbs and grasses and on seeds. Thus, degus experience geographical and seasonal changes in the availability of water and nutrients in their food (Meserve, 1981; Meserve et al.

1983, 1998). Bozinovic (1995) indicated that in the dry summer months the plants consumed by degus (*Vulpea* sp. and *Erodium* sp.) contained nearly 60% neutral detergent fiber with a range of water content from approximately 3% to 6% (C. Veloso, personal communication), while during the wet winter these plant species contained 37% neutral detergent fiber and 70–80% water (Bozinovic and Torres-Contreras, 1998; C. Veloso, personal communication). Although small mammals such as degus may select sparsely distributed, high-quality plants or use coprophagy to recycle water and nutrients (Kenagy et al., 1999), during nutritional-water bottlenecks they must consume low-quality food out of necessity rather than choice (Bozinovic, 1995, 1997; Bozinovic et al., 1997; Torres-Contreras and Bozinovic, 1997). Indeed, the differences in seasonal water flux in *O. degus* are probably a product of differences in consumption of preformed water in plants. A similar phenomenon was reported in fat sand rats by Degen et al. (1991). Seasonal differences in water flux as a result of dietary shifts from a dry diet in summer to a relatively humid diet in winter and spring are common in degus, and such fluxes have also been reported in several Negev desert rodents (Degen et al., 1997). Also, water influx and efflux rates reflecting dietary preformed water were reported in two diurnal species, the pocket gopher *Thomomys bottae* and the antelope ground squirrel *Ammospermophilus leucurus*, by Gettinger (1984) and Karasov (1983), respectively. As observed in degus, the dietary items consumed by these rodents exhibited seasonal availability, thus influencing the homeostasis of the consumers.

Classical studies have traditionally viewed the capacity to concentrate urine as an indicator of the efficiency of water regulation (Schmidt-Nielsen, 1964; Abbott, 1971; Prosser, 1991; McNab, 2002), as well as an advantage for colonization and survival in deserts (Degen, 1997). According to MacMillen and Hinds (1983), this view is probably correct for rodents that lose body mass when deprived of water and are kept on a dry diet. Under such conditions, rodents should exercise maximal capabilities of water conservation, reflected in maximal urine osmolalities. Bozinovic et al. (1995) reviewed studies dealing with renal performance and maximal urine osmolality among rodent species inhabiting semixeric and xeric regions of Chile, southwestern USA and Australia. For the five species inhabiting semixeric and xeric habitats in Chile, the maximal capacity to concentrate urine (recorded under laboratory conditions) ranged from ~3.3 mosmol kg^{-1} in the fossorial octodontid rodent *Spalacopus cyanus* to nearly 4.5 mosmol kg^{-1} in the sigmodontine rodent *Phyllotis darwini.* The mean value for the species inhabiting the matorral was 4.138 mosmol kg^{-1}, a value similar to that found for nine western North American rodent species, including the wood rat *Neotoma albigula* (3.930 mosmol kg^{-1}). In the laboratory, the maximum U attained by degus is 4.338 mosmol kg^{-1} (Cortés et al., 1988). Our observed mean U during summer was 27.7% lower than the maximum U recorded in degus, probably because the diets used in experimental laboratory conditions were drier than those consumed by degus in the field.

What are the cellular mechanisms that allow degus to concentrate urine and to be able to save water during the dry season? AQP water channels are membrane-integral proteins that mediate facilitated water transport across cell membranes of a wide variety of cells, including epithelia. In the renal tubule, several AQPs are expressed with a very specific pattern. AQP-1 is expressed in apical and basolateral membranes of proximal tubule and thin descending limb cells (Nielsen et al., 1993a). AQP-2 is expressed in apical membrane of connecting, principal and inner medullary collecting duct cells of the distal nephron (Nielsen et al., 1993b). AQP-3 and AQP-4 are basolateral aquaporins expressed in the same cells that express AQP-2 (Ecelbarger et al., 1995; Terris et al., 1995). These water channels play a key physiological role in the urine-concentrating mechanism (Ma et al., 2000; Schnermann et al., 1998; Promeneur et al., 2000; Chou et al., 1998). The role of vasopressin in the urine-concentrating mechanism is related to the regulation of AQP-2 and AQP-3. Vasopressin regulates AQP-2 through an acute and long-term two-part mechanism. In the first part, vasopressin stimulates AQP-2 insertion in the apical membrane. In the second part, vasopressin increases AQP-2 gene transcription (Nielsen et al., 2002).

In *O. degus*, AQP-1 is expressed in apical and basolateral membranes of surface absorptive and crypt epithelium of the distal colon (Gallardo et al., 2002). Studies of water absorption in the distal colon of this rodent have shown that absorption is dramatically decreased by the presence of the mercurial agent, a known inhibitor of water channels. Thus, AQP-1 may be involved in water absorption and fecal dehydration (Gallardo et al., 2002), allowing water conservation in degus (Cortés et al., 1988). As far as we know, not much information is available concerning the regulation of AQPs in desert-dwelling rodents; in fact, this appears to be the first field study reporting AQP-2 acclimatization, but see Huang et al. (2001).

The degu kidney expresses several AQPs involved in the urine-concentrating mechanism: AQP-1, AQP-2 and AQP-3. Their distribution is quite similar to that described for the laboratory rat (P. A. Gallardo, unpublished results). Indeed, in this study, we observed that degus captured during the winter (i.e. with free access to water) do not express a high capacity for renal water reabsorption and excrete a dilute urine. On the other hand, degus captured during summer (i.e. during the dry season) have a limited supply of free water, feed on dry food (see above) and therefore must conserve water. Hence, during the summer, degus excrete a hyperosmotic urine.

The functional state of the urine-concentrating mechanism can be easily associated with the urine using the U/p ratio. The main concept of water balance is the regulation of plasma osmolality, which is achieved by the mechanism of thirst and renal water reabsorption. This mechanism is vasopressin dependent, and its activation results in the excretion of hypertonic urine. In fact, in degus captured during the dry season the U/p ratio was significantly higher than in winter degus. This increased U/p ratio also suggests that vasopressin levels are higher in animals from the dry season compared with those of the rainy season. This hormone should be measured in plasma and under field conditions.

At the molecular stage, high levels of vasopressin imply short- and long-term mechanisms of action. In fact, AQP-2 immunolabeling in the apical membrane, as well as AQP-2 protein levels, was increased in degus captured during summer; conversely, winter animals had less AQP-2 immunoreactivity and lower protein levels. Thus, the shift in vasopressin secretion, initially triggered by an increase in plasma osmolality, allows (together with other water-conserving mechanisms) the maintenance of the water balance through changes in the subcellular distribution and abundance of AQP-2. Although animals captured during the dry season did not attain the maximal urine osmolality recorded under artificial laboratory conditions, our results clearly demonstrate a degree of flexibility for the kidney, which allows degus to conserve water.

What are the implications of the flexibility of osmoregulation and AQP expression on survival? As mentioned before, since the semi-arid areas of Chile are characterized by a seasonal regime of temperature and precipitation, small mammals experience a strongly seasonal environment, with warm dry summers and cool wet winters. In this habitat, the populations of rodent species exhibit numerical fluctuations associated with high and low rainfall years (Yunger et al., 2002). Indeed, climate-related variables (mainly rainfall) exhibited more influence on reproductive variables than on survival.

From our study, we can suggest that the observed in-field phenotypic flexibility of water 'economy and management' at organismal, systemic and cellular levels of desert-dwelling rodents is critically important and may allow animals to successfully overcome the physiological challenges of arid environments. Consequently, the observed molecular and physiological flexibility reported here (which allow for a high water economy) may explain values of desert survival and colonization at the individual, as well as the population, level during low rainfall seasons and years in semi-arid habitats. In summary, the observed acclimatization for dealing with water economy may serve as a way to budget survival in the face of the hot, arid and unpredictable habitat of desert rodents.

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