

THE EFFECT OF WATER DEPRIVATION ON SIGNALLING MOLECULES THAT UTILISE cGMP IN THE SPINIFEX HOPPING MOUSE *NOTOMYS ALEXIS*

RACHEL A. HEIMEIER, RAY C. BARTOLO AND JOHN A. DONALD

Heimeier RA, Bartolo RC and Donald JA, 2004. The effect of water deprivation on signalling molecules that utilise cGMP in the spinifex hopping mouse *Notomys alexis*. *Australian Mammalogy* **26**: 191-198.

In mammals the natriuretic and guanylin peptides influence renal and intestinal fluid content and electrolyte transport by binding to and activating guanylyl cyclase (GC) receptors that in turn stimulate production of the intracellular second messenger guanosine 3':5'-cyclic monophosphate (cGMP). However, the role of natriuretic and guanylin peptides in desert mammals is not understood. The spinifex hopping-mouse (*Notomys alexis*), has a suite of behavioural and physiological mechanisms that permits survival for extended periods without access to free water. Because signalling molecules that generate cGMP are known to promote water excretion, it was predicted that natriuretic and guanylin peptide synthesis would be down regulated in water-deprived *N. alexis*, and thus reduce the amount of water lost in the urine and faeces. However, in the kidney ANP and GC-A mRNA levels were increased in water-deprived mice, but CNP and GC-B mRNA levels were decreased. Water deprivation increased guanylin and uroguanylin mRNA expression in the distal colon, but it remained unchanged in the kidney and proximal colon. The expression of GC-C mRNA increased in the proximal colon but not in the distal colon. This study shows that water deprivation differentially affects the expression of regulatory molecules that stimulate cGMP production, and that a down-regulation associated with water conservation does not uniformly occur.

Key words: cGMP, natriuretic peptides, guanylin peptides, *Notomys alexis*, kidney, colon.

RA Heimeier, RC Bartolo and JA Donald, School of Biological and Chemical Sciences, Deakin University, Geelong, Vic 3217, Australia. Email: heimeier@deakin.edu.au.

THE high temperatures and low humidity of desert regions pose problems for endothermic animals, because their high metabolism and body temperatures challenge water conservation. Despite this, some desert mammals can survive for extended periods without drinking by utilizing preformed water from food, and metabolic water that is produced upon the oxidation of the food (Degen 1997). The spinifex hopping mouse (*Notomys alexis*) is a small rodent that is well adapted for life in the deserts of central and western Australia. *N. alexis* has a range of behavioural and physiological adaptations that permit individuals to live for extended periods without access to free water (MacMillen and Lee 1969; Weaver *et al.* 1994). Two important physiological adaptations that enable *N. alexis* to conserve water are the production of highly concentrated urine and dry faeces. The production of concentrated urine is dependent on the regulation of two processes, ultrafiltration of plasma in the glomerulus and the reabsorption of water in the renal tubules. Accordingly, desert mammals have a reduced

glomerular filtration rate and enhanced tubular water reabsorption (Degen 1997). The ability of mammals to reabsorb water is dependent on the medullary osmotic gradient created by the loop of Henle, which drives the reabsorption of water from the collecting duct. A higher osmotic gradient enables more water to be reabsorbed, thus producing concentrated urine. *N. alexis* have elongated loops of Henle that can extend as far as the urethra and are therefore capable of excreting very concentrated urine. In fact, they have been reported to produce the most concentrated urine of any mammal (9370 mOsm/l) (MacMillen and Lee 1969; Hewitt 1981). Furthermore, the colon of *N. alexis* has a greater absorptive area than that of non-desert rodents, which facilitates increased water reabsorption and the production of relatively dry faeces. A larger surface area of the colon also serves to increase the metabolism of lipids, thereby producing more water (Degen 1997), which is seen as an important adaptation of desert mammals such as *N. alexis* (MacMillen and Lee 1969; Murray *et al.* 1995).

The role of vasopressin and renin-angiotensin systems in desert rodents

The endocrine regulation of fluid balance in desert rodents has focussed on the roles of vasopressin and the renin-angiotensin systems, because of their importance in regulating mechanisms that conserve water. In mammals, vasopressin plays a crucial role in the maintenance of plasma osmolality and blood volume. The posterior pituitary secretes vasopressin into the circulation when an increase in plasma osmolality and/or a decrease in blood volume are detected by specific receptors. Vasopressin binds to vasopressin 2 (V2) receptors in the collecting ducts of the kidney, which causes an increase in the reabsorption of water from the filtrate, thereby increasing the concentration of the urine; this results in the restoration of plasma osmolality and blood volume to normal levels. In comparison to non-desert rodents, the plasma levels of vasopressin in desert rodents such as the Cairo spiny mouse (*Acomys cahirinus*) and golden spiny mouse (*Acomys russatus*) (Castel *et al.* 1974), Egyptian gerbil (*Gerbillus gerbillus*) and lesser Egyptian jerboa (*Jaculus jaculus*) (El Hussein and Haggag 1974) and greater Egyptian jerboa (*Jaculus orientalis*) (Baddouri *et al.* 1984) are much higher. For example, the plasma vasopressin levels of *J. orientalis* are 100 - 200 times greater than those of a laboratory rat and twenty times higher than that of laboratory rats water-deprived for two days (Baddouri *et al.* 1984). *N. alexis* have a large posterior pituitary that contains three times the amount of vasopressin per unit of body weight as compared with a laboratory rat (Bridges and James 1982), indicating that *N. alexis* has a greater capacity to synthesise and release vasopressin. However, in *N. alexis* which were water-deprived for seven and 14 days, haematocrit and plasma osmolality did not (Table 1), which indicates that water deprivation did not cause dehydration in *N. alexis*. Therefore, the stimulus for vasopressin release is not increased in water-deprived animals (Heimeier *et al.* 2002; Donald and Bartolo 2003). We have recently shown that after three days of water deprivation, renal V2 receptor mRNA expression increases in *N. alexis* (Donald and Bartolo, unpubl. data), which may lead to an increase in the number of V2 receptors in the collecting ducts and an increased response to vasopressin. This finding suggests that in *N. alexis* the vasopressin system may be an important regulator of water balance during the early stages of water deprivation.

A decrease in blood volume stimulates the release of renin from the kidney, which converts angiotensinogen to angiotensin I. Angiotensin I is converted to angiotensin II, which mediates vasoconstriction and stimulates thirst and the release

of aldosterone from the adrenal glomerulosa, as well as vasopressin from the posterior pituitary. Aldosterone targets the distal tubule and collecting ducts of the kidney and increases the reabsorption of sodium. As water osmotically follows sodium, the retention of sodium also promotes water retention, and a subsequent increase in blood volume. The kangaroo rat (*Dipodomys spectabilis*) and the dryland desert gerbil (*Meriones unguiculatus*) have higher basal plasma angiotensin II levels than the laboratory rat. However, water deprivation for four days caused a five-fold (500%) increase in plasma angiotensin II in laboratory rats, but only a 50% increase in *D. spectabilis* and *M. unguiculatus* (Wright and Harding 1980). In addition, *N. alexis* have been shown to have higher basal levels of plasma renin and angiotensinogen than laboratory mice (Weaver *et al.* 1994). When *N. alexis* are water-deprived for seven days plasma angiotensinogen and renin concentrations increase, but after 28 days of water deprivation, the levels were not different from those measured in animals with access to water (Weaver *et al.* 1994). Higher basal plasma angiotensin II levels in desert rodents may lead to a decrease in blood flow to the kidney, thereby decreasing the glomerular filtration rate. It would also lead to higher plasma aldosterone and vasopressin levels, thus increasing water reabsorption in the kidney.

Therefore, it appears that the basal expression levels of the vasopressin and renin-angiotensin systems in desert mammals are much higher than those of non-desert mammals, which may be an adaptation for survival in xeric environments. However, when non-desert mammals are water-deprived they have a much greater capacity to up-regulate these hormone systems than desert-adapted mammals.

Period	Group	Hct (%)	PO (mOsm)
7 Days	Control	48.3 ± 1.3 (7)	341.7 ± 5.0 (7)
	WD	50.2 ± 0.6 (7) (<i>p</i> = 0.190)	336.0 ± 6.0 (7) (<i>p</i> = 0.544)
14 Days	Control	48.6 ± 1.8 (8)	346.0 ± 3.6 (8)
	WD	52.6 ± 2.6 (9) (<i>p</i> = 0.458)	342.3 ± 5.0 (9) (<i>p</i> = 0.544)

Table 1. Haematocrit (Hct) and plasma osmolality (PO) in control and water deprived (WD) *N. alexis*. Water deprivation periods were for three, seven and 14 days. Numbers in brackets represent the number in each study group. * indicates statistical significance (*p* ≤ 0.05) from control values

Cyclic GMP signalling systems and fluid homeostasis

Within the past two decades interest has arisen in two new regulatory molecules that control salt and water handling by the kidney and the intestine. The natriuretic and guanylin peptides are signalling

molecules that are activated by an increase in the extracellular fluid volume caused by excess salt and water. In mammals the peptides act by binding to and activating guanylyl cyclase (GC) receptors on the apical surface of target cells; three distinct domains are involved (Fig. 1). Upon peptide binding to the ligand-binding domain, the GC domain converts cytosolic guanosine triphosphate (GTP) to the intracellular second messenger, guanosine 3':5'-cyclic monophosphate (cGMP) (Lucas 2000). The natriuretic and guanylin peptides bind to specific GC receptors based on relative affinities for the ligand-binding domain of the GC receptor (Fig. 1). An increase in the production of cGMP leads to an increase in the excretion of water (diuresis) and salt (natriuresis) by the kidney and inhibits the absorption of water and salt by the intestine (Lucas 2000). Therefore, these peptides oppose the actions of the vasopressin and renin-angiotensin systems; this will be discussed below.

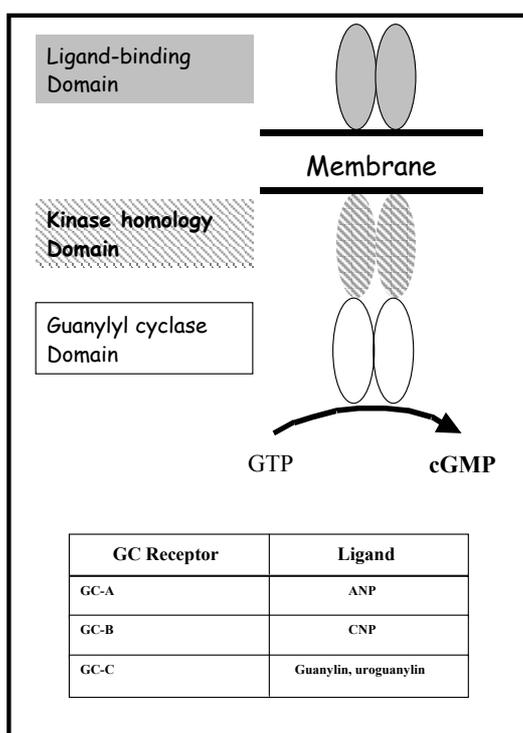


Fig. 1. General structure of the plasma membrane GC-receptor, including a list of the GC receptors and their ligands

Natriuretic peptides

The natriuretic peptide family includes atrial natriuretic peptide (ANP) (De Bold 1981), brain natriuretic peptide (Sudoh *et al.* 1988) and C-type natriuretic peptide (CNP) (Sudoh *et al.* 1989). In

mammals, atrial stretch caused by hypervolemia stimulates the release of ANP from the atria into the circulation (Espiner 1994; Takei 1999). ANP reduces blood volume and pressure by increasing renal natriuresis and diuresis, relaxing vascular smooth muscle, suppressing renin secretion and inhibiting vasopressin-mediated water reabsorption (Brenner *et al.* 1990; Takei 1999). ANP has also been identified in a number of tissues including the kidney. It is now believed that renal ANP may be more important than cardiac ANP as the regulator of sodium and water excretion (Goetz 1991). In addition to renal ANP, CNP is expressed in specific regions of the renal tubules and blood vessels. Therefore, it is now considered that natriuretic peptide regulation of renal function occurs via paracrine actions of endogenous ANP and CNP. The physiological effects induced by ANP and CNP are mediated via two particulate GC receptors, GC-A and GC-B, respectively. In the kidney, natriuresis and diuresis occurs due to a combination of effects in the glomerulus and renal tubules. In addition, ANP, CNP and their receptors are present in the intestine and have been shown to inhibit the absorption of water, sodium and chloride (Bosc *et al.* 2000).

Very little is known about the expression and function of the natriuretic peptide system in desert mammals. One study found that the levels of cardiac and plasma ANP in two North African desert rodents, the fat sand rat (*Psammomys obesus*) and the Libyan jird (*Meriones libycus*), were lower in comparison to laboratory rats (Lacas *et al.* 1998). In addition, ANP levels in various tissues such as the kidney, adrenal gland and liver, are lower in *P. obesus* compared to the laboratory rat (Bachar and Lichstein 1993), which suggests that ANP-mediated diuresis and natriuresis are lower in desert rodents. This situation may be an appropriate adaptation to survival in the absence of free water. In a follow up study, Lacas *et al.* (2000) observed that water deprivation for eight days increased cardiac ANP, but decreased plasma ANP levels in the slender gerbil (*Taterillus gracillus*) which is a desert rodent. However, in another species, the northwestern fat mouse (*Steatomys caurinus*), cardiac and plasma ANP levels were unaffected by water deprivation. As natriuretic peptide regulation of renal function may occur independently of cardiac and plasma ANP, it is important to examine the endogenous peptides of the kidney rather than the heart alone.

Guanylin and uroguanylin peptides

Activation of guanylyl cyclase C (GC-C) receptors in the colon by heat stable enterotoxins leads to the secretion of chloride and bicarbonate ions, causing severe diarrhoea (Field *et al.* 1989; Joo *et al.* 1998). Currie *et al.* (1992) performed cGMP bioassays in

search of a potential ligand that activated intestinal GC-C, and showed that rat jejunum and kidney tissue extracts contained a material that was called guanylin. As the kidney contained GC-C receptors and kidney extracts stimulated GC-C activity, it was then thought that the urine would also contain guanylin. Using a cGMP bioassay, the bioactivity of opossum urine was tested and it was found to contain a peptide that stimulated GC-C resulting in an increase in intracellular cGMP (Hamra *et al.* 1993). The peptide was related to guanylin and was subsequently called uroguanylin based on its site of discovery (Hamra *et al.* 1993).

The physiological actions of guanylin peptides (guanylin and uroguanylin) in the intestine are to regulate the secretion of chloride and bicarbonate ions into the lumen during digestion. Increasing the secretion of these ions neutralizes hydrochloric acid in the small intestine, and organic acids produced by enteric bacteria in the colon (Joo *et al.* 1998). Guanylin peptides can also inhibit the absorption of salt and water throughout the gastrointestinal tract (Forte *et al.* 1999). Uroguanylin is released into the blood stream after salt is ingested and it is also released locally from the cells lining the renal tubules (Fan *et al.* 1997; Forte and Hamra 1996). Guanylin is also produced in the kidney but it appears that uroguanylin is the predominant peptide mediating salt and water excretion from the kidney (Forte *et al.* 2000).

Study rationale

In mammals that live in desert environments, it may be predicted that the natriuretic and guanylin peptide systems that generate cGMP would be down-regulated when water is limited or absent because cGMP activates cellular processes that promote water excretion. However, the role of the peptides in fluid homeostasis in desert species is yet to be determined. To address this we have been examining the effect of water deprivation on the mRNA expression of the natriuretic and guanylin peptide systems in *N. alexis*. The transcription of mRNA is an indicator of the activity of the signalling systems because mRNA production reflects the protein levels of the signalling molecules and their receptors.

MATERIALS AND METHODS

Water deprivation was performed for seven and 14 days on a 12:12 h photoperiod. For the water deprivation experiment, *N. alexis* were housed in sand filled glass aquaria that provided a medium for burrowing and were fed fresh millet seed daily. Prior to the commencement of each study sexually mature *N. alexis* were selected and placed into groups of four in order for the animals to familiarise themselves with the new housing and the other animals. All

animals in each group were of the same sex and there was a control (access to water) and experimental group of each sex. For individual identification animals were ear tagged. The animals were weighed on a daily basis and their health was assessed. The temperature was kept constant at 21°C, and the relative humidity was between 45 - 60%.

The effect of water deprivation on mRNA expression was performed using a semi-quantitative polymerase chain reaction (PCR), which detects changes in mRNA expression by the incorporation of dCTP α -³²P into PCR products. As dCTP α -³²P is randomly incorporated into the PCR products, the amount of radiation emitted by a PCR product can be used to detect changes in mRNA expression. Quantification of the mRNA was determined by normalising the levels of amplification of the gene of interest against that of a house-keeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Stuzenbaum and Kille 2001). The mean expression of mRNA in control *N. alexis* were adjusted to represent 100%, \pm standard error (SE) and the mean level of mRNA expression in WD *N. alexis* was calculated as a percentage of the control, \pm SE.

RESULTS AND DISCUSSION

During water deprivation *N. alexis* steadily lose weight until about day nine, when weight stabilises until day 14 (Fig. 2). Longer-term water deprivation studies have shown that *N. alexis* will begin to increase body weight after 14 days to pre-deprivation levels (Weaver *et al.* 1994; Heimeier, 2004). Table 1 shows the haematocrit and plasma osmolality data from control and water deprived *N. alexis* after seven and 14 days of water deprivation; no significant difference was observed between water-deprived and control animals.

Natriuretic peptide system

In the kidney the response to water deprivation was ligand/receptor specific, because the mRNA expression of ANP/GC-A and CNP/GC-B were affected differentially (Heimeier *et al.* 2002; Heimeier and Donald 2003). Water deprivation for seven and 14 days had no effect on ANP mRNA expression in the heart but the expression of ANP mRNA in the kidney was clearly affected; however, it did not show a consistent change (Fig. 3). Seven days of water deprivation significantly increased renal ANP mRNA expression, while 14 days of water deprivation decreased the expression of renal ANP mRNA (Heimeier *et al.* 2002). These data suggest that there is differential transcriptional control of cardiac and renal ANP mRNA expression during water deprivation and that the renal ANP system may be primarily responsible for regulating osmotic balance. The increase in renal ANP mRNA

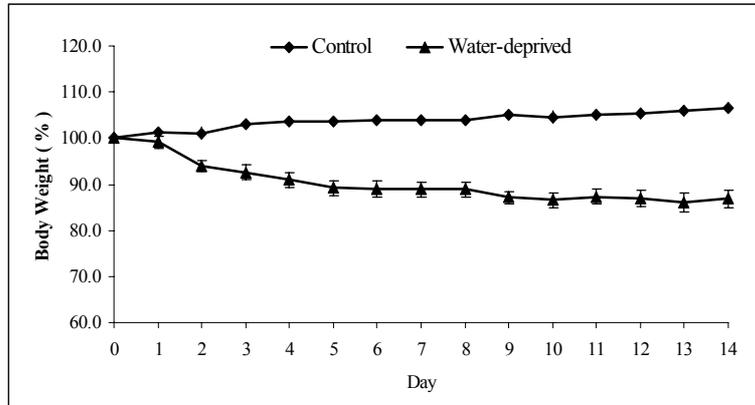


Fig. 2. Percentage weight variation of *N. alexis* during the 14-day water deprivation study period, values expressed as means \pm SE.

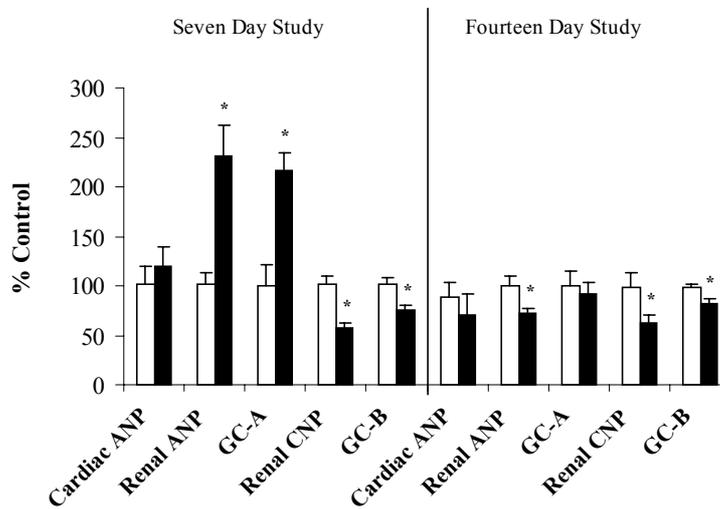


Fig. 3. Histograms showing the relative level (% of total change of mRNA expression) of cardiac and renal ANP, renal GC-A (A), renal CNP and renal GC-B (B) mRNA in seven and 14 day water-deprived *N. alexis* compared with controls. The ANP, GC-A, CNP and GC-B to GAPDH ratios for the control animals were set at 100%. * indicates statistical significance ($p \leq 0.05$) from control values. After seven days of water deprivation there was a significant increase in renal ANP and GC-A mRNA expression and a significant decrease in renal CNP and GC-B mRNA expression. After 14 days of water deprivation there was a significant decrease in renal ANP, CNP and GC-B mRNA expression.

expression after seven days of water deprivation suggests that there is an increase in the production of ANP in the kidney. This result is surprising because a water-deprived animal would benefit by reducing urinary water loss. In addition to ANP there is also an increase in GC-A mRNA expression in the kidney after seven days of water deprivation. However, after 14 days of water deprivation the GC-A mRNA levels remain the same as control animals with access to water. These data show that transcriptional regulation of GC-A mRNA is also affected by the early stages of water deprivation. In addition, the ability of ANP to stimulate cGMP production in kidney membranes is significantly up-regulated after seven days of water deprivation, as compared with control animals with

access to water (data not shown). Taken together, the increase in ANP/GC-A mRNA expression and ANP-mediated GC activity after seven days of water deprivation was in the opposite direction to what was predicted, which indicates that down-regulation of this system is not part of the physiological response to an absence of free water. In contrast, the decrease in ANP mRNA expression after 14 days of water deprivation may reflect a reduction in ANP mediated effects in the kidney during extended water deprivation.

The response of the renal CNP/GC-B system in *N. alexis* following seven and 14 days of water deprivation showed that both CNP and GC-B mRNA

expression were down regulated (Fig. 3) (Heimeier and Donald 2003). In non-desert animals, the physiological role of the CNP/GC-B system in the kidney is not fully understood, but it appears to be important for regulating renal blood flow (Bonhomme *et al.* 1998). During periods of water deprivation a decrease in CNP/GC-B mRNA levels may reflect a reduction in the CNP tone influencing blood flow to the glomeruli and inner medulla. This would lead to a reduction in blood flow that would decrease the glomerular filtration rate, and consequently urine production. In addition, a reduction in blood flow to the inner medulla could be important in maintaining the medullary osmotic gradient, thereby ensuring that osmolytes are not removed from the inner medulla (Cowley 1997).

Guanylin system

Guanylin and uroguanylin mRNA expression were found in the proximal and distal colon, caecum, small intestine, kidney and heart. The expression of GC-C mRNA was found in the small intestine, caecum, and the proximal and distal colon, but not in the heart and kidney. The absence of GC-C mRNA expression in the kidney was surprising since GC-C expression has been clearly demonstrated in the rat kidney (Carrithers *et al.* 2000). Given that both guanylin and uroguanylin are expressed in the kidney of *N. alexis*, further analysis on the presence or absence of GC-C proteins in the kidney is required.

Notomys alexis water-deprived for seven days showed an increase in the expression of guanylin mRNA in the proximal and distal colon but an increase in uroguanylin mRNA expression was only observed in the distal colon (Fig. 4). An increase in

guanylin and uroguanylin mRNA expression suggests that there is also an increase in the production of guanylin and uroguanylin peptides in the distal colon, which may compromise the ability of the colonic epithelium to reduce water loss in the faeces. In contrast, GC-C mRNA expression was significantly increased in the proximal colon but no change was observed in the distal colon (Fig. 4). Thus, it appears that in *N. alexis* there is differential regulation of guanylin peptides and the GC-C receptor in the colon following seven days of water-deprivation (Donald and Bartolo 2003).

It was predicted that there would be a decrease in renal guanylin and uroguanylin mRNA expression to reduce the level of cGMP-mediated excretion of salt and water, if indeed GC-C proteins are present in the kidney of *N. alexis* (see above). However, the expression of guanylin and uroguanylin mRNAs in the kidney was not affected by water deprivation when compared to control animals with access to water. This result is consistent with data from a water-deprivation experiment in laboratory mice in which no effect on renal guanylin and uroguanylin expression was found (Potthast *et al.* 2001). However, a salt load in drinking water mediated an increase in renal uroguanylin mRNA expression but not guanylin expression (Potthast *et al.* 2001). Salt loads given in drinking water cause dehydration by increasing plasma osmolality. During water deprivation, the plasma osmolality of *N. alexis* does not increase from control animals with access to water, thus the stimulus that causes changes in uroguanylin expression is absent (Donald and Bartolo 2003).

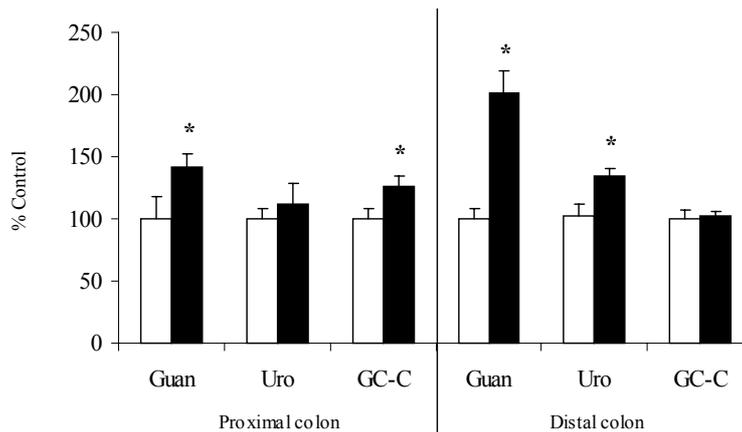


Fig. 4. Histogram showing the relative level of mRNA expression of guanylin (Guan), uroguanylin (Uro) and GC-C in the proximal colon and distal colon of control and seven day water-deprived *N. alexis*. The guanylin, uroguanylin or GC-C to GAPDH ratios for the control mice were set at 100%. * indicates statistical significance ($p \leq 0.05$) from control values. Significant increases in guanylin and uroguanylin mRNA expression were found in the distal colon but only guanylin and GC-C mRNA expression was significantly increased in the proximal colon.

In conclusion, it is known that in mammals regulatory molecules that activate GC receptors and generate cGMP cause an increase in the excretion of water from the kidney and colon. Therefore, we had expected that the expression of two peptide families that signal via cGMP would be down-regulated in *N. alexis*, during water deprivation. However, our data show that a uniform down-regulation does not occur, and in fact some peptides and receptors are up-regulated. However, it must also be considered that other peptide systems could be acting to conserve water during periods of water deprivation. Clearly, further research is required to precisely elucidate the biological role of the natriuretic and guanylin peptides in desert mammals such as *N. alexis*.

REFERENCES

- BACHAR H AND LICHTEN D, 1993. Distribution of atrial natriuretic peptides in the sand rat (*Psammomys obesus*) in comparison to that in the rat. *Journal of Basic and Clinical Physiology and Pharmacology* **4**: 47-56.
- BADDOURI K, BUTLEN D, IMBERT-TEBOUL M, BOUFFANT FLE, MARCHETTI J, CHARBARDES D AND MOREL F, 1984. Plasma antidiuretic hormone levels and kidney responsiveness to vasopressin in the jerboa, *Jaculus orientalis*. *General and Comparative Endocrinology* **54**: 203-215.
- BONHOMME MC, GROVE KL, CARON S, CRILLEY CT, THIBAUT G, DESCHEPPER CF AND GARCIA R, 1998. Immunolocalisation of natriuretic peptide receptor B in the rat kidney. *Journal of the American Society of Nephrology* **9**: 1777-1786.
- BOSC LVG, MAJOWICZ MP AND VIDAL NA, 2000. Effects of atrial natriuretic peptide in the gut. *Peptides* **21**: 875-887.
- BRENNER BM, BALLERMAN BJ, GUNNING ME AND ZEIDEL ML, 1990. Diverse biological actions of atrial natriuretic peptide. *Physiological Reviews* **70**: 665-699.
- BRIDGES T AND JAMES N, 1982. The hypothalamo-neurohypophysial system of native Australian desert rodents. The vasopressin and oxytocin contents of hypothalamus and posterior pituitary of *Notomys alexis* and *Pseudomys australis* compared with those of the laboratory rat and mouse in different states of water balance. *Australian Journal of Biological Medical Science* **60**: 265-283.
- CARRITHERS SL, TAYLOR B, CAI WY, JOHNSON BR, OTT CE, GREENBERG RN AND JACKSON BA, 2000. Guanylyl cyclase-C receptor mRNA distribution along the rat nephron. *Regulatory Peptides* **95**: 65-74.
- CASTEL M, BORUT A AND HAINES H, 1974. Blood titres of vasopressin in various murids (Mammalian: Rodentia). *Israel Journal of Zoology* **23**: 208-209.
- COWLEY AW, 1997. Role of the renal medulla in volume and arterial pressure regulation. *American Journal of Physiology* **273**: R1-R15.
- CURRIE MG, FOK KF, KOTO J, MOORE RJ, HAMRA FK, DUFFIN KL AND SMITH CE, 1992. Guanylin: an endogenous activator of intestinal guanylate cyclase. *Proceedings of the National Academy of Sciences of the United States of America* **89**: 947-951.
- DE BOLD AJ, 1981. Tissue fractionation studies on the relationship between an atrial natriuretic factor and specific atrial granules. *Canadian Journal of Physiology and Pharmacology* **60**: 324-330.
- DEGAN AA, 1997. *Ecophysiology of small desert mammals*. Springer-Verlag: Germany.
- DONALD JA AND BARTOLO RC 2003. Cloning and mRNA expression of guanylin, uroguanylin, and guanylyl cyclase C in the Spinifex Hopping mouse, *Notomys alexis*. *General and Comparative Endocrinology* **132**: 171-179.
- EL HUSSEINI M AND HAGGAG G, 1974. Antidiuretic hormone and water conservation in desert rodents. *Comparative Biochemical Physiology* **47**: 347-350.
- ESPINER EA, 1994. Physiology of natriuretic peptides. *Journal of Internal Medicine* **235**: 527-541.
- FAN X, WANG Y, LONDON RM, EBER SL, KRAUSE WJ, FREEMAN RH AND FORTE LR, 1997. Signalling pathways for guanylin and uroguanylin in the digestive, renal, central nervous, reproductive, and lymphoid systems. *Endocrinology* **138**: 4636-4648.
- FIELD M, RAO MC AND CHANG B, 1989. Intestinal electrolyte transport and diarrheal disease. *New England Journal of Medicine* **321**: 879-883.
- FORTE LR, FREEMAN RH, KRAUSE WJ AND LONDON RM, 1999. Guanylin peptides: cyclic GMP signalling mechanisms. *Brazilian Journal of Medical and Biological Research* **32**: 1329-1336.
- FORTE LR AND HAMRA FK, 1996. Guanylin and Uroguanylin: Intestinal peptide hormones that regulate epithelial transport. *News Physiological Science* **11**: 17-24.
- FORTE LR, LONDON RM, KRAUSE WJ AND FREEMAN RH, 2000. Mechanisms of guanylin action via cyclic GMP in the kidney. *Annual Review of Physiology* **62**: 673-695.
- GOETZ KL, 1991. Renal natriuretic peptide (urodilatin?) and atriopeptin: Evolving concepts. *American Journal of Physiology* **261**: F921-F932.

- HAMRA KF, FORTE LR, EBER SL, PIDHORODECKYJ NV, KRAUSE WJ, FREEMAN RH, CHIN DT, TOMPKINS JA, FOK KF AND SMITH CE, 1993. Uroguanylin: structure and activity of a second endogenous peptide that stimulates intestinal guanylate cyclase. *Proceedings of the National Academy of Sciences of the United States of America* **90**: 10464-10468.
- HEIMEIR RA, 2004. Natriuretic peptides: Do they regulate water balance in desert-adapted rodents? Ph.D. thesis, Deakin University, Victoria.
- HEIMEIR RA, DAVIS BJ AND DONALD JA, 2002. The effect of water deprivation on the expression of atrial natriuretic peptide and its receptors in the Spinifex Hopping mouse, *Notomys alexis*. *Comparative Biochemistry and Physiology* **132A**: 893-903.
- HEIMEIR RA AND DONALD JA, 2003. Renal C-type natriuretic peptide and natriuretic peptide receptor B mRNA expression are affected by water deprivation in the Spinifex Hopping mouse. *Comparative Biochemistry and Physiology* **136A**: 565-575.
- HEWITT S, 1981. Plasticity of renal function in the Australian desert rodent *Notomys alexis*. *Comparative Biochemistry and Physiology* **69A**: 297-304.
- JOO NS, LONDON RM, KIM HD, FORTE LR AND CLARKE LL, 1998. Regulation of intestinal Cl⁻ and HCO₃⁻ secretion by uroguanylin. *American Journal of Physiology* **274**: 633-644.
- LACAS S, ALLEVARD AM, AG'ATTEININE S, GALLO-BONA N, GAUQUELIN-KOCH G, HARDIN-POUZET H, GHARIB C, SICARD B AND MAUREL D, 2000. Cardiac natriuretic peptide response to water restriction in the hormonal adaptation of two semidesert rodents from west Africa (*Steatomys caurinus*, *Taterillus gracilis*). *General and Comparative Endocrinology* **120**: 176-189.
- LACAS S, BENTCHIKOU M, GAGRION J, GALLO-BONA N, GAUQUELIN-KOCH G, GHARIB C AND ALLEVARD AM 1998. Presence of atrial natriuretic peptide in two desert rodents - comparison with rat. *Peptides* **19**: 715-726.
- LUCAS KPS, KEZEROUNIAN S, RUIZ-STEWART I, PARK J, SCHULZ S, CHEPENIK K AND WALDMAN S, 2000. Guanylyl cyclases and signalling by cyclic GMP. *Pharmacological Reviews* **52**: 375-413.
- MACMILLEN RE AND LEE AK, 1969. Water metabolism of Australian hopping mice. *Comparative Biochemistry and Physiology* **28**: 493-514.
- MURRAY BR, HUME ID AND DICKMAN CR, 1995. Digestive tract characteristics of the Spinifex Hopping-mouse, *Notomys alexis* and the Sandy Inland mouse, *Pseudomys hermannsburgensis* in relation to diet. *Australian Mammalogy* **18**: 93-97.
- POTTHAST R, EHLER E, SCHEVING LA, SINDIC A, SCHLATTER E AND KUHN M, 2001. High salt intake increases uroguanylin expression in mouse kidney. *Endocrinology* **142**: 3087-3097.
- SUDOH T, KANGAWA K, MINAMINO N AND MATSUI H, 1988. A new natriuretic peptide in porcine brain. *Nature* **332**: 78-81.
- STURZENBAUM SR AND KILLE P, 2001. Control genes in quantitative molecular biological techniques: the variability of invariance. *Comparative Biochemistry and Physiology* **130B**: 281-289.
- SUDOH T, MAEKAWA K, KOJIMA N, KANGAWA K AND MATSUI H, 1989. Cloning and sequence analysis of cDNA encoding a precursor for human brain natriuretic peptide. *Biochemical and Biophysical Research Communications* **159**: 1427-1434.
- TALEI Y, 1999. Structural and functional evolution of the natriuretic peptide system in vertebrates. *International Review of Cytology* **194**: 1-66.
- WATTS CHS AND ASLIN HJ, 1981. *The rodents of Australia*. Angus and Robertson: Australia.
- WEAVER D, WALKER L, ALCORN D AND SKINNER S 1994. The contributions of renin and vasopressin to the adaptation of the Australian Spinifex Hopping-mouse (*Notomys alexis*) to free water deprivation. *Comparative Biochemistry and Physiology* **108A**: 107-116.
- WITHERS PC, LEE AK AND MARTIN RW, 1979. Metabolism, respiration and evaporative water loss in the Australian Hopping-mouse *Notomys alexis* (Rodentia: Muridae). *Australian Journal of Zoology* **27**: 195-204.
- WRIGHT JW AND HARDING JW, 1980. Body dehydration in xeric adapted rodents: Does the renin-angiotensin system play a role? *Comparative Biochemistry and Physiology* **66A**: 181-188.