

Antidiuretic responses to osmotic cutaneous stimulation in the toad, *Bufo arenarum*

A possible adaptive control mechanism for urine production

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Summary. Osmotic stimulation of the skin of the toad *Bufo arenarum* with isotonic (115 mM) or hypertonic (400 mM) NaCl solutions produced a marked and reversible antidiuresis within 5 min. No changes in plasma osmolarity were detected in the course of this response.

Hypophysectomized animals exhibited a lower and delayed antidiuresis when exposed to a hypertonic environment (400 mM NaCl). This antidiuretic response was drastically reduced in normal toads after ten consecutive days of administration of the sympatoplexic guanethidine.

The existence of a feed-forward control of urine production initiated by cutaneous osmotic sensors and involving an adrenergic component is proposed.

Key words: Antidiuresis – Reflex – Control – Adaptive – Toad

Introduction

The model of the control of water and electrolytes in vertebrates is based primarily on the concept of error detection in the corresponding parameters of the internal environment (Fitzsimmons 1980; McFarland 1971). According to this model, changes in plasma osmolarity, sodium concentration or blood volume (or a combination of these) are detected by central or peripheral sensors, producing the signal error that actuates different organs (hypothalamus, neurohypophysis, adrenals, kidneys, etc.) and promotes the release of hormones

that tend to correct the shift (Rolls and Rolls 1981; Sawyer and Pang 1975).

Data of different authors (Gallardo et al. 1980; Segura et al. 1982a, b, 1984, 1987; Yokota and Hillman 1984; Reboreda et al. 1987) have shown that the central nervous system has a substantial role in the water balance of anurans. Adrenergic factors are involved in the control of glomerular and tubular mechanisms of urine production in *Rana catesbeiana* (Gallardo et al. 1980). In previous papers the existence of central and peripheral nervous components in the regulation of the water economy of the toad, *Bufo arenarum* Hensel has been clearly established (Segura et al. 1982, a, b; 1984; 1987). Moreover, Reboreda and Segura (1984) also described the remarkable sensitivity of the skin of *B. arenarum* to salt solutions.

This evidence led us to analyse a possible feed-forward control of water uptake and urine production in the toad *Bufo arenarum* (Petriella et al. 1986).

This paper describes a series of experiments to investigate the existence of a reflex control of urine production by the kidney in response to changes in the osmolarity of the external environment in the toad, *Bufo arenarum*. The role played by the adrenergic and the hypophyseal system in this response is also analysed.

Material and methods

Adult male toads (*Bufo arenarum*), weighing 90–150 g were collected near Buenos Aires and used between 1 and 3 weeks after capture. During this period they were kept in large cages with free access to water. They were transferred 24 h before the experiments to opaque individual plastic boxes (15 × 15 × 10 cm) containing 150 ml of distilled water. The following groups were studied:

Group 1. Normal: Animals adapted to distilled water and then successively maintained for 30 min in the following media: (a)

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distilled water ($n=7$), (b) 115 mM NaCl ($n=7$), and (c) 400 mM NaCl ($n=10$).

Group 2. Normal recovery ($n=8$). The following sequence was used to assess complete recovery after osmotic stimulation of the skin: (a) distilled water, 30 min immersion, (b) 400 mM NaCl 30 min immersion, and (c) return to distilled water until complete recovery of the control rate of urine production.

Group 3. Normal animals submitted to sequence a–b of Group 2 and to simultaneous measurement of plasma osmolarity every 10 min (see Fig. 2).

Group 4. Hypophysectomized ($n=11$): animals adapted to distilled water and then submitted to the sequence a–b of Group 2.

Group 5. Guanethidine treated groups. Animals received single doses of $10 \text{ mg} \cdot \text{kg}^{-1}$ subcutaneously of Ismelin (Ciba) for 10 consecutive days. Urine production was then measured after 30 min in distilled water ($n=4$), or in 400 mM NaCl ($n=5$).

The animals were undisturbed in the experimental boxes and the changes of solutions were performed manually by means of a simple hydraulic mechanism.

Surgery. Toads were deeply anesthetized with MS222 (tricaine metanosulphonate, Sigma), $0.3 \text{ mg} \cdot \text{kg}^{-1}$ subcutaneously. Both ureters were exposed laterally to the urostyle. Polyethylene canulae (1.3 mm external bore) were placed into the ureters and sutured to muscle and skin. Hypophysectomies and retrochiasmatic sections were made as described previously (Segura et al. 1982, a).

Animals were not used in experiments for at least 24 h after ureteral cannulation or 10 days after hypophysectomy. There is clear evidence that in the latter case, a 10-day period is sufficient for complete atrophy of the ADH-producing system in anurans (Shoemaker and Waring 1968; Rodriguez and Dellman 1970).

Plasma osmolarity measurements. Plasma osmolarity was measured with a vapour pressure osmometer (Wescor 5500). Blood samples were obtained from 11 intact toads 0, 10, 20 and 30 min after distilled water had been replaced by (a) distilled water ($n=6$) or (b) 400 mM NaCl ($n=5$).

These samples (50–70 μl) were taken through a polyethylene cannula (1 mm external bore) placed in the dorsal aorta (lateral cannulation). The blood was heparinized with Lique-mine (Roche) at a final concentration of 50 U/ml and then centrifuged for 15 min at 1000 rpm.

Analysis of results. All results are presented as mean $\pm$ standard error of the mean. Comparisons between and within groups were made using two-way or one-way analysis of variance for repeated measures (Keppel 1973), respectively. Comparisons were made using the Scheffe test.

Results

The changes in urine production ($\text{ml}/100 \text{ g} \cdot \text{h}^{-1}$) following the change in the osmolarity of the external bath are illustrated in Fig. 1. During the period in distilled water the animals exhibited a stable diuresis (C). The replacement of water by a 400 mM NaCl solution induced a significant decrease in urine production (54.2%; $P<0.01$) within 10 min, and a maximum reduction of 84.3%

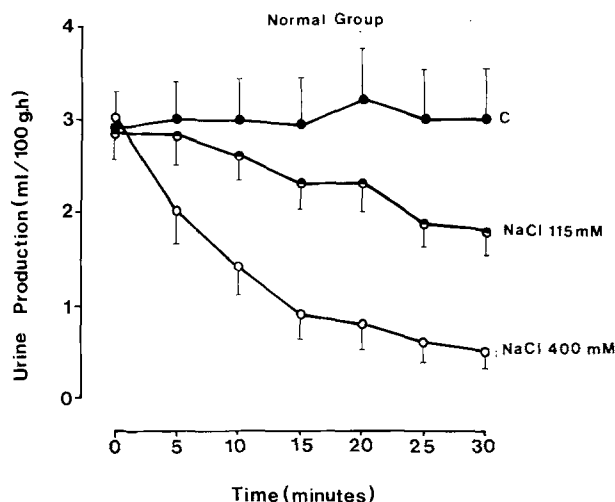


Fig. 1. Responses of urine production to changes in the osmolarity of the external medium in the intact toad. C control in distilled water. In the other groups the water was exchanged for other solutions indicated at time 0. Note that a significant decline of urine production takes place within 5–15 min after bath replacement in the experimental groups. See text for further information

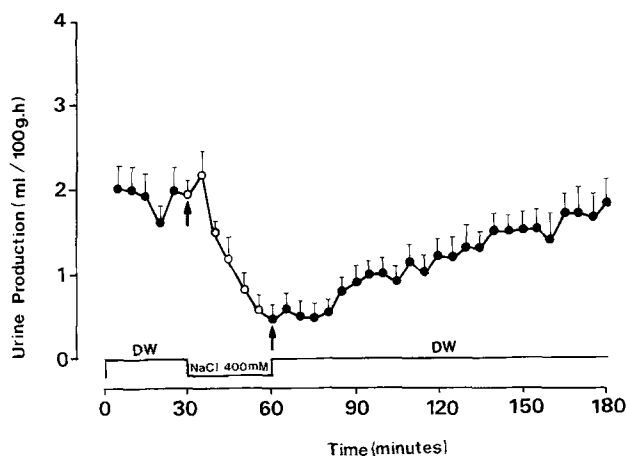


Fig. 2. Response of urine production to a change of the external medium from distilled water (DW) to 400 mM NaCl and the subsequent recovery

in 30 min ($P<0.01$) in intact toads. It should be noted that the replacement of distilled water by isotonic (115 mM) NaCl also resulted in a significant antidiuresis in normals. A decrease in urine production of 22.7% ($P<0.01$) was observed 15 min after the change (Fig. 1, NaCl 115 mM) and a maximum of 39.1% ($P<0.01$) at 30 min.

The reversibility of these effects is illustrated in Fig. 2, where the changes in the urine flow of a group of normal animals following transfer from distilled water to 400 mM NaCl and back are shown. The recovery of normal levels of urine production within 2 h of the return to distilled water

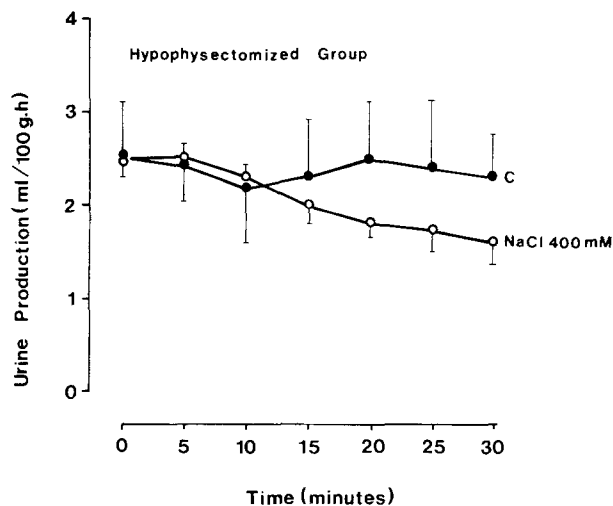


Fig. 3. Response of urine production to changes in the osmolarity of the external medium in the hypophysectomized toad. A significant decrease in production takes place 20 min after bath replacement at time 0. Note the delay of this response when compared with those of intact animals showed in Fig. 1. C control in distilled water

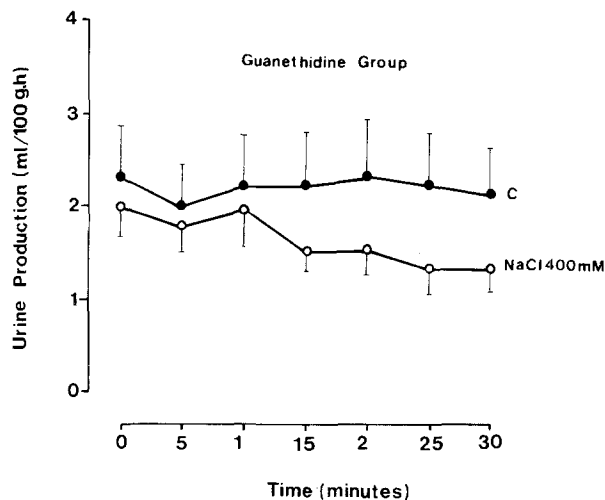


Fig. 5. Absence of antidiuretic response to cutaneous stimulation with 400 mM NaCl in toads after 10 consecutive days of treatment with guanethidine $10 \text{ mg} \cdot \text{kg}^{-1}$ subcutaneously. C, control in distilled water

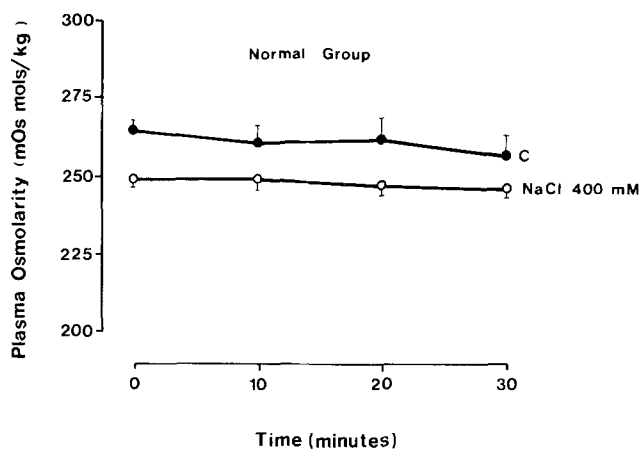


Fig. 4. Temporal course of the plasma osmolarity of intact toads submitted to rapid changes in the osmolarity of the external medium from distilled water to 400 mM NaCl at time 0. Note that the plasma osmolarity remains constant both in distilled water (C) and in 400 mM NaCl. Ordinates: plasma osmolarity in mosmoles. Abscissae: time in minutes

is clear. When hypophysectomized animals were moved from distilled water to 400 mM NaCl a significant, although less marked, oliguria was also observed (Fig. 3). In this experiment the decreases of urine production were 23.9% ($P < 0.01$) within the first 20 min, and 32.3% ($P < 0.01$) 30 min after the bath was changed. As shown in Fig. 4, no difference was observed when the plasma osmolarity of animals maintained in distilled water (C) was compared with that exhibited by animals in NaCl

400 mM. No body weight decreases greater than 3% of initial values were observed.

No significant changes were detected in urine production when guanethidine-treated animals were successively exposed to distilled water (C) or to 400 mM NaCl (Fig. 5).

Discussion

The possible involvement of nervous factors in the control of the water economy of anurans has been considered for a long time. However, the experimental evidence has always been indirect and obtained under extreme physiological conditions. The drastic increases in the uptake of water observed after midbrain lesions in *Rana catesbeiana* (Adolph 1934) and in *Bufo arenarum* Hensel (Segura et al. 1982a), for instance, are strong arguments for this hypothesis. Even more persuasive are the experiments involving the reversible blocking of brain areas, the midbrain tegmentum in particular, with microinjections of 1 M KCl, which induce massive water intake and antidiuresis both in normal and hypophysectomized animals (Segura et al. 1982a). Furthermore, a series of clear demonstrations of the basic role played by brain structures in the control of water uptake across the skin and urine production have also been provided (Segura et al. 1982b; 1984; 1987). All these data suggest the existence of a neuroneural or neuroendocrine mechanism controlling the water economy of the toad.

The hypothesis of a feed-forward loop of control initiated in the skin and allowing the animal to anticipate a lack of water also appeared particularly attractive (Segura et al. 1986).

It is essential to design an experiment which preserves the functional integrity of the preparation. To accomplish this unanesthetized animals were employed and rapid changes in the osmolarity of the environment were used as stimuli. The anti-diuresis observed in this way was striking for its rapid development and its magnitude. The effectiveness of both hyperosmotic and iso-osmotic stimuli, in triggering this response was also clear.

The first and most simple explanation is to consider the existence of a neuroendocrine mechanism involving the ADH producing system in the efferent pathway. However, the persistence of the anti-diuretic response, even after total hypophysectomy, demonstrates the need for an alternative explanation.

Clear evidence of adrenergic innervation of the anuran kidney was provided by histochemical techniques (Morris and Gibbins 1983). Using *Bufo marinus* they were able to demonstrate the existence of a dense network of sympathetic fibres covering all preglomerular vessels, from the level of the dorsal aorta to the termination of each afferent arteriole at the glomerular vascular pole. In addition, histological studies by Tsuneki et al. (1984) established the existence of nerve fibres in close contact with the epithelium of renal tubules in *Rana catesbeiana*.

Concurrently, both electrical stimulation and arterial perfusion with catecholamines have a remarkable effect on renal vascular resistance in different species of anurans (Richards and Schmidt 1924; Gallardo et al. 1980; Morris 1983). Moreover, removal of tonic renal vasoconstriction by destruction or blockade of the renal nerves also results in increased glomerular flow and diuresis (Richards and Schmidt 1924; Gallardo et al. 1980). On this basis, the possible existence of a sympathetic efferent component in the production of the phenomenon was considered. This presumption was reinforced when the sustained oliguresis induced by osmotic stimulation of the skin was suppressed by guanethidine administration. Both glomeruli and tubuli could be involved in this adrenergic mechanism, since Mayer (1969) observed decreases in both glomerular filtration rate and tubular water reabsorption in frogs after a long exposure to a saline environment.

In our experiments, simultaneous measurement of systemic blood pressure (data not shown) demonstrated that the oliguresis described could not

be attributed to general circulatory changes, such as sustained hypotension. According to McBean and Goldstein (1970), no significant variations in either the extracellular or vascular compartment of *Xenopus laevis* were evident after 21 days in a hypertonic environment. Therefore, changes in the relative volume of body compartments following the short-term exposure to a hypertonic bath as employed in our experiments would be negligible. Consequently, it is evident that mechanisms such as circulatory disturbances or increases in plasma osmolarity cannot be invoked as direct causes of this oliguria, which is primarily related to exteroceptive stimulation. These facts suggest that there is an interplay of neural and endocrine mechanisms in the control of urine production in the toad.

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References

- Adolph EF (1934) Influences of the nervous system on the intake and excretion of water by the body. *J Cell Comp Physiol* 5:123-139
- Fitzsimmons JT (1980) Thirst and sodium appetite. *Endeavour, New Series* 4:97-101
- Gallardo R, Pang PKT, Sawyer WH (1980) Neural influences on bullfrog renal functions. *Proc Soc Exp Biol Med* 165:233-240
- Keppel G (1973) Design and analysis. A researcher's handbook. Prentice Hall, NJ
- Mayer N (1969) Adaptation de *Rana esculenta* a des milieux varies. Etude speciale a l'excretion renale de L'eau et des electrolytes au cours des changements. *Comp Biochem Physiol* 29:27-50
- McBean RL, Goldstein L (1970) Renal function during osmotic stress in the aquatic toad *Xenopus laevis*. *Am J Physiol* 219:1115-1123
- McFarland DJ (1971) Feedback mechanisms in animal behaviour. Academic Press, London, pp 1-279
- Morris JL (1983) Effects of renal nerve stimulation on vascular resistance in the toad kidney. *Arch Pharmacol* 323:335-340
- Morris JL, Gibbins IL (1983) Innervation of the renal vasculature of the toad (*Bufo marinus*). *Cell Tissue Res* 231:357-376
- Petriella, Silvia, Brennan Margarita C, Segura ET (1986) Cambios precoces en la morfología y función renales debidos a la estimulación osmótica de la piel en *Bufo arenarum* Hensel. *Medicina* 46:254
- Reboreda JC, Segura ET (1984) Electrolyte sensitivity of the skin in the toad *Bufo arenarum* Hensel. *Acta Physiol Pharmacol Lat Am* 34:37-43
- Reboreda JC, Petriella Silvia, Segura ET (1987) Short-term changes in natriphoric and hydrosmotic water fluxes across the skin and in urine production due to increases in the osmolarity of the external environment in the toad. *Comp Biochem Physiol* 88A:571-575
- Richards AN, Schmidt CF (1924) A description of the glomerular circulation in the frog's kidney and observations concerning the action of adrenal and various other substances upon it. *Am J Physiol* 178-208

- Rodriguez EM, Dellmann HD (1970) Hormonal content and ultrastructure of the disconnected neural lobe of the grass frog (*Rana pipiens*). Gen Comp Endocrinol 15:272–288
- Rolls BJ, Rolls ET (1981) The control of drinking. Br Med Bull 37:127–130
- Sawyer WH, Pang PKT (1975) Endocrine adaptation to osmotic requirements of the environment. Endocrine factors in osmoregulation by lung fishes and amphibian. Gen Comp Endocrinol 25:224–229
- Segura ET, Bandsholm Ulla C, Bronstein A, Woscoboinik D (1982a) Role of the CNS in the control of the water economy of the toad *Bufo arenarum* Hensel. I. Effects of handling, brain lesions, anesthesia and reversible coma upon water uptake, urine production and overall water balance. J Comp Physiol 146:95–100
- Segura ET, Bandsholm Ulla C, Bronstein A (1982b) Role of the CNS in the control of the water economy of the toad *Bufo arenarum* Hensel. II. Adrenergic control of water uptake across the skin. J Comp Physiol 146:101–106
- Segura ET, Reboreda JC, Skorka A, Cuello Maria E, Petriella Silvia (1984) Role of the CNS in the control of the water economy of the toad *Bufo arenarum* Hensel. III. Skin permeability increases to raised osmotic pressure of the external 'milieu'. J Comp Physiol B 154:573–578
- Segura ET, Reboreda JC, Petriella Silvia, Brennan Margarita C, Lemoine AP (1986) Adaptive control of the water balance in the toad, *Bufo arenarum* Hensel. Proc Int Union Physiol Scs vol XVI, p 508 (P471.13)
- Segura ET, Cuello Maria E, Petriella Silvia, Orti G (1987) Anti-diuretic responses to osmotic, ionic or volume stimulation of the brain in the unanesthetized toad, *Bufo arenarum* Hensel. Comp Biochem Physiol 87A:107–110
- Shoemaker VH, Waring H (1968) Effect of hypothalamic lesions on the water response of a toad *Bufo marinus*. Comp Biochem Physiol 24:47–54
- Tsuneki Kazuhiko, Kobayashi H, Gallardo R, Pang PKT (1984) Electron microscopic study of the innervation of the renal tubules and urinary bladder epithelium in *Rana catesbeiana* and *Necturus maculosus*. J Morphol 181:143–153
- Yokota SD, Hilman SS (1984) Adrenergic control of the anuran cutaneous hydrosmodic response. Gen Comp Endocrinol 53:309–314