SHORT-TERM CHANGES IN NATRIPHERIC AND HYDROSMOTIC WATER FLUXES ACROSS THE SKIN AND IN URINE PRODUCTION DUE TO INCREASES IN THE OSMOLARITY OF THE EXTERNAL ENVIRONMENT IN THE TOAD*

JUAN C. REBOREDA,[†] SILVIA PETRIELLA[†] and E. T. SEGURA[‡]

Laboratorio de Fisiologia del Comportamiento, Instituto de Biologia y Medicina Experimental, Obligado 2490, 1428 Buenos Aires, Argentina

(Received 19 March 1987)

Abstract—1. Sudden decreases in the osmotic gradient across the skin due to the replacement of water of the bath by 115 mM NaCl had no effect on water uptake of intact or hypophysectomized toads. 2. A concomitant decrease in the urine production was observed in intact but not in hypophysectomized

2. A concomitant decrease in the unne production was observed in infact but not in hypophysectomized animals.

3. Addition of amiloride chlorydrate (0.25 mM) to the 115 mM NaCl bath induced a significant decrease in water uptake both in intact and in hypophysectomized toads.

4. The osmotic permeability coefficient (L_{PD}) increased significantly during the osmotic gradient reduction with 115 mM NaCl plus 0.25 mM amiloride or 230 mM sucrose in both groups.

5. No changes in the plasmatic osmolarity were detected during the development of these responses to the osmotic gradient reduction.

6. These results are consistent with the hypothesis of short-term changes in the natripheric and hydrosmotic fluxes of water across the skin and in urine production triggered by the osmotic gradient reduction. The possible participation of arginine vasotocin in these responses is discussed.

INTRODUCTION

The homeostasis of body fluids is a complex physiological process in amphibians in which the skin, the kidney, and the urinary bladder are mainly involved.

As is well known, the neurohypophysis participates as a basic regulatory factor of these phenomena. For instance, the administration of neurohypophyseal extracts to toads and frogs kept in water, results in an increase of the body weight, the so-called "Brunn" or "water balance effect" (Brunn, 1921; Heller, 1941). This phenomenon is dependent on the presence of arginine vasotocin (AVT) which is known to act in several ways. It promotes urinary water reabsorption across the wall of the urinary bladder and renal tubules of the kidney; it increases the hydrosmotic and natripheric water flux across the skin, and it reduces the glomerular filtration rate (Bentley, 1974; Pang et al., 1980; De Sousa and Grosso, 1981). The circulating levels of this hormone are regulated by feedback mechanisms due to changes in blood volume and in a lesser degree by changes in the plasmatic osmolarity (Bentley, 1969; Sawyer and Pang, 1975).

However, several works suggested the participation of extrahypophyseal-hypothalamic mechanisms in the control of body fluids. For instance, hypophysectomized or hypothalamic lesioned toads showed antidiuretic responses and changes in skin and bladder permeability due to dehydration, hemorrage, or salt load (Middler *et al.*, 1967; Shoemaker and Waring, 1968; Jorgensen *et al.*, 1969; Christensen and Jorgensen, 1972; Bakker and Bradshaw, 1977).

In the same way, lesions of the medulla oblongata (Adolph, 1934) or the midbrain (Segura *et al.*, 1982a) produce an irreversible increase in the rate of water uptake across the skin in frogs and toads with independence of the neurohypophyseal integrity.

Moreover, cutaneous permeability appears to be also modulated by adrenergic mechanisms. Isoproterenol induces an *in vitro* (De Sousa and Grosso, 1982) and *in vivo* skin hydrosmotic response (Yokota and Hillman, 1984). Phenoxybenzamine also increases the water uptake of toads (Segura *et al.*, 1982b).

Besides, some external factors like the osmolarity of the external environment that are also involved in the water uptake and urine production in toads have been only scarcely studied at present. Most references in this sense are related to long-lasting changes in body fluids and ionic plasmatic concentrations of toads adapted to highly saline environments (Ferreira and Jesus, 1973; Katz, 1975; Garland and Henderson, 1975; Liggins and Grigg, 1985). Only two references deal with rapid changes in water balance triggered by the increase of the osmolarity of the external bath (Mayer, 1969; Segura et al., 1984). However, changes in natripheric or hydrosmotic fluxes of water and the involvement of AVT in these changes have not been studied yet. Consequently, this paper deals with short-term changes in natripheric and hydrosmotic fluxes of water and the urine production of toads submitted to a sudden change in the osmolarity of the external bath and the degree

^{*}This work was supported by a grant from the Consejo Nacional de Investigaciones Cientificas y Tecnicas

[†]Fellow of the Consejo Nacional de Investigaciones Científicas y Tecnicas.

[‡]Established investigator of the Consejo Nacional de Investigaciones Científicas y Tecnicas.

of involvement of the neurohypophysis in these phenomena.

MATERIAL AND METHODS

Animals

Adult male toads (*Bufo arenarum*) weighing 100-150 g were collected near Buenos Aires and used after 2–3 weeks of captivity. During this period, they were kept in large cages with free access to water. Twenty-four hours before the experiments, they were transferred to opaque individual plastic boxes ($15 \times 15 \times 10$ cm) containing 150 ml of deionized water.

Water balance measurements

Water uptake across the skin and urine production were measured gravimetrically (Segura *et al.*, 1982a). Toads were weighed every 15 min, before and after bladder emptying, for 2 hr. The results are expressed as ml/hr/100 g body weight with respect to the standard weight (the weight of the fully hydrated animal with its bladder empty) (Ruibal, 1962). Water balance was estimated as the difference between water uptake and urine production for a given period. The experiments were performed in individual plastic boxes containing 150 ml of deionized water. At the end of the first hour of measurements water was replaced by NaCl 115 mM (experiment 1), NaCl 115 with amiloride 0.25 mM (experiment 2), or sucrose 230 mM (experiment 3).

Osmotic permeability coefficient (L_{PD}) was calculated from the non-equilibrium thermodynamics relationship (Katchalsky and Curran, 1965)

$$J_{\rm w} = L_{\rm P} \,\,\Delta P + L_{\rm PD} \Delta \pi \tag{1}$$

where J_w is the water flow across the skin; L_P is the hydraulic permeability coefficient; ΔP , the hydrostatic pressure; and $\Delta \pi$ the osmotic gradient across the skin. $\Delta \pi = (O_i - O_e)RT$ where R is the gas constant; T, the absolute temperature (293 K); and O_e the internal and external osmolarity respectively. An O_i value of 245 mosmol/kg H₂O was used in these calculations. As it follows from equation (1)

$$L_{\rm PD} = (J_{\rm w}/\Delta\pi)$$
$$\Delta P = 0.$$

 $J_{\rm w}$ was estimated according to Barker-Jorgensen (1950) by the formula

$$J_w = WU/S = WU/9bw^{2/3}$$

where WU represents the water uptake in g/hr; S, the body surface in cm²; and bw the body weight in grams.

Surgery

HR groups (hypophysectomized, postchiasmatic sectioned, infundibulum lesioned toads). Toads were anesthetized by exposure to ether. A medial incision was made in the roof of the buccal cavity below the parasphenoid bone. A small round opening was then made by means of a dental drill and the hypothalamic-hypophyseal area was exposed. The whole hypophysis was removed and the infundibulum lesioned by light suction with a glass capillary (0.8 mm external bore) connected to a vacuum pump. A section across the width of the diencephalon just behind the optic chiasma was then made with a sharp blade. The round was gently covered with a small square of absorbable gelatin sponge (Gelfoam, Upjohn) and the mucus membrane sutured.

The toads were used not before 20 days postoperation. It is assumed that a complete atrophy of the magnocellular complex occurs during this period (Rodriguez and Dellman, 1970).

Histology

At the end of each experiment, the HR toads were sacrificed by decapitation, the brain removed and fixed in 25% chloral hydrate, silver impregnated, embedded in paraffin and cut in serial sections of 10μ . Complete degeneration of the preoptic magnocellular nucleus of the hypothalamus and its neurohypophyseal connections were certified in all cases.

Plasmatic osmolarity measurements

Plasmatic osmolarity was measured with a vapour pressor osmometer Wescor 5120C. The blood samples were taken at the end of the first and second hour from 6 intact and 6 hypophysectomized unesthetized toads which had gone through the sequence of experiment 1. These samples $(50-70 \ \mu$ l) were taken through a polyethylene cannulae (1 mm external bore) placed in the dorsal aorta (lateral cannulation). The blood was heparinizated with Liquemine (Roche) at a final concentration of 50 U/ml and then centrifugated 15 min at 1000 rpm.

Analysis

WU

All the results are presented as mean \pm standard error of the mean (SEM). Comparison within groups are made using paired *t*-test unless otherwise stated.

RESULTS

The decrease of the osmotic gradient due to the replacement of water by 115 mM NaCl in the external bath did not produce significant differences in the water uptake of intacts (P < 0.3) or hypophysectomized toads (P < 0.2). At the same time, this procedure resulted in a significant decrease in the urine production in intact (P < 0.001) but not in hypophysectomized toads (P < 0.2) (Fig. 1).

No changes in plasmatic osmolarity consecutive to immersion in 115 mM NaCl were observed in intact toads. By the end of the first hour (water), the plasmatic osmolarity was $242.7 \pm 4.41 \mod k$ (mean \pm SEM) whereas by the end of the second (115 mM NaCl) it was $246.69 \pm 4.04 \mod k$. In the hypophysectomized toads the values observed

Fig. 1. Rate of cutaneous water uptake (WU) and urine production (UP) (ml H₂O/100 g/hr) of toads immersed in water (open bars) and in NaCl 115 mM (striped bars). N: intact group (n = 18). HR: hypophysectomized group (n = 6). The horizontal dotted line corresponds to the water balance (i.e. the difference between water uptake and urine prouction). Statistical significance of differences (paired *t*-test) between the values in water and in NaCl 115. *** P < 0.001.



Fig. 2. Rate of cutaneous water uptake and urine production of intact (n = 12) and hypophysectomized (n = 6) toads immersed in water (open bars) and in NaCl 115 mM plus amiloride 0.25 mM (striped bars). Statistical significance of differences (paired *t*-test) between the values in water and in NaCl 115. *P < 0.05; **P < 0.01; ***P < 0.001

were 175.42 ± 7.50 and 209.75 ± 6.33 mosmol/kg, respectively (paired *t*-test 5.74, P < 0.001).

When 115 mM NaCl plus amiloride (0.25 mM) was used to reduce the osmotic gradient a significant decrease in the water uptake could be seen in both intact (P < 0.001) and hypophysectomized toads (P < 0.01) (Fig. 2). In intact toads the water uptake during the osmotic gradient reduction was 47.18% of the value in water whereas in hypophysectomized it was 41.87%. In this case (experiment 2) a significant antidiuresis in the intact (P < 0.05) but not in the hypophysectomized group was also noticed.

Similar results to the ones obtained in experiment 2 were observed when 230 mM sucrose was used (Fig. 3). In this case the water uptake in intact toads during the osmotic gradient reduction was



Fig. 3. Rate of cutaneous water uptake and urine production of intact (n = 6) and hypophysectomized (n = 8)toads immersed in water (open bars) and in sucrose 230 mM (striped bars). Statistical significance of differences (paired *t*-test) between the values in water and in NaCl 115. *P < 0.05;*** P < 0.001.



Fig. 4. Osmotic permeability coefficient (L_{PD}) (cm/sec atm $\times 10^6$) of intact (N) and hypophysectomized (HR) toads immersed in water (W); NaCl 115 mM plus amiloride 0.25 mM (A) and sucrose 230 mM (Suc). Statistical significance of differences (paired *t*-test) between the values in water and the osmotic gradient reduction. *P < 0.05. ***P < 0.001

38.5% of the control value (P < 0.001), and in hypophysectomized it was 27.8% of the control value (P < 0.001).

As regards the urine production, a significant antidiuresis was observed for both, the intact group (P < 0.001) and the hypophysectomized group (P < 0.05).

When L_{PD} was calculated in experiments 2 and 3 it was seen that it increased significantly in both groups (Fig. 4). During the 115 mM NaCl plus amiloride 0.25 mM osmotic gradient reduction the L_{PD} increased to 9 times the control value (P < 0.001) in intact toads and 7 times in hypophysectomized ones (P < 0.001) whereas when the osmotic gradient was reduced by adding sucrose to the bath it increased 3.7 times (P < 0.001) and 2.6 times (P < 0.05), respectively.

DISCUSSION

The results reported in this paper on the terrestrial amphibian *Bufo arenarum* are consistent with the hypothesis of short-term changes in the hydrosmotic and natripheric fluxes of water across the skin and in the urine production which are triggered by the osmotic gradient reduction. Changes in water fluxes appear to be independent of the hypothalamicneurohypophyseal integrity whereas changes in urine production appear to be dependent.

The development of natripheric fluxes of water when the osmotic gradient is reduced is supported by several evidences. First of all, there was no correlation between the water uptake and the osmotic gradient across the skin. The water uptake did not decrease significantly when the water is replaced by 23, 46, 69, and 92 mM NaCl (data not shown); and 115 mM NaCl (Fig. 1) neither in normal toads nor in hypophysectomized ones. Besides, when amiloride chlorhydrate 0.25 mM was added to the 115 mM NaCl solution, a significant decrease in the water uptake in intact as well as in hypophysectomized toads was observed. Finally, the rate of cutaneous water uptake of dehydrated toads was similar when they were rehydrated in water or in 115 mM NaCl, but it was significantly lower when rehydration took place in 115 mM NaCl sucrose plus amiloride or in 230 mM sucrose (data not shown).

On the other hand, the hydrosmotic flux across the skin decreased during the osmotic gradient reduction with 115 mM NaCl plus amiloride or 230 mM sucrose, but this decrease was not proportional to the osmotic gradient reduction. This appears quite clear when the L_{PD} is calculated. These increases in L_{PD} , as well as in the natripheric flux, are not dependent on AVT. The absence of correlation between the cutaneous water uptake and the circulating arginine vasotocin in anurans is supported in previous works. It was shown that administration of AVT had no significant effect on the rates of cutaneous water uptake nor in intact (Bakker and Bradshaw, 1977; Yokota and Hillman, 1984) or in hypothalamic lesioned toads (Bakker and Bradshaw, 1977). Furthermore, previous experiments in the intact toad, Bufo arenarum, showed that the necessary doses for increasing the rate of cutaneous water uptake had to be 100 times higher $(1-10 \mu g/kg)$ than those necessary for producing a significant antidiuretic response (10 ng/kg).

Moreover, it is known that the administration of adrenergic drugs increases the water uptake *in vitro* (Bastide and Jard, 1968; Jard *et al.*, 1968; De Sousa and Grosso, 1982) and *in vivo* (Elliot, 1968; Hillyard, 1979; Yokota and Hillman, 1984). In the same way the administration of 6-hydroxydopamine produces a body weight increase (Clavers and Morris, 1983) and the administration of guanethidine or phenoxibenzamine an increase of the rate of water uptake (Segura *et al.*, 1982b) in intact and hypophysectomized toads. This would provide an alternative mechanism to AVT that could be responsible for the changes in the water fluxes shown in this paper.

With reference to the changes in urine production due to the osmotic gradient reduction, they appear to be mediated by AVT. Plasmatic AVT levels in toads are regulated by dehydration and hypovolemic stimuli (Bentley, 1969; Sawyer and Pang, 1975; Pang, 1977). However, in this paper no changes in volemia or in plasma osmolarity were detected during the experiments. This is in agreement with results reported by Mayer (1969) who observed changes in the tubular reabsorption of water after osmotic gradient reduction with Ringer solution or 0.7% NaCl, long before any changes in plasma osmolarity occurred.

The existence of changes in the hydrosmotic and natripheric fluxes of water and in the diuresis without changes in the blood volume or the plasmatic osmolarity brings up the question about the control mechanism involved in this change. A negative feed-back mechanism controlling the error of an internal variable (for example, plasmatic osmolarity or volemia) appears not to be enough as explanation in this case; for this reason an alternative mechanism that is able to prevent future errors in the internal variable could be present.

In a previous paper (Reboreda and Segura, 1984) a proportional sensitivity of the skin to electrolytes was demonstrated in the same species. This would provide a physiological basis for a possible feedforward control mechanism.

In the same way, anticipatory drinking behaviour in response to changes in the osmolarity of the external medium has also been reported in the eel (Hirano, 1974), and the participation of homeostatic and non-homeostatic mechanisms involved in drinking behaviour has been thoroughly discussed by Toates (1979).

Further studies on the involvement of the skin sensitivity of the toad and its relationship with the changes in natripheric flux, L_{PD} and diuresis are needed.

REFERENCES

- Adolph E. F. (1934) Influences of the nervous system on the intake and excretion of water by the frog. J. Cell. comp. Physiol. 5, 123–139.
- Bakker H. R. and Bradshaw D. (1977) Effect of hypothalamic lesions on water metabolism of the toad Bufo marinus. J. Endocr. 75, 161-172.
- Bastide F. and Jard S. (1968) Actions de la noradrenaline et de l'ocytocine sur le transport actif de sodium et la permeabilite à l'eau de la peau de grenouille. Role du 3',5'-AMP cyclique. Biochim. Biophys. Acta 150, 113-123.
- Bentley P. J. (1969) Neurohypophyseal function in amphibia: hormone activity in the plasma. J. Endocr. 43, 359-369.
- Bentley P. J. (1974) Actions of neurohypophyseal peptides in amphibians, reptiles and birds. In *Handbook of Phys*iology, Section 7: Endocrinology, Chap. 20, pp. 545–563.
- Brunn F. (1921) Beitrag zur kenntis der wirkung von hipophysenextrak auf den wasserhaushalt des froshes. Z. Ges. Exptl. Med. 25, 170–175.
- Christensen C. U. and Jorgensen C. B. (1972) Role of pars distalis and pars nervosa of the hypophysis in the water economy of the toad Bufo bufo bufo. Gen. comp. Endocr. 18, 169-174.
- Clevers Jeni and Morris Judith L. (1983) 6-Hydroxydopamine produces a weight increase in toads. Comp. Biochem. Physiol. 76C, 339-344.
- De Sousa R. C. and Grosso A. (1981) The mode of action of vasopressin: membrane microstructure and biological transport. J. Physiol., Paris 77, 643-669.
- De Sousa R. C. and Grosso A. (1982) Osmotic water flow across the abdominal skin of the toad *Bufo marinus*: effect of vasopressin and isoprenaline. J. Physiol. **329**, 281–296.
- Elliot A. B. (1968) Effect of adrenaline on water uptake in *Bufo. J. Physiol.* 197, 87-88P.
- Ferreira H. G. and Jesus C. H. (1973) Salt adaptation in Bufo bufo. J. Physiol. 228, 583-600.
- Garland H. O. and Henderson I. W. (1975) Influence of environmental salinity on renal and adrenocortical function in the toad, *Bufo marinus. Gen. comp. Endocr.* 27, 136-143.
- Heller H. (1941) Differentiation of an (amphibian) water balance principle from the antidiuretic principle of the posterior pituitary gland. J. Physiol., Lond. 100, 125-141.
- Hillyard S. D. (1979) The effects of isoproterenol on the anuran water balance response. *Comp. Biochem. Physiol.* 62C, 93-95.
- Hirano T. (1974) Some factors regulating water intake by the eel, Anguilla Japonica. J. exp. Biol. 61, 737-747.
- Jard S., Bastide F. and Morel F. (1968) Analyse de la relation dose-effet biologique' pour l'action de l'ocytocine et de la noradrenaline sur la peau et la vessie de la grenouille. *Biochim. Biophys. Acta* **150**, 124–130.

- Jorgensen C. B. (1950) The amphibian water economy with special regard to the effect of neurohypophyseal extracts. *Acta Physiol. Scand.* 22, Suppl. 78, 1–79.
- Jorgensen C. B., Rosenkilde P. and Wingstrand K. G. (1969) Role of the preoptic-neurohypophyseal system in the water economy of the toad *Bufo bufo* (L). *Gen. comp. Endocr.* 12, 91-98.
- Katz U. (1975) Salt-induced changes in the sodium transport across the skin of the euryhahline toad, *Bufo viridis*. J. Physiol. 247, 537-550.
- Katchalsky A. and Curran P. F. (1965) In Non Equilibrium Thermodynamics Biophysics. Harvard University Press, Cambridge, MA.
- Liggins G. W. and Grigg G. C. (1985) Osmoregulation of the cane toad, *Bufo marinus*, in salt water. *Comp. Biochem. Physiol.* 82A, 13-619.
- Mayer N. (1969) Adaptation de *Rana esculenta* a des milieux varies. Etude speciale de l'excretion renale de l'eau et des electrolytes au cours des changements de milieux. *Comp. Biochem. Physiol.* **29**, 27-50.
- Middler S. A., Kleeman C. R. and Edwards E. (1967) Neurohypophyseal function in the toad *Bufo marinus*. *Gen. comp. Endocr.* 9, 38–48.
- Pang P. K. T. (1977) Osmoregulatory functions of neurohypophyseal hormones in fishes and amphibians. Am. Zool. 17, 739-749.
- Pang P. K. T., Galli-Gallardo S. M., Collie N. and Sawyer D. H. (1980) Renal and peripheral vascular responsiveness to arginine vasotocin in the bullfrog, *Rana* catesbiana. Am. J. Physiol. 239, R156-R160.
- Reboreda J. C. and Segura E. T. S. (1984) Electrolyte sensitivity of the skin in the toad *Bufo arenarum* Hensel. *Acta physiol. pharmacol. latinoam.* 34, 37–43.
- Rodriguez E. M. and Dellman H. D. (1970) Hormonal

content and ultrastructure of the disconnected neural lobe of the grass frog (*Rana pipiens*). Gen. comp. Endocr. 15, 272–288.

- Ruibal R. (1962) The adaptive value of bladder water in the toad, Bufo cognatus. Physiol. Zool. 35, 218–223.
- Sawyer W. H. and Pang P. K. T. (1975) Endocrine adaptation to osmotic requirements of the environment: Endocrine factors in osmoregulation by lungfishes and amphibians. Gen. comp. Endocr. 25, 224–229.
- Segura E. T., Bandsholm Ulla Carmen, Bronstein A. and 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. B146, 95-100
- Segura E. T., Bandsholm U. C. and 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. B146, 101-106.
- Segura E. T., Reboreda J. C., Skorka A., Cuello M. E. and Petriella S. (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. B154, 573-578.
- Shoemaker V. H. and Waring H. (1968) Effect of hypothalamic lesions on the water balance response of a toad Bufo marinus. Comp. Biochem. Physiol. 24, 47-54.
- Toates F. M. (1979) Homeostasis and drinking. Behav. Brain Sci. 2, 95-136.
- Yokota S. D. and Hillman S. S. (1984) Adrenergic control of the anuran cutaneous hydrosmotic responses. *Gen. comp. Endocr.* 53, 309–314.