Comp. Biochem. Physiol. Vol. 100C, No. 3, pp. 433–437, 1991
Printed in Great Britain

0306-4492/91 $3.00 + 0.00

β-ADRENERGIC CONTROL OF THE WATER PERMEABILITY OF THE SKIN DURING REHYDRATION IN THE TOAD *BUFO ARENARUM*

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(*Received* 4 *December* 1990)

Abstract—1. Toads dehydrated to 80% of their standard weight (% SW) were rehydrated during 3 hr in distilled water.

2. Water permeability of the skin was positively correlated with the degree of dehydration in the range 80–100% SW.

3. Systemic administration of the β-adrenergic agonist isoproterenol (5 mg/kg) 90 min after rehydration started (animals fully hydrated) increased skin permeability to the values observed in 80% SW dehydrated animals.

4. The administration of the β-adrenergic blocker propranolol (5 mg/kg) 15 min before rehydration started produced a long-lasting decrease in water permeability during the 3 hr of rehydration.

5. The results are consistent with the hypothesis of a β-adrenergic control of the water permeability of the skin during rehydration.

INTRODUCTION

Dehydrated amphibians normally do not drink, but rehydrate by absorbing water osmotically across their permeable skin (Bentley and Yorio, 1979). This water uptake is proportional to both, the osmotic gradient between the body fluids and the environment, and the skin permeability to water.

It is generally accepted that in amphibians, skin permeability to water is under control of arginine vasotocin (AVT) (Bentley, 1974; DeSousa and Grosso, 1981). In accordance with this view, the administration of exogenous AVT produces an increase in the water flow across the skin both *in vitro* and *in vivo* (Bentley, 1974). However, hypothalamic lesions (Bakker and Bradshaw, 1977) or the section of the preoptic-neurohypophyseal tract (Jørgensen *et al.*, 1969; Christensen and Jørgensen, 1972) do not modify the rate of water uptake of dehydrated toads. In addition, although the administration of AVT always increases the water permeability of the bladder and collector duct epithelia (Pang *et al.*, 1980), its effects on skin permeability are not always present (Bentley, 1974) or are present at concentrations between 10 and 100 times higher than the ones which produce renal or vesical effects (Reboreda and Segura, 1991; Yokota and Hillman, 1984). Thus, factors other than AVT may be involved in the control of permeability to water in amphibian skin.

It has been shown that the adrenergic system is involved in the regulation of skin permeability to water. Bastide and Jard (1968) showed that norepinephrine increases the permeability in the isolated frog skin and Rajerison *et al.* (1972) demonstrated that this effect is produced by the interaction of norepinephrine with the β-adrenergic receptor. In a similar way, the β-agonist isoprenaline increases the permeability to water in the isolated skin of *Bufo marinus* (DeSouza and Grosso, 1982) and *Bufo arenarum* (Gamundi *et al.*, 1984). In addition, isoproterenol stimulates water gain and reduces urine production in fully hydrated toads *Scaphopus couchi* (Hillyard, 1979) and mimics the cutaneous hydrosmotic response in *Bufo cognatus* (Yokota and Hillman, 1984).

The aim of this work is to study the changes in skin permeability during rehydration and the involvement of AVT and β-adrenergic receptors in these changes in the toad, *Bufo arenarum*.

MATERIALS AND METHODS

Animals

Adult male toads (*Bufo arenarum*) weighing 80–180 g were collected locally during spring and summer (October–February). Experiments were conducted within 3 weeks of their arrival to the laboratory. During this period the toads were kept in large cages with free access to water and fed with ox liver twice a week.

Water uptake and permeability measurements

Animals were kept in opaque individual plastic boxes (12 × 12 × 8 cm) containing 150 ml of distilled water for 24 hr to ensure hydration. At this time the standard weight (the weight of the hydrated animal with its bladder empty; Ruibal 1962) was recorded. Toads were weighed every 15 min, after their urinary bladders were emptied by inserting a glass probe into the cloaca and applying suprapubic pressure. This procedure was followed until the difference in weight between 2 consecutive measurements was less than 1% of the body weight. This value was assumed as the standard weight (SW).

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The animals were dehydrated to 80% SW in individual open containers (mesh 2 cm) at room temperature (18–22°C). In these conditions they lost mass at rates of 8–15% SW per day. Rehydration was performed for 3 hr in opaque individual plastic boxes containing 150 ml of distilled water at 20°C. During this period they were weighed every 15 min in a Ohaus Dial-O-Gram balance with a precision of 0.1 g. The gross weight gain over each period was assumed to be due to water uptake (WU) and was expressed as ml/hr. Generally, toads do not urinate during rehydration. However, those animals which showed body weight decrease at any time during the experiment were excluded from the analysis. In all cases, rehydration experiments were made between 48 and 72 hr after dehydration started.

Osmotic permeability coefficient (L_{PD}) was calculated following Katchalsky and Curran (1965)

$$J_w = L_P * \Delta P + L_{PD} * \Delta \Pi$$

where J_w is the water flux across the skin; L_P is the hydraulic permeability coefficient; and ΔP and $\Delta \Pi$ the hydrostatic and osmotic pressures across the skin respectively.

As ΔP was equal to 0, then:

$$L_{PD} = \frac{J_w}{\Delta \Pi}.$$

J_w was estimated as WU/S, where S is the body surface in cm^2. The body surface was assumed to be equal to 9 * body weight (g)$^{2/3}$ (Jørgensen, 1950). Water uptake in toads is limited to the ventral skin which contributes approximately 10% of the total surface area (McClanaham and Baldwin, 1969). Therefore, it was assumed that all water intake was through 10% of body surface.

The osmotic pressure across the skin was calculated as:

$$\Delta \Pi = (O_i - O_e) * R * T$$

where R is the gas constant; T, the absolute temperature (293°K); and O_i and O_e the internal and external osmolarity respectively. As rehydration was performed always in distilled water, O_e was equal to 0. The internal osmolarity was calculated according to Shoemaker (1964) from the initial osmolarity and body water content and the percentage of dehydration (%D). Plasma osmolarity in fully hydrated *Bufo arenarum* is 245 mOsm/kg and body water content in *Bufo* is 80% (Ferreira and Jesus, 1973).

Hence

$$O_i = \frac{245 * 80}{80 - \%D}.$$

Treatments

In the experiment of β-adrenergic stimulation, the subjects were injected 90 min after rehydration started with 115 mM NaCl ($N = 6$), vasotocin (Arg^8 Vasotocin, Sigma) 10 μg/ml ($N = 5$) or isoproterenol (Isoproterenol, Sigma) 5 mg/ml ($N = 5$). In the experiment of β-adrenergic blockade, the subjects were injected 15 min before rehydration started with 115 mM NaCl ($N = 5$) or propranolol (Propranolol, Sigma) 5 mg/ml ($N = 6$). The drugs were dissolved in 115 mM NaCl and administrated through the dorsal lymphatic sac. The volume injected was 1 ml/kg body weight.

Plasmatic osmolarity measurements

Blood samples (50 μl) were taken either during dehydration or during rehydration from a group of toads submitted to the same experimental dehydration–rehydration sequence mentioned before. The samples were taken through a polyethylene tube (o.d. = 1 mm) placed and fixed (lateral cannulation) in the dorsal aorta 72 hr before dehydration started. The blood was heparinized with liquemine (Roche) at a final concentration of 50 U/ml and then centrifuged for 15 min at 1000 rpm. Plasma osmolarity was measured by duplicate with a vapour pressure osmometer Wescor 5120C.

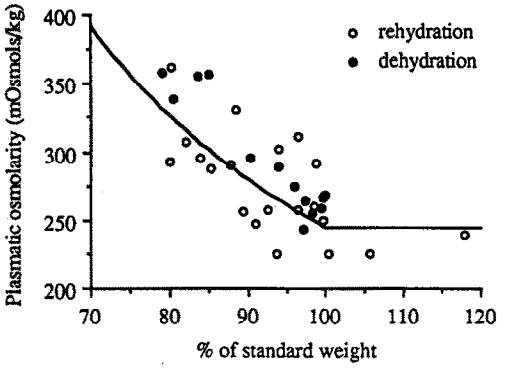

Fig. 1. Plasmatic osmolarity values for different percentages of the standard weight (% SW). Blood samples were taken either during dehydration (filled circles) or rehydration (empty circles). The line shows the osmolarity values predicted from the homeostatic value of osmolarity, percentage of body water content, and % SW (see Materials and Methods).

Analysis

Averaged results are presented as mean ± standard error of the mean (SEM). Comparison within and between groups were done using one-way and two-way analysis of variance for repeated measures.

RESULTS

Plasmatic osmolarity values during dehydration, as well as during rehydration, were similar to the ones predicted from the initial osmolarity, initial body water content and percentage of dehydration (Fig. 1). Therefore, water permeability in every 15 min interval was estimated from the water uptake in that interval and the estimated value of plasmatic osmolarity at the beginning of that interval.

During the 3 hr of rehydration, water permeability decreased from $35.43 \pm 3.83 * 10^{-6}$ cm/seg * atm in 80.13 ± 0.48% SW animals to $7.59 \pm 0.91 * 10^{-6}$ cm/seg * atm in fully hydrated animals (% SW = 105.3 ± 1.33) ($F_{11,239} = 29.49$, $P < 0.0001$). After 90 min of rehydration ($T = 90$), the toads had recovered most of the water lost during dehydration. At this time, the % SW was 99.41 ± 1.29 whereas the value of the skin

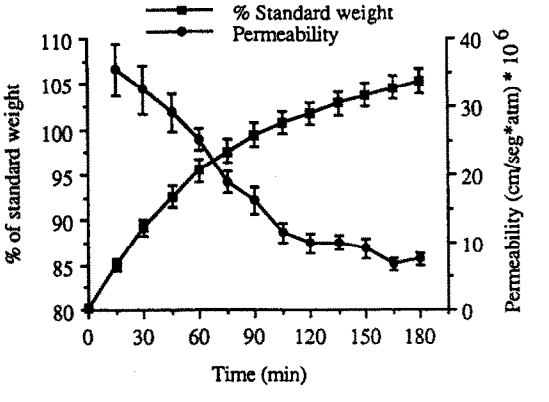

Fig. 2. Changes in the % SW and in the skin permeability in toads dehydrated to 80% SW and rehydrated during 3 hr in distilled water. The values correspond to the mean ± SEM of 20 toads.

permeability was 16.2 ± 2.05 (Fig. 2). No significant changes in skin permeability were observed between $T = 105$ and $T = 180$ (Fisher PLSD test).

In order to evaluate the involvement of β-adrenergic receptors in these changes, we studied the effect of the administration of the β-adrenergic agonist, Isoproterenol (5 mg/kg), in fully hydrated animals (90 min after rehydration started). Two other control groups received 115 mM NaCl or AVT (10 μg/kg). The changes in water permeability during rehydration differed between groups ($F_{2,143} = 7.79$; $P < 0.01$). There were no significant differences during the first 90 min of rehydration ($P > 0.05$ for $T = 15$ to $T = 90$, 1W AOV and Scheffe F-test) but after the administration of the drugs, the isoproterenol-treated group showed higher values of water permeability than the other two groups at $T = 105$, $T = 120$, $T = 135$, $T = 150$ and $T = 180$ (1W AOV and Scheffe F-test). Thus, the administration of isoproterenol in fully hydrated animals (% SW = 103.5 ± 1.42) increased the skin permeability to values similar to the ones observed at the beginning of the rehydration (37.29 ± 2.82 at $T = 0$ and 29.96 ± 6.46 at $T = 120$). As a result of this, after 3 hr of rehydration, the % SW of the isoproterenol-treated group was 118 ± 2.19 whereas in the 115 mM NaCl and in the AVT-treated groups it was 108.7 ± 1.43 and 109.4 ± 3.13 respectively (Fig. 3).

In another experiment, we studied the effect of the β-adrenergic antagonist, propranolol (5 mg/kg), on the water permeability of the skin. The blocker was injected in 80% SW dehydrated toads 15 min before rehydration started. Another group received the same volume of 115 mM NaCl. The changes in water permeability differed in both groups ($F_{1,99} = 60.19$; $P < 0.0001$) being significantly lower in the propranolol treated group than in the control group from $T = 15$ to $T = 180$. As a result of this, after 3 hr of rehydration, the % SW in the propranolol treated group was 88.5 ± 0.46 whereas in the control group it was 107 ± 2.18 (Fig. 4).

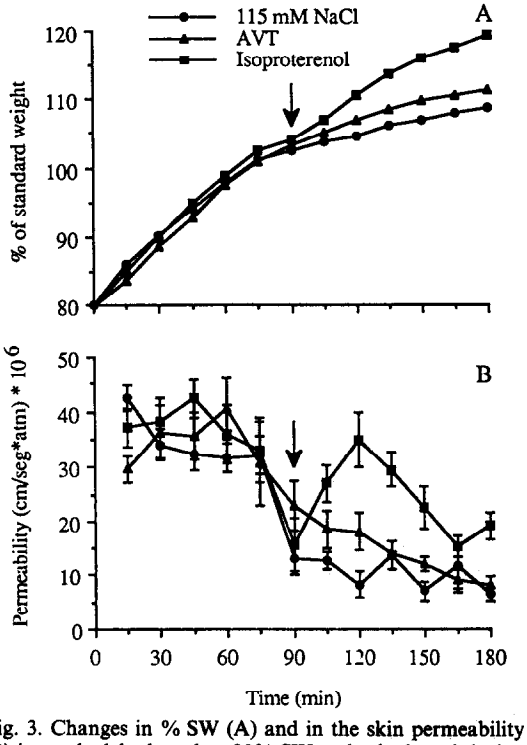

Fig. 3. Changes in % SW (A) and in the skin permeability (B) in toads dehydrated to 80% SW and rehydrated during 3 hr in distilled water. The animals were injected 90 min after rehydration started with: 115 mM NaCl (circles, $N = 6$), arginine vasotocin 10 μg/kg (triangles, $N = 5$) and isoproterenol 1 mg/kg (squares, $N = 5$).

DISCUSSION

The results presented before are consistent with the hypothesis of a β-adrenergic control of the permeability to water of the toad skin during rehydration. Although the administration of a β-adrenergic agonist increased the permeability to water in fully hydrated animals, the administration of a β-adrenergic antagonist decreased the permeability to water in dehydrated animals. On the contrary, the administration of AVT to fully hydrated animals was unable to produce an increase in the permeability to water. This lack of effect of AVT on skin permeability cannot be attributed to the use of sub-umbral concentrations of the drug because doses between 10 and 100 times smaller than the amount injected produced both cardiovascular and antidiuretic responses (Reboreda and Segura, 1991). In addition to these results, dehydrated animals treated with isoproterenol or fully hydrated animals treated with propranolol show similar values of permeability to the ones shown by the control groups treated with 115 mM NaCl (Reboreda and Segura, 1991).

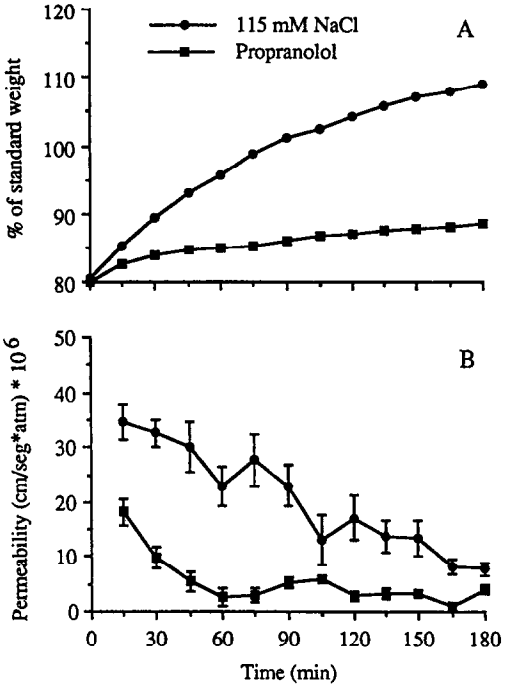

Fig. 4. Changes in % SW (A) and in the skin permeability (B) in toads dehydrated to 80% SW and rehydrated during 3 hr in distilled water. The animals were injected 15 min before rehydration started with: 115 mM NaCl (circles, $N = 5$) and propranolol 1 mg/kg (squares, $N = 6$).

Beta-adrenergic receptors in amphibians appear to function in heart rate regulation. Isoproterenol elevated heart rate in the bullfrog *Rana catesbeiana* in a dose-dependent manner and that response could be blocked with propranolol (Herman and Sandoval, 1983). It has been shown that circulatory changes can affect osmotic water flow in amphibian skin. Christensen (1974, 1975) found that water flow through isolated frog skin depended on the rate of perfusion of fluid through the skin vasculature. In a similar way, water exchange *in vivo* was shown to be more sensitive to circulatory changes than to AVP-induced permeability changes (Mahany and Parsons, 1978). In our experiment, the fully hydrated animals injected with isoproterenol showed a clear vasodilatation of the skin in the region of the "pelvic patch," similar to the one shown during rehydration by dehydrated animals. In contrast, the animals injected with propranolol showed no evidence of vasodilatation and the appearance of the skin in the "pelvic patch" was similar to the skin of fully hydrated animals. Thus, the increase in water uptake produced by isoproterenol would be the result of two processes; the increase in the permeability to water and the increase of the rate of perfusion of fluid through the skin vasculature.

During rehydration, the permeability to water was positively correlated with the degree of dehydration. This result suggests a feedback relationship between permeability and either plasmatic osmolarity or volemia or both. However, in toads there is not a clear correlation between these variables and the plasmatic levels of catecholamines. In *Bufo marinus*, plasma catecholamines increase only after hemorrhage of 7–9% of mass loss or dehydration of 15% of mass loss (Withers *et al.*, 1988). Catecholamine levels in amphibians are determined by both adrenal gland secretion and sympathetic nerve terminal release, but adrenalectomy experiments suggest that nerve-terminal release may be the primary source of circulating catecholamines (Withers *et al.*, 1988). Thus, the changes in permeability to water mediated by β-adrenergic receptors should be the result of a local effect of nerve terminal release either in the epithelial cells or in the blood vessels surrounding the skin. Related to this, Tsuneki *et al.* (1984) described nerve endings in the renal tubules and urinary bladder epithelium of *Rana catesbeiana* and *Necturus maculosus* and suggested that these endings could be involved in the control of permeability to water.

The presence of a direct nerve control of the skin permeability to water could provide an adaptative mechanism for rapid adjustments in water uptake. Thus, when toads are dehydrated in an environment without water, they could maintain a low permeability, minimizing the evaporative water losses. Then, they raise it when water is present and finally decrease it rapidly when rehydrated, avoiding the problem of dilution of the body fluids by overshooting.

Acknowledgements—This study was supported by a research grant (P.I.D 3-039100/85) from the Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentina (CONICET).

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