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'

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Summary. 1. The water uptake (WU), urine production (U), overall water balance (WB) and body weight variations (bwv) were measured in normal (N) as well as in totally hypophysectomized, retrochiasmatic sectioned, infundibulum lesioned (HR) toads. Groups of animals were studied while adapted to tap water and during immersion in sucrose solutions of different concentrations (46, 92, 138, 220 and 230 mM). Subsequently the rate of water uptake (J_w) and the osmotic permeability coefficient (L_{PD}) were calculated. Also the effect on these variables of the subcutaneous administration of a non-competitive α -blocker (phenoxybenzamine: POB) was tested.

2. No correlation was found in normal controls between the osmotic pressure outside and WU, U, WB, bwv or J_w . J_w remained relatively stable from 46 to 184 mOsm of external osmotic pressure, apparently due to a compensatory increase of L_{PD} in the same group.

3. At a higher concentration of the external environment (220 mM) a drastic increase in L_{PD} was observed in normal animals but the effect was markedly attenuated in HR animals.

4. Alpha-adrenergic blockade with POB induced a significant and almost constant increase in WU, WB and J_w in normal animals in solutions up to 138 mM sucrose. These effects ceased when the external concentration reached 170 mM. A concomitant and stable increment in L_{PD} was also observed. These values provide an indirect estimate of the adrenergic factor controlling water movements across the skin of the toad in vivo (Segura et al. 1982b), which appear to be osmotically independent within this range. The copious increase in WU due to adrenergic blockade was unaffected by amiloride (10^{-4} M), so water transport cannot be linked with sodium transport.

5. The present results appear to establish that: (a) Permeability of the skin to water is related to the osmolarity of the external environment in the normal toad; (b) hypothalamic-hypophyseal mechanisms seem only in part to account for this responsiveness; (c) normal toads are able to compensate in some degree for the reduction in water uptake caused by an increase in the osmolarity of the external environment by an increase in the osmotic permeability of the skin.

Introduction

In previous papers (Segura et al. 1982a, b) we have described a central nervous mechanism involved in the control of the water economy of toads mediated by the adrenergic system. The nervous system appeared to limit water inflow across the skin, and this action to be independent of hypothalamohypophyseal factors. After complete destruction of the ADH (antidiuretic hormone) producing system, however, animals displayed a much stronger response of water influx and balance to adrenergic blockade, hence the existence of complex interactions between adrenergic and neurohypophyseal mechanisms on skin permeability had to be considered.

Abbreviations: WU water uptake; U urine production; WB overall water balance; bwv body weight variations; J_w influx of water; L_{PD} osmotic permeability coefficient; N normal (control) toads; HR hypophysectomized, retrochiasmatic sectioned, infundibulum lesioned toads; POB phenoxybenzamine

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Fig. 1. Photomicrograph of a parasagittal section of the midbrain showing degeneration of the magnocellularis preoptic nucleus (po) in a toad 4 weeks after total hypophysectomy, retrochiasmatic section and infundibular lesion. Note intense nuclear pycnosis, excentricity and vacuolization, severe damage of plasmatic membranes and retrograde degeneration of axons (a). V third ventricle

This paper describes the quantitation of adrenergic influences on water uptake in terms of osmotic permeability coefficient, and their relation to water permeability of the skin. A concurrent effect of increased osmolarity on skin permeability to water is also demonstrated, which partially depends on the integrity of the hypothalamo-hypophyseal axis.

Material and methods

Eighty adult male toads Bufo arenarum Hensel, weighing 110-130 g, were used. Experiments were carried out on unanesthetized normal and on totally hypophysectomized, postchiasmatic sectioned animals both in winter and in summer. Maintenance and the measurement of water uptake and urine production were as described previously (Segura et al. 1982a).

The rate of water uptake (J_w) (μ l·cm⁻²·h⁻¹) was estimated according to Rey (1937) and Barker-Jørgensen (1950) by the formula:

$$J_{\rm w} = \frac{\rm WU}{\rm S} = \frac{\rm WU}{9i/\rm bw^2}$$

where WU represents the water uptake in $g \cdot h^{-1}$, S the initial body surface in cm², and bw the body weight in g.

Concurrently, the osmotic permeability coefficient (L_{PD}) was calculated from the equation developed by Kedem and Katchalsky (1958):

$$J_{\rm w} = L_{\rm P} \varDelta P + L_{\rm PD} \varDelta \pi$$

where $L_{\rm P}$ is the hydraulic permeability coefficient, $L_{\rm PD}$ the osmotic permeability coefficient, ΔP the hydrostatic pressure and $\Delta \pi$ the osmotic pressure difference across the skin, i.e. $RT(O_i - O_e)$; R being the gas constant, T, the absolute temperature (293 °C, since all experiments were done at 20 °C) and O_i and O_e, the internal and external osmolarity, respectively. A O_i value of 230 mOsm was used in all calculations.

As
$$L_{\rm P} \Delta P$$
 was always zero, this reduces to
 $J_{\rm w} = L_{\rm PD} R T({\rm O_i} - {\rm O_e})$
and

$$L_{\rm PD} = \frac{J_{\rm w}}{R T({\rm O}_{\rm i} - {\rm O}_{\rm e})}.$$

а

These parameters were investigated in the following experimental groups:

Normal animals: (a) Intact toads individually caged were kept for 72 h in tap water, then WU and U were measured every 15 min for 1 h. The bath was then replaced by a solution of sucrose, and a second series of measurements lasting 2 h was done. Finally a fourth hour of observations was made after replacing the animals into tap water. (b) The same series of experiments was carried out in the same group of animals but a single dose of POB (phenoxybenzamine, 5 mg/kg subcutaneously) was injected at the end of the 1st h in sucrose. The concentrations of sucrose used were 46, 92, 138, 184, and 220 mM. The possible role of sodium on the effect of adrenergic blockers was investigated by adding amiloride (10^{-4} M) to the external medium. (c) A series of experiments using 150, 160 and 170 mM of sucrose was also carried out in order to define more precisely the osmolarity which would counteract the antiadrenergic positive effect on water uptake.

Totally hypophysectomized postchiasmatic sectioned and infundibulum lesioned toads were prepared according to the technique described by Segura et al. (1982a) and used 4 weeks after surgery. Complete degeneration of the preoptic magnocellular nucleus of the hypothalamus and its neurohypophyseal connections were histologically certified in all cases (Fig. 1). These animals were submitted only to the procedure (a) applied to normal animals. In all cases, groups of 6 toads were used in each experiment.

Statistical differences were established by means of the oneway analysis of variance and the Tukey test for group contrast or the paired t-test. The family regression analysis was also employed when necessary.



Fig. 2. Interactions of increased osmolarity of the external bath with the hydrosmotic response to phenoxybenzamine (P) in toads. S mM Sucrose molarity. Ordinates: WU water uptake. U urine production of normal toads. W tap water; S sucrose. Black or white dots: changes in water content in g% of body weight. Note that effect of the α -blocker was present in tap water, 46, 92 and 138 mM sucrose, whereas in 184 mM sucrose it disappeared. ***, P < 0.001. *, P < 0.05. Same abbreviations were used in all figures. Means \pm SE. Columns represent consecutive observation periods (hours) from left to right



Fig. 3. Effect of changes in the osmolarity of the bathing solution (range 140–170 mM sucrose) upon water uptake, urine production and water balance (changes in water content as g% body weight). Note that in 170 mM sucrose the blocking of increased water influx after addition of phenoxybenzamine (P) is clear. Other abbreviations as in Fig. 2

Results

Osmotic equivalence of the adrenergic action on water uptake

The copious water uptake induced by adrenergic blockers in normal and HR toads has already been described (Segura et al. 1982b). In order to obtain a convenient measure of this effect, the osmolarity of the bath was progressively increased. As Fig. 2 shows, the effect of phenoxybenzamine observed in water (W) persisted with 46, 92 and 138 mM



Fig. 4. L_{PD} changes in the skin of the toad in response to increases in the osmolarity of the external environment. *C*, control group. *POB*, group treated with phenoxybenzamine. *O_e*, external osmolarity in milliosmoles. Ordinate: percent of basal value. Note the significant differences between the C and POB groups from 46 to 138 mOsm. *, *P*<0.05; **, *P*<0.01. See text for further information

of sucrose outside and disappeared only when the external osmolarity reached 184 mM. An external solution of 170 mM exactly counteracted the adrenolytic facilitation of water uptake (Fig. 3).

Concurrently, increases in $L_{\rm PD}$ associated with the increased water uptake induced by POB were observed in concentrations of sucrose up to 184 mM (Fig. 4). Regression analysis showed that the data fitted a parabolic model (n=24, DF: 22; r=0.68, P<0.001). This gives significant and stable $L_{\rm PD}$ differences between normal and POB treated animals, in the range of 46 to 138 mM, with an average value of $68.69 \pm 4.19\%$. Similar differences were observed when changes in $J_{\rm w}$ were examined within the same range (Fig. 5). The hydrosmotic action of adrenergic blocking remained unaffected when 10^{-4} M of amiloride chloride was added to the water (Fig. 6, A and B).

L_{PD} and J_w changes in response to increased osmolarity of the environment

No differences in J_w nor in L_{PD} of normal animals were found when compared with HR animals maintained in tap water. J_w ranged from 7.44 ± 1.32 to $16.64\pm2.88 \,\mu l \, cm^{-2} \, h^{-1}$ (mean \pm SD) in four groups of normal controls and from 8.70 ± 1.4 to $17.01\pm2.41 \,\mu l \, cm^{-2} \, h^{-1}$ (mean \pm SD) in four groups of HR toads. Similarly the



1000 $rac{m}{2}$ 400- $ac{m}{2}$ 400- $ac{m}{2}$ 400- $ac{m}{2}$ 400- $ac{m}{2}$ 46 92 138 184 220 $ac{m}{2}$ 0, $ac{m}{2}$ 0, $ac{m}{2}$ mosm.

Fig. 7. Differences in the osmotic permeability coefficient (L_{PD}) responses (in percent of the basal value) to the external osmolarity ($O_e mOsm$) between control (C) and HR toads. Note that up to 138 mM of sucrose outside, there is no difference between both groups, whereas beginning with 184 mM sucrose a significant increase is evident

Fig. 5. Changes in water influx J_w in percent of the basal value, in relation to external osmolarity ($O_e mOsm$). Note the progressive change and negative correlation between O_e and J_w in HR animals, whereas normal animals (C) and those treated with phenoxybenzamine (*POB*) maintain a stable value of J_w up to 184 mOsm



Fig. 6. Lack of effect of amiloride addition (10^{-4} M) to the external medium on increased water uptake after administration of adrenergic blockers. a, Changes in water uptake (WU)and urine production (U) in toads maintained during four consecutive one hour-periods in tap water (W), tap water plus amiloride (A), water plus amiloride but toad injected with phenoxybenzamine (P, left) or guanethidine (G, right), and again in tap water (W). Black dots, negative overall water balance; white dots, positive balance. **b**, Changes in water flux (J) across the skin of toads maintained for two successive one hour-periods in tap water (W) or tap water plus 10^{-4} M amiloride, the toads being injected with phenoxybenzamine, (P, left) or guanethidine (G, right). Note that in no case amiloride (that inhibits sodium transport) is able to block increased water uptake brought about by the adrenergic blockers. Asterisks denote significant differences to preceding column: *, P < 0.05; **, P<0.01; ***, P<0.001

 $L_{\rm PD}$ did not differ significantly, 13.46 ± 2.39 units to 32.66 ± 5.57 units in normal, against 15.73 ± 2.53 to 22.69 ± 2.13 in lesioned toads.

The most interesting result was that normal toads reacted to the rise in external osmolarity with a significant increase in $L_{\rm PD}$. Regression analysis of these values fitted well with the parabolic model (n=24, DF: 22; r=0.83; P<0.001) (Fig. 4 and 7, C). $J_{\rm w}$ was constant over the range of 46 to 220 mM in normal animals and decreased abruptly in higher concentrations (Fig. 5, C). This was at variance with the results in HR toads which exhibited an almost perfect inverse correlation between $J_{\rm w}$ and the sucrose concentration in the bath (Fig. 5, HR). Regression analysis gave a good fit with a linear model (n = 31, DF: 29; r = 0.87; P <0.001). This increase on $L_{\rm PD}$ to raised external osmolarity was drastically affected but not completely abolished after destruction of the hypothalamichypophyseal system (Fig. 7, HR).

Finally, in no case were significant changes in overall water balance or in the body weight due to variations in the external osmolarity observed within the 4 h period of study.

Discussion

The water economy of anurans appears to depend upon complex physiological, chronobiological and behavioral factors (Schmajuk and Segura 1982). Physiological mechanisms are mainly related to the automatic resetting of variables like blood pressure and volume to reference levels by means of negative feedback actions, that depend basically on the osmolarity of the body fluids.

Biological rhythms and behavior have also been shown to be involved in the maintenance of water stores in several cases (Schmajuk and Segura 1982). In this context, toads and frogs have been intensively explored as suitable models for studying water and salt homeostasis, and the view that they behave as biological osmometers seems to be widely accepted. Hypothalamic and extrahypothalamic endocrine factors involved in the hydromineral metabolism have also been exhaustively studied in these species (Shoemaker and Nagy 1977). Nevertheless, neural and behavioral influences on the water economy of anurans have only recently been seriously taken into consideration (Gallardo et al. 1980; Segura et al. 1980, 1982a, b; Schmajuk and Segura 1982; Pang et al. 1982). The role played by the CNS and the adrenergic mechanisms involved in the control of the water economy of the toad, Bufo arenarum Hensel, have been well established by Segura et al. (1982a, b).

The experiments described in this paper were planned primarily to provide a simple but accurate measurement of adrenergic action upon water interchanges in the intact toad. The existence of an adrenergic tonus opposed to water inflow and equivalent to an osmotic pressure of 170 mOsm was established.

For a better understanding of the mechanisms involved, standard formulae developed for calculating the osmotic permeability coefficient of the skin ($L_{\rm PD}$) in vitro were applied to the intact toad. The fact that the mean basal $L_{\rm PD}$ found in our experiments, 5.99 ± 0.94 (SD) $\times 10^{-7}$ cm s⁻¹ atm⁻¹ is similar to that reported by De Sousa and Grosso (1982) for the skin of another species of toad, *Bufo marinus* (ca. 15×10^{-7} cm s⁻¹ atm⁻¹) in vitro, deserves to be emphasized. By this procedure a significant increase of $L_{\rm PD}$ was detected, coinciding with the copious water uptake produced by adrenergic blockers, and a close dependence of skin permeability on adrenergic influences clearly revealed.

Concurrently no differences were observed in the water uptake of HR toads when compared to normal controls adapted to tap water. This is in agreement with previous results (Segura et al. 1982a).

Values of J_w and L_{PD} were also similar in both groups, whereas the response to increased osmolarity of the environment was clearly different. Thus the rate of water uptake of HR decreased in close correlation with the raised osmolarity of the external bath, whereas normal toads were able to maintain a significantly higher level of water uptake without any demonstrable correlation with the environmental osmotic pressure, within the tested range. This response of the intact toads was unexpected since it is at variance with that observed in other species such as Bufo marinus and Bufo melanostictus when bathed in non-ionic solutions. Bentley (1957) and Dicker and Elliot (1967) found a close and inverse correlation between the external osmolarity and the rates of water uptake in vivo, the toads behaving as perfect osmometers. Unfortunately these authors did not estimate L_{PD} in their experiments. A similarly negative correlation between $J_{\rm w}$ and the concentration of sucrose in vitro, without changes in L_{PD} of the skin of *Bufo marinus*, has been described by Benedictis and Lacaz-Vieira (1982). However, as they worked in a lower range of hypotonic concentrations (less than 140 mM) the results cannot be considered in the present discussion. The question to which degree in vitro and in vivo techniques may be compared is debatable. The suppression of neurohumoral influences in isolated skin might account for these differences. The in vivo response of our HR group (i.e. toads deprived of their hypothalamic-hypophyseal hormone producing system) with regard to water influx is similar to that described by Benedictis and Lacaz-Vieira (1982) in vitro, in normal skins.

According to our results the modulatory action of adrenergic factors on water flow across the skin of the intact toad is clear. It has also been shown that this modulation coincides with changes in the osmotic permeability coefficient.

Recently, De Sousa and Grosso (1982) reported a significant hydrosmotic response to a β -adrenergic agonist (isoprenaline) in the skin of *Bufo marinus*, in vitro. This is in agreement with previous results from this laboratory (Segura et al. 1982b).

Since the hydrosmotic response to adrenergic blocking agents persists after complete exclusion of the ADH producing system, it is concluded that it does not depend on this hormone. On the other hand, experiments with amiloride demonstrate that the observed increases in J_w and L_{PD} are not dependent on Na fluxes either. Consequently, water channels or pores other than those affected by ADH or Na must be engaged in the hydrosmotic response described in this paper.

These data seem to confirm the view that adrenergic receptors of the skin act as limiting elements for water uptake in the toad. Moreover, their role in the adaptive control of water uptake across the skin in response to dessication should be investigated.

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