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 (Jw) and the osmotic permeability coefficient (Lpd) 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 Jw. Jw remained relatively stable from 46 to 184 mOsm of external osmotic pressure, apparently due to a compensatory increase of Lpd in the same group.

3. At a higher concentration of the external environment (220 mM) a drastic increase in Lpd 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 Jw 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 Lpd 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.

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

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 hypothalamo-hypophyseal 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.
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 \( J_u \) (\( \mu l \cdot cm^{-2} \cdot h^{-1} \)) was estimated according to Rey (1937) and Barker-Jørgensen (1950) by the formula:

\[
J_u = \frac{WU}{S + \frac{1}{bw^2}}
\]

where \( WU \) represents the water uptake in \( g \cdot h^{-1} \), \( S \) the initial body surface in \( cm^2 \), and \( bw \) the body weight in g.

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

\[
J_u = L_{wp}AP + L_{po}AP - \frac{J_u}{RT(O_e - O_i)}
\]

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

As \( L_{wp}AP \) was always zero, this reduces to

\[
J_u = L_{po}RT(O_e - O_i)
\]

and

\[
L_{po} = \frac{J_u}{RT(O_e - O_i)}
\]

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 one-way analysis of variance and the Tukey test for group contrast or the paired t-test. The family regression analysis was also employed when necessary.

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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, eccentricity and vacuolization, severe damage of plasmatic membranes and retrograde degeneration of axons (a). V third ventricle