ASYMMETRICAL PERMEABILITY OF THE INTEGU-MENT OF TREE FROGS (HYLIDAE)

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SUMMARY

The skin from four species of North American tree frogs (Hylidae) was found *in vitro*, to be remarkably permeable to water in the presence or absence of the neurohypophysial hormone vasotocin. This property was, however, only seen in preparations from the ventral integument; osmotic water movement across the dorsal skin was negligible. The ionic permeability, reflected by the electrical resistance, was also much greater in the ventral skin. Unidirectional fluxes of ^{22}Na, ^{36}Cl, ^{14}C-urea and tritiated water were measured *in vitro* in a single species, *Agalychnis dacnicolor* Cope. The ventral skin was 10–20 times more permeable to these molecules than the dorsal skin. The short-circuit current (usually reflecting active transmural Na transport) across the ventral skin was increased by aldosterone and vasotocin but these responses were absent on the dorsal side. The lipid content of the dorsal skin was four times greater than that on the ventral surface.

INTRODUCTION

The Amphibia generally possess skin which, compared to other tetrapods, is highly permeable to respiratory gases, water and solutes. This property is often considered to be a phyletic character that may have a considerable influence on their physiology and the selection of habitats where they can survive. It is now known that the permeability of the skin of amphibians is far from uniform and may display both inter-specific variation (Bentley, Lee & Main, 1958; Bentley, 1971) and differences between areas of the integument of the same animal (McClanahan & Baldwin, 1969; Bentley & Main, 1972; Bentley & Yorio, 1976).

The Hylidae (tree frogs) have a cosmopolitan distribution in temperate, tropical and even desert regions of both hemispheres (Darlington, 1957). The success of the geographical radiation of this family is probably only matched by the Ranidae and Bufonidae. Most hylids are arboreal, an ecological niche which may be expected to impose special osmoregulatory problems due to potential exposure to desiccating conditions and possibly a more limited access to free water. There are sporadic reports of appropriate cutaneous adaptations to such conditions. These adaptations include an ability to rehydrate rapidly (Main & Bentley, 1964), a pronounced rate of water uptake in response to neurohypophysial hormones (Heller & Bentley, 1965) and a low rate of evaporative water loss (Shoemaker *et al.* 1972).

In the present study we have measured osmotic water movement across the dorsal and ventral skin (*in vitro*) of several species of tree frogs, in the presence and absence of the neurohypophysial hormone vasotocin. There were considerable differences in the responses of skin from the two integumental surfaces. We have made a detailed comparison in a single species (*Agalychnis dacnicolor*) and measured unidirectional fluxes of Na, Cl, urea and water. The skin of this tree frog displays a number of unique characteristics which appear to be consistent with its manner of life.

METHODS

The tree frogs were obtained from local sources during the summer season (May to August). *Hyla arenicolor* Cope were kindly collected by Dr W. A. Calder of the University of Arizona in Tucson. The others were obtained commercially, *Agalychnis dacnicolor* from Southwestern Scientific, Tucson, Arizona, and *Hyla squirrella* Latreille and *Hyla femoralis* Latreille from Prince Biologicals, Silver Springs, Florida. They were kept in the laboratory at 19–20 °C, and used within 1 week after they were received.

In vitro preparations of the dorsal and ventral surface skin were made following double pithing of the frogs. For the initial experiments (Tables 1 and 2) pieces of skin were tied on to the end of a glass tube to form a diaphragm with a surface area of either 1·6 cm² or (for *A. dacnicolor*) 3·8 cm². The epidermal side (outside the frog) faced inwards and contained 3 ml of Ringer's solution or (for the measurement of osmotic permeability) a 1:10 dilution of this. The corium side (inside the frog) was bathed by 30 ml of Ringer's solution.

The electrical potential difference (PD) was measured by connecting each side of the skin preparations to a voltmeter (Keithley model 610C) through saturated KCl agar bridges and calomel cells. The short-circuit current (SCC) was applied from an external battery connected to each side of the skin through a similar pair of bridges and Ag–AgCl cells and the current was read with an ammeter.

Osmotic water movement was measured by weighing the tubes, to 1 mg, at intervals of 1 h.

For the determination of diffusional water flow (with tritiated H_2O) and urea permeability (with ^{14}C-urea), the dorsal and ventral surface skins of *A. dacnicolor* were tied to the end of a lucite cylinder (to form a diaphragm with a surface area of 10 cm²). The epidermal surface faced inwards and was bathed with 10 ml of Ringer's solution while the volume on the corium side was 20 ml. Vigorous mixing was accomplished with magnetic stirring bars, one of which was suspended on the epidermal side of the skin by a nylon thread attached to a stainless-steel fishline spinner. Isotopes (1 $\mu Ci/ml$) were added to the inside, and serial samples were taken from the cold side every 0·5 min for THO, and every minute for urea, for five successive periods. At no time did the activity of the 'cold' side exceed 2% that of the 'hot' solution. The unidirectional flux was determined from the specific activity of the 'hot' solution and was used to calculate the permeability coefficient, K_{trans}.

Unidirectional fluxes of Na and Cl were also determined on *A. dacnicolor* using ^{22}Na and ^{36}Cl as tracers. For these experiments, the dorsal and ventral surface skins were mounted in Ussing-type lucite chambers (surface area 3 cm²) and were maintained

Table 1. *Effects of vasotocin (4×10^{-8} M) on water transfer across the dorsal and ventral skin of tree frogs (Hylidae)*

	$\mu l \ cm^{-2} \ h^{-1}$	
	Control	Vasotocin present
H. arenicolor		
Dorsal (5)	0·1 ± 1	0·7 ± 1
Ventral (5)	18 ± 2	164 ± 8
H. squirrella		
Dorsal (6)	6 ± 1	4 ± 1
Ventral (6)	35 ± 4	110 ± 13
H. femoralis		
Dorsal (6)	1 ± 3	3 ± 2
Ventral (6)	20 ± 3	254 ± 11
Agalychnis dacnicolor		
Dorsal (5)	3 ± 2	2 ± 2
Ventral (5)	78 ± 15	182 ± 27

Results are as means ± S.E. Number of experiments in parentheses.

under short-circuited conditions with an automatic voltage clamp with the SCC continually recorded on a strip chart recorder. At least 30 min was allowed for isotope equilibration and samples were then collected from the side not exposed to isotopes at 15 min intervals, for four successive periods. A stable flow of isotope was an indication of isotope equilibration. The fluxes were determined from the specific activity of the radioactive solution.

Total lipid content of the dorsal and ventral skins of *A. dacnicolor* were determined according to the method of Schmid & Barden (1965).

The Ringer's solution had the following composition (mM): NaCl, 111; KCl, 3·35; $CaCl_2$, 2·7; $NaHCO_3$, 4·0; and glucose, 5·0. This solution was aerated and the pH was about 8·0.

^{14}C-urea, THO, ^{22}Na and ^{36}Cl were purchased from New England Nuclear Corp., Boston, Mass., and 8-arginine vasotocin from Schwartz/Mann, Orangeburg, N.Y.

RESULTS

Water transfer across the skin: responses to vasotocin

Osmotic water transfer across the skin from the dorsal and ventral surfaces of the frogs was measured *in vitro* in four species. Skin from the ventral surface was quite permeable to water in contrast to that from the dorsal side, where in some instances it was negligible (Table 1). The neurohypophysial hormone vasotocin increases the permeability of amphibian skin to water but this response could not be detected in dorsal integument of the tree frogs. The ventral skin, however, displayed a response which is larger than that which has been observed *in vitro* in other anurans.

Transcutaneous PD, short-circuit current and electrical resistance of the skin

Amphibian skin is permeable to ions and the active transport of these results in a transcutaneous PD and SCC. It can be seen (Table 2) that the SCC was greater (*in vitro*) in the ventral skin of the frogs. The electrical resistance, which reflects the

Table 2. PD, SCC and electrical resistance of dorsal and ventral
skin of tree frogs (Hylidae)

	PD (mV)	SCC (μA cm^{-2})	Electrical resistance (ohms cm^2)
H. arenicolor			
Dorsal (5)	66±5	15±2	4400
Ventral (5)	43±4	56±5	768
H. squirrella			
Dorsal (6)	26±2	17±3	1529
Ventral (6)	32±9	38±7	824
H. femoralis			
Dorsal (6)	29±4	15±2	1933
Ventral (6)	13±2	21±2	619
Agalychnis dacnicolor			
Dorsal (5)	20±3	8±2	2500
Ventral (5)	61±17	118±81	508

Electrical resistance was calculated as PD/SCC.

Table 3. Influx (J_{in}) and efflux (J_{out}) of Na and Cl in ventral and
dorsal skin of the tree frog Agalychnis dacnicolor

	Ventral	Dorsal
I. Sodium J_{in} (8) (μ-equiv. cm^{-2} h^{-1})	1·52±0·08	0·40±0·08*
PD (mV)	22±5	28±5
I_{scc} (μA cm^{-2})	51±5	15±4*
Sodium J_{out} (6)	0·20±0·02	0·08±0·02*
PD	22±3	28±3
I_{scc}	68±8	16±4*
II. Chloride J_{in} (6)	2·45±0·36	0·11±0·01*
PD	17±3	25±5
I_{scc}	52±4	15±5*
Chloride J_{out} (6)	3·07±0·64	0·23±0·06*
PD	20±3	27±4
I_{scc}	64±6	16±4*

* $P < 0.001$ for differences between the means, dorsal versus ventral.

membrane's permeability to ions, can be calculated from the PD and SCC and was found to be much greater across the dorsal than the ventral part of the integument in all of the species studied.

Unidirectional fluxes of solutes and water across the skin of Agalychnis dacnicolor

Additional specimens of *Agalychnis dacnicolor* became available so that it was possible to do a more detailed study of the permeability of its skin.

(i) *Sodium.* The influx of Na exceeded the efflux in both dorsal and ventral skin, which is consistent with the presence of an active Na transport mechanism. The fluxes in both directions were, however, much less in the dorsal than in the ventral skin. Influx and efflux measurements were made using skin preparations obtained from different animals so that the net flux across either the dorsal or ventral skin can only be estimated. It appeared, however, to be substantially less than that calculated from the

Table 4. *Passive permeability of the ventral and dorsal skin of the tree frog*
A. dacnicolor to water (as THO) and urea (as ^{14}C-urea)

	Flux (μl cm^{-2} h^{-1})	$K_{trans} \times 10^7$ cm s^{-1}
Water		
Dorsal (5)	26 ± 7	263 ± 74
Ventral (5)	657 ± 55	6640 ± 580
Urea	$K_{trans} \times 10^7$ cm s^{-1}	
Dorsal (5)	8 ± 0·7	
Ventral (5)	122 ± 19	

The unidirectional flux for water measured in the direction epidermis to corium
(outside to inside) and for urea corium to epidermis.

Table 5. *Effects of aldosterone (10^{-6} M) and vasotocin (4×10^{-8} M)*
on PD (mV) and I_{SCC} (μA cm^{-2}) across the dorsal and ventral skin of the
tree frog A. dacnicolor

		Initial	17 h after 10^{-7}M aldosterone	Mean increase I_{SCC}
Ventral (5)	PD	20 ± 2	52 ± 14*	—
	I_{SCC}	61 ± 5	110 ± 8*	50 ± 8**
Dorsal (6)	PD	37 ± 3	58 ± 5*	—
	I_{SCC}	21 ± 6	31 ± 6	10 ± 6
			30 min after vasotocin	
Ventral (5)	PD	61 ± 17	72 ± 18	—
	I_{SCC}	118 ± 36	242 ± 44	124 ± 29**
Dorsal (5)	PD	20 ± 3	22 ± 2	—
	I_{SCC}	8 ± 2	10 ± 2	2 ± 2

** $P < 0.01$, * $P < 0.05$ for differences between the means.

SCC (net Na flux × 26·8 = SCC in μA), suggesting that there may also be a net transport of other ions across the skin.

(ii) *Chloride*. The permeability of skin from the ventral surface to chloride was 10–20 times greater than seen in the dorsal skin (Table 3). Efflux of Cl was generally greater than influx but this difference was not statistically significant. However, it is known that the skin glands of anurans can secrete Cl in an outward direction (Koefoed-Johnsen, Ussing & Zerahn, 1952). Such a Cl extrusion would tend to increase the SCC and could account for the disparity between this value and that calculated from the net Na flux.

(iii) *Water*. The unidirectional flux of water using tritiated water (the diffusion permeability) was measured in the absence of an osmotic gradient and in the direction epidermis to corium. The permeability of the ventral side was 20–30 times greater than that of the dorsal skin (Table 4).

(iv) *Urea*. Amphibian skin is permeable to urea and in these tree frogs the ventral skin was about 15 times more permeable than that from the dorsal surface (Table 4).

Table 6. *Permeability of the skin of* Agalychnis dacnicolor *to water and urea as compared to observations on other anurans*

| | Permeability coefficient ($K_{trans} \times 10^7$) | | | |
| | Ventral skin | | Dorsal skin | |
	Water	Urea	Water	Urea
Agalychnis dacnicolor	6640	122	263	8
Bufo marinus[*]	1673	1·3	872	1·3
Rana pipiens[*]	5217	4	4241	5

[*] From Bentley & Yorio (1976).

Effects of vasotocin and aldosterone on short circuit current

The hormones aldosterone and vasotocin increase Na transport, and its reflected SCC, across anuran skin. These responses were also seen in *A. dacnicolor* (Table 5) but were only apparent in skin from the ventral surface.

Lipid content

Lipids play an important role in determining the permeability of biological membranes. Skin from the dorsal surface of *A. dacnicolor* contained $0·64 \pm 0·13$ mg lipid cm^{-2} whereas that from the ventral surface contained only $0·17 \pm 0·02$ mg cm^{-2} (means $\pm$ S.E. for 5 skins, $P < 0·01$ for differences between the means). The dorsal skin was also somewhat thicker than the ventral tissue, weighing $39 \pm 1·5$ mg cm^{-2} compared to $31 \pm 1·4$ mg cm^{-2} for the ventral side (7 skins, $P < 0·01$ for mean difference). Thus the dorsal skin lipid content was also greater on a unit weight basis.

DISCUSSION

The integument of four species of North American tree frogs displayed an asymmetrical permeability to water and solutes. The dorsal region of the integument was much less permeable than the ventral one, and it also lacked responses to the hormones vasotocin and aldosterone. Such differences in cutaneous permeability have been observed previously in species from other families of the Anura (see Introduction) but these were not as great as described in the present observations nor have they usually been correlated with unidirectional fluxes.

The rates of osmotic water movement across the ventral skin of the tree frogs appear to be greater than have been previously observed *in vitro* in other anurans (see Bentley, 1971). Thus in *H. femoralis* a water transfer of $250 \mu l$ cm^{-2} h^{-1} was observed in the presence of vasotocin while comparable values in the Ranidae are only $20–60 \mu l$ cm^{-2} h^{-1}. A rate of $420 \mu l$ cm^{-2} h^{-1} has been observed in the pelvic skin of the desert toad *Bufo punctatus in vivo* (McClanahan & Baldwin, 1969). The rate of water transfer across the ventral skin of the tree frogs used in the present study is consistent with rates of water uptake seen *in vivo* in dehydrated Australian tree frogs (Main & Bentley, 1964) and the European *Hyla hyla* following injection with vasotocin (Heller & Bentley, 1965).

The permeability of the skin of *Agalychnis* to Na is in a range which is similar to

that observed in other anurans such as *Rana pipiens* and *Bufo marinus* (Bentley & Yorio, 1976). However, the movement of Cl^- across the ventral skin is much more rapid than that observed in most other species, where it is more similar to that seen in the dorsal skin from *Agalychnis*. This high Cl permeability would appear to be the principal determinant of the observed low electrical resistance of this region of the skin, though the transfer of Na may also contribute.

A comparison of the permeability of the skin of *Agalychnis dacnicolor* to water and urea, with that of *Rana pipiens* and *Bufo marinus* is shown in Table 6. These measurements were all made under identical conditions, including the rate of stirring of the bathing solutions. The permeability of the ventral skin to water was not remarkable compared to the other species but the dorsal skin was the least permeable. Although the permeability to water and urea in the skin of the tree frog appear to be parallel in the dorsal and ventral surfaces, this relationship is probably fortuitous as it is clearly not seen interspecifically. The cutaneous permeability to each molecular species appears to be reflecting different processes (Levine, Franki & Hays, 1973).

The reasons for the differences in permeability of the skin from the dorsal and ventral surfaces of the tree frogs are unknown. The dorsal skin is about 30 % thicker than that of the ventral surface but it is unlikely that this can account for the differences in permeability. The lipid content of the skin of *A. dacnicolor* was about four times greater on the dorsal than on the ventral surface. It has been suggested (Schmid & Barden, 1965) that the lipids in frog skin may restrict its permeability to water. The present observations are consistent with such a possibility, though it could also be related to the permeability to other species, such as Cl and urea. The integument of *Rana pipiens* does not display an asymmetrical permeability (Bentley & Yorio, 1976) and it is interesting that the lipid content is the same in the skin from both the dorsal and ventral surfaces of this frog (Yorio & Bentley, unpublished observations). It should, however, be emphasized that these relationships between the lipid content of the skin and its permeability are only correlations and do not exclude other mechanisms.

One can only speculate about the possible biological significance of an asymmetrically permeable integument. The dorsal surface of tree frogs is probably more generally exposed than the ventral side. The latter, on the other hand, may be apposed to moisture on the surfaces of trees and bushes so that a high permeability may facilitate water uptake across this otherwise more protected surface. The possible physiological significance of the difference in ionic permeability is unknown.

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