

## Basal and Amiloride-Induced Short-Circuit Current Across Isolated Toad Skin (*Bufo arenarum*)

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**Abstract.** We have previously demonstrated that amiloride (amil) addition to the isolated ventral pelvic (VPel) skin of *Bufo arenarum* toad induces negative short-circuit current values, which are equivalent to the isotopically measured net chloride transport. In the present work, we found that exposure of various regions of toad skin to amil yielded different values of short-circuit current (aSCC): negative aSCC was found in the VPel and ventral pectoral skin, while those of the dorsal one were not different from zero. The distinct values of aSCC found show a regional difference in the active chloride absorption, probably related to postural adaptations. A possible role of this adaptation would be related to chloride participation in the saline balance of the animals, or the maintenance of epithelial integrity.

**Key words:** Toad skin — Short-circuit current — Reversed SCC — Zonal differences — Amphibia — *Bufo arenarum*

### Introduction

Functional differences between different regions of the toad skin have been described both in vivo (McClanahan and Baldwin 1969) and in vitro (Bentley and Main 1972; Baldwin 1974; Christensen 1974; Marrero and Hillyard 1985). These differences may be attributed to an adaptation related with toad posture (Stille 1958).

*Bufo arenarum* Hensel has terrestrial habits, and its isolated pelvic skin actively transports sodium and chloride ions (Berman et al. 1986). After addition of amiloride — a blocker of apical sodium channels (Bentley 1968) — the short-circuit current (SCC), a measure of net ion transport, goes down to negative values, which are equal to the isotopically measured net chloride flux (Berman et al. 1987).

In the present paper we compare the SCC values in skin isolated from different regions of the toad body, under basal conditions (bSCC) and after amiloride addition (aSCC), and the relationship between bSCC and aSCC for each zone.

## Materials and Methods

*Bufo arenarum* toads of either sex, collected in Tucumán, were kept in tap water overnight (moulting animals were not used). After double pithing, symmetrical pieces of skin from different regions of the toad body were dissected according to Bentley and Main (1972): from the ventral pelvic (VPel), ventral pectoral (VPect) and dorsal (D) region. Each skin was rinsed and mounted as a flat sheet (3.1 cm<sup>2</sup> in area) between two plastic chambers. The solutions in each chamber (5 ml) were stirred and aerated by bubbling with air. Standard Ringer solution used contained (mmol/l): NaCl 90; KCl 3; CaCl<sub>2</sub> 2; MgSO<sub>4</sub> 1; glucose 5; Tris-HCl buffer 25; pH 7.4; osmolality 220 mOsm/kg H<sub>2</sub>O. Experiments were performed at room temperature (20–24°C), and conducted during all seasons in 1986. Although seasonal variations were observed in the measured values, the transport patterns across the different regions were similar through the different seasons, so the mean value of SCC is reported here.

The short-circuit current (SCC) was measured, as described by Ussing and Zerahn (1951), with an automatic device (Iglesias et al. 1983). The skins were kept short-circuited continuously, except for brief intervals for potential difference measurements. Results are expressed as individual values of the SCC and as the mean  $\pm$  SEM ( $\mu$ A/cm<sup>2</sup>). Student's *t* test for paired data was used for statistical analysis and significance probabilities for the correlation coefficient.

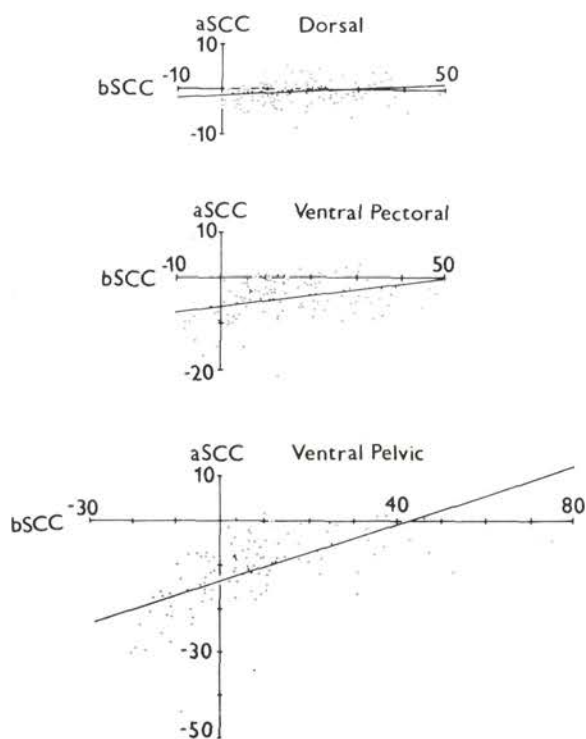
## Results

Table 1 shows the mean values of SCC ( $n = 115$ ) under basal conditions (bSCC) and 5 min after amiloride addition (amil, 0.08 mmol/l) to skin patches isolated from VPel, VPect and D regions. The bSCC of VPel are lower than those for other zones. Amil reversed VPel and VPect SCC to negative values. The mean differences between VPel and VPect in amil-induced SCC (aSCC) is  $7.0 \pm 0.5 \mu\text{A}/\text{cm}^2$ ,  $n = 115$ ,  $p < 0.01$ . The aSCC of D skin did not go down to negative values. Moreover, as all the amil-blocked SCC is equivalent to the active sodium transport (Bentley 1968), we have calculated the SCC before and after amil addition, i. e. the effect of amil (delta SCC) in the different regions used. It can be seen in Table 1 that there was no difference in the amil-induced delta SCC (basal sodium transport) across the regions tested.

Fig. 1 shows the relationship between the individual values of bSCC and aSCC for each skin region. Spontaneously negative bSCC occurred in VPel skin (35 % frequency) and in VPect skin (9 % frequency) while no negative bSCC was observed across D skin. There was a positive correlation between bSCC and aSCC in skin of the three regions tested (Table 2) and a variation in the values of correlation parameters as well (VPel > VPect  $\geq$  D). The difference between the negative aSCC across the three zones tested and the correlation between bSCC and aSCC support the participation of chloride transport, the component that originates negative SCC in VPel and VPect region in bSCC.

**Table 1.** Effect of amiloride (amil, 0.08 mmol/l) on basal SCC (bSCC). Amil was added to the epidermal bath of skin isolated from different regions of the toad body. Values after 5 min of amil exposure (aSCC) and the amil effect (delta, bSCC + aSCC) are shown. Values are mean  $\pm$  SEM of SCC ( $\mu\text{A}/\text{cm}^2$ ). Number of experiments = 115.

	Ventral Pelvic	Ventral Pectoral	Dorsal
bSCC	$6.8 \pm 1.6$	$14.9 \pm 1.2$	$15.9 \pm 1.0$
aSCC	$-11.6 \pm 0.8$	$-4.6 \pm 0.5$	$-0.6 \pm 0.2$
bSCC + aSCC	$18.4 \pm 1.3$	$19.5 \pm 1.1$	$16.7 \pm 0.9$



**Fig. 1.** Relationship between SCC in the basal state (bSCC) and amiloride-induced SCC (aSCC, 0.08 mmol/l) across isolated skin from different regions of the toad body. Each point represents individual basal SCC values and those after (5 min) amiloride; SCC values are expressed in  $\mu\text{A}/\text{cm}^2$ . The regression lines were calculated and coefficients are given for 1:1 relationship. Number of experiments = 115.

**Table 2.** Correlation values for the relationship between basal SCC and amiloride-induced SCC across skin isolated from different regions of the toad body. The values shown correspond to those shown in Figure 1. *p* represents statistical probability of significance for *r*.

	Ventral Pelvic	Ventral Pectoral	Dorsal
<i>r</i>	0.57	0.27	0.20
slope	0.30	0.11	0.05
<i>p</i>	< 0.001	< 0.005	< 0.05

## Discussion

In the present work, we show differences in the bSCC across isolated skin from VPel, VPect and D regions of the toad. By means of amil, we could dissect the components originating bSCC, and found that the different values were due to regional differences in net chloride — but not sodium — transport.

The fact that the highest values of chloride transport are localized in the VPel region would indicate a postural adaptation, as that region is the one of greatest exposure to the soil. The probable role of this adaptation would be related to chloride participation in the saline balance of the animals, or to the maintenance of epithelial integrity (by means of cell volume regulation).

The results reported here point out the importance of specifying the body regions selected when transport studies through isolated amphibian skin are conducted. Moreover, the different cutaneous regions may offer different models for studying transport phenomena.

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