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CHLORIDE CONDUCTANCE ACROSS TOAD SKIN: EFFECTS OF IONIC ACCLIMATIONS AND CYCLIC AMP AND RELATIONSHIP TO MITOCHONDRIA-RICH CELL DENSITY

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Summary

The anionic conductance across toad (*Bufo viridis*) skin was studied using the voltage-clamp technique following long-term (more than 10 days) acclimation to NaCl and KCl solutions. The non-specific baseline conductance was approximately 0.6 mS cm^{-2} and was similar in skins from all acclimation conditions. The voltage-activated Cl$^-$ conductance (G_{Cl}) was maximal in skins from distilled-water- and KCl-acclimated toads (>3 mS cm^{-2}) and was greatly reduced following acclimation to NaCl solutions. Cyclic AMP (EC_{50}=13 μmol l^{-1}) and isobutylmethyl xanthine (IBMX) (EC_{50}=69 μmol l^{-1}) exerted different effects on the activated conductance. IBMX only sensitized the activated conductance, whereas cyclic AMP (CPTcAMP) at high concentrations induced an increase in anionic conductance that was insensitive to electrical potential. Furthermore, external Cl$^-$ was not required for the stimulatory effect of cyclic AMP, and the conductive pathway had low selectivity. The effects of the two agonists were reversible and depended on the acclimation conditions. Following electrical measurements, the skin of the toads was removed and stained with silver to measure mitochondria-rich cell density (D_{mrc}). There was no correlation between D_{mrc} and Cl$^-$ conductance in the present study.

Key words: toad, *Bufo viridis*, skin, anion conductance, IBMX, NaCl, KCl, NO_3^-, amiloride, mitochondria-rich cell.

Introduction

The Cl$^-$ conductance (G_{Cl}) across amphibian skin epithelium has been studied extensively in the past two decades (for reviews, see Larsen, 1991; Katz and Nagel, 1994). The transport of Cl$^-$ is passive, but a selective transepithelial conductance is activated by an inwardly directed (inside-positive) electrical potential. The voltage-activated conductance shows a characteristic, non-linear pattern of time-dependent increase. The pathway is assumed to be localized to the mitochondria-rich (MR) cells of the epithelium, but this is still under debate because recent studies could not localize more than 20 % of the total Cl$^-$-dependent current over MR cells (Nagel et al., 1998). Cyclic AMP and IBMX (isobutylmethyl xanthine) exert different effects on transepithelial anionic conductance (Katz and Nagel, 1995), but the molecular mechanisms involved have not been resolved. The Cl$^-$ conductance is greatly decreased in the skin of toads acclimated to high external [NaCl] (Katz and Larsen, 1984), and this was paralleled by a decrease in MR cell density. However, later studies found that, in both frog and toad skin after acclimation to KCl, the density of mitochondria-rich cells (D_{mrc}) is maintained or increased (Ehrenfeld et al., 1989; Katz and Gabbay, 1995). Direct methods have failed to solve the problem of localization of G_{Cl} in amphibian skin. Therefore, an indirect approach using the conventional voltage-clamp technique was undertaken, taking advantage of the large variability in G_{Cl} and D_{mrc} in *Bufo viridis* (Katz and Gabbay, 1995) acclimated to different conditions. In the present study, the effects of long-term acclimation to NaCl and KCl, the effects of cyclic AMP and IBMX on the anionic conductance and the density of MR cells in the skin of the toad under these conditions were investigated.

Materials and methods

Toads (*Bufo viridis*) were of local origin. They were maintained in the laboratory (19–22 °C) with free access to tapwater (40–50 mosmol kg^{-1}; approximately 15 mmol l^{-1} Cl$^-$) and fed mealworms once a week. For acclimation, the toads were kept in a solution 2–3 cm deep of appropriate ionic composition; acclimation to high [NaCl] (200 mmol l^{-1}) was achieved in steps over 6–8 days. No mortality occurred over the course of the experiments. Plasma (collected from the vena angularis in the mouth) and urine (collected through the cloaca in plastic tubes) composition were determined using a Wescor 5520 vapour pressure osmometer (osmolality), a Corning 480 flamephotometer ([Na^+] and [K^+]) and a Radiometer (Copenhagen) chloridometer ([Cl$^-$]).

Electrophysiological measurements were performed on

pieces of abdominal skin, as described previously (Katz and Nagel, 1995). Briefly, the skin was mounted in a modified Ussing chamber (area 0.5 cm^2) continuously perfused at more than 4 ml min^{-1} with Ringer's solution on both sides. Normal Ringer had the following composition (in mmol l^{-1}): Na^+, 115; K^+, 2.5; Ca^{2+}, 1; Mg^{2+}, 1; Cl^-, 117; Hepes, 3.5; pH 7.6. Ionic replacements were made on an equimolar basis. After elimination of Na^+ transport by the addition of 10 μmol l^{-1} amiloride to the mucosal fluid, tissues were depolarized to −30 mV to deactivate the Cl^- conductance (G_{Cl}). To activate G_{Cl}, transepithelial potential was clamped intermittently to +80 mV (serosa-positive). Reported values of G_{Cl} reflect the difference between the total conductance (G_t) at −30 and +80 mV.

Dose–response relationships for the effects of cyclic AMP and IBMX on the deactivated conductance and on voltage-activated G_{Cl} and G_t were measured after normalization of the values to that under the respective control conditions. A sigmoidal regression function was obtained by data fitting using a commercial software package (Origin, Microcal Inc.). CPTcAMP (8-(4-chlorophenylthio) cyclic AMP), the permeable, non-hydrolysable derivative of cyclic AMP, and IBMX were purchased from Sigma.

The density of MR cells (D_{mrc}) was estimated by counting silver-stained cells in the pieces of skin used in the electrophysiological analyses. Briefly, the skin was rinsed with distilled water, incubated for 5 min with 0.25 % $AgNO_3$ and then washed and exposed to strong illumination for approximately 30 min. The diameter of the apical aperture of these cells was measured using a scaled eyepiece micrometer.

Statistical analyses were performed using analysis of variance (ANOVA). Results are presented as means ± S.E.M.

Results

The toads used in the present study were from two series of experiments (40 toads in winter and 12 toads in summer). The animals were acclimated to tapwater and distilled water, to 50 and 200 mmol l^{-1} NaCl and to 50 mmol l^{-1} KCl for more than 12 days. Table 1 summarizes the plasma and urine ionic composition in toads (winter series) acclimated to tapwater, 50 mmol l^{-1} KCl and 200 mmol l^{-1} NaCl. The plasma osmolality remained relatively high in control, tapwater conditions in *Bufo viridis*, which is a characteristic of this species, compared with the value in most other amphibians (Table 1). Urea accounts for the difference between the measured osmolality and that calculated from measurements of the ionic composition. In all conditions, plasma ion levels remained fairly stable, but the urine was always hypotonic to the plasma. Plasma [K^+] nearly doubled in toads acclimated to KCl and 200 mmol l^{-1} NaCl (although the difference is not significant; P=0.053), and plasma [K^+] in the urine was significantly higher than the control value only in the KCl-acclimated toads (P<0.001).

The physiological state of the skin can be judged from the short-circuit current (I_{sc}; active Na^+ transport) recorded at the start of each experiment. I_{sc} was 21±4 μA cm^{-2} in skins from distilled-water-acclimated toads, 21±3 μA cm^{-2} in skins from tapwater-acclimated toads and 36±7 μA cm^{-2} in skins from KCl-acclimated toads. The I_{sc} of skins from NaCl-acclimated toads tended to reduce (P=0.5) for those acclimated in 50 mmol l^{-1} NaCl (18±5 μA cm^{-2}) and diminished for those acclimated to 200 mmol l^{-1} (8±5 μA cm^{-2}) compared with tapwater-acclimated toads. Only in the last group was the value significantly lower than in the other groups (P<0.05).

Movements of Cl^- across the skin were analyzed in non-transporting conditions after apical application of 10 μmol l^{-1}

Table 1. *Plasma and urine composition in toads acclimated to tapwater, NaCl and KCl solutions*

	Acclimation condition		
	Tap water	50 mmol l^{-1} KCl	200 mmol l^{-1} NaCl
Plasma			
Osmolality (mosmol kg^{-1})	362±15	384± 6	416±12
Na^+ (mmol l^{-1})	134± 9	142±10	189± 9
K^+ (mmol l^{-1})	4.9±0.8	7.2±0.4	7.0±0.7
Cl^- (mmol l^{-1})	115± 6	116± 2	220±11
Urine			
Osmolality (mosmol kg^{-1})	128±11	203±14	324±22
Na^+ (mmol l^{-1})	9.3±3.0	3.1±0.6	136±20
K^+ (mmol l^{-1})	4.8±1.5	26±1.6	7.3±2.2
Cl^- (mmol l^{-1})	15± 3.2	26±1.5	141±21

Values are means ± S.E.M. for 4–6 toads from the winter series.

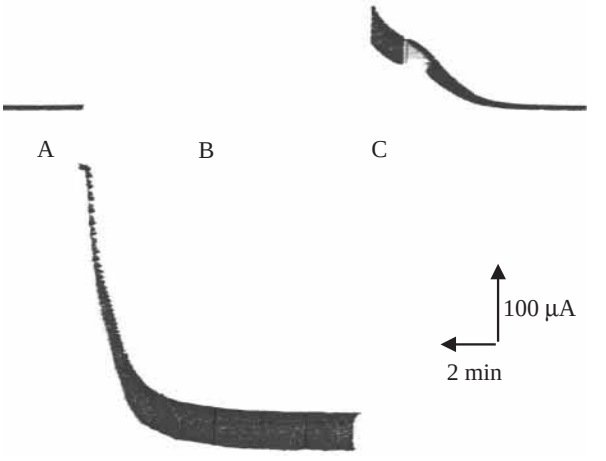

Fig. 1. Typical recording of the current response to transepithelial voltage perturbation across toad skin (tapwater-acclimated toad), mounted in an Ussing chamber with normal Ringer's solution on both sides. The voltage was stepped from −30 mV (A) to +80 mV (B) and back to −30 mV (C; deactivation). Note the time-dependent increase in current (activated conductance) and the time course of deactivation. The transepithelial conductance is calculated from the small current responses to constant external 10 mV pulses.

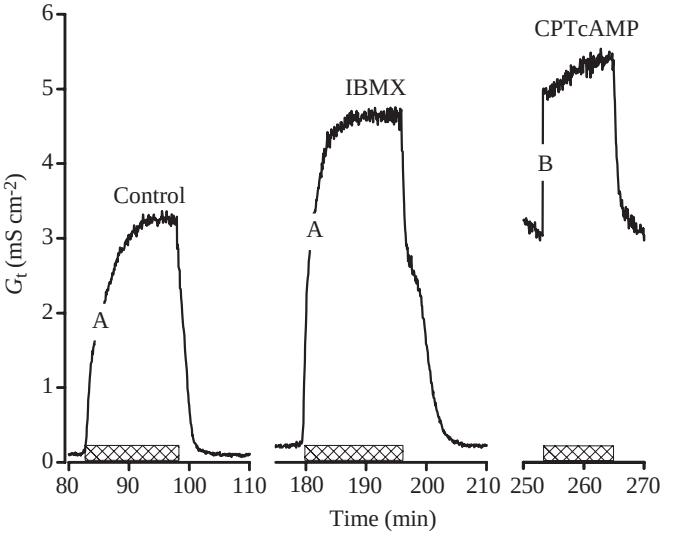

Fig. 2. The effects of IBMX (100 μmol l^{-1}, apical) and CPTcAMP (200 μmol l^{-1}, serosal) on transepithelial and voltage-activated (+80 mV, crossed-hatched bars) conductances. (A) Voltage-activated conductance; (B) voltage-induced conductance. This piece of skin was taken from a KCl-acclimated toad. G_t, total anion conductance.

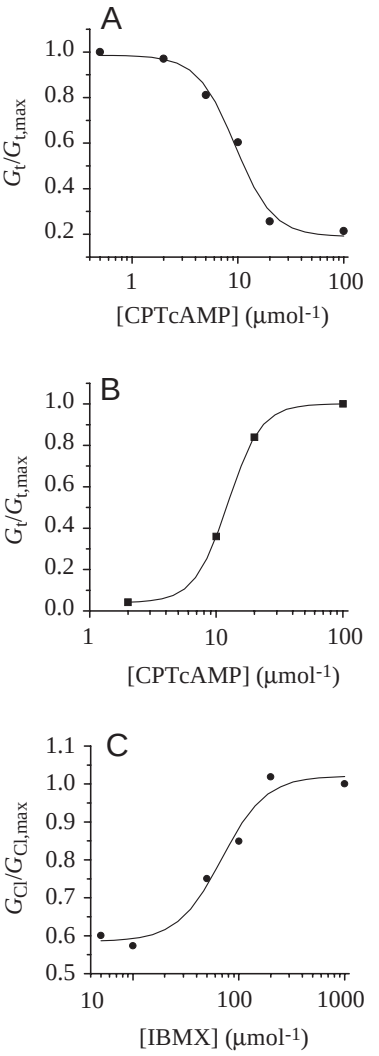

Fig. 3. Dose–response relationships for the effects of CPTcAMP and IBMX on the anion conductance across toad skin (see Fig. 2). (A) Reduction of voltage-activated total conductance (G_t) by CPTcAMP, IC_{50}=9 μmol l^{-1}; (B) stimulation of voltage-induced G_t by CPTcAMP, EC_{50}=13 μmol l^{-1}; (C) sensitization of the voltage-activated Cl$^-$ conductance (G_{Cl}) by IBMX, EC_{50}=69 μmol l^{-1}. Values (G_t, G_{Cl}) are normalized to the maximal value ($G_{t,max}$, $G_{Cl,max}$).

amiloride. Similar results were obtained in the two series, and the data were pooled. Numerical values of current and conductance were obtained from recordings such as that shown in Fig. 1. The effects of IBMX and CPTcAMP on the total conductance (G_t) are shown for a representative skin in Fig. 2. Voltage activation (+80 mV) was sensitized reversibly by IBMX, whereas high concentrations of cyclic AMP led to an elevation of the baseline conductance. Moreover, following the addition of cyclic AMP, transepithelial conductance was almost insensitive to voltage activation, and became time-independent within the resolution of the system (Katz and Nagel, 1995). External Cl$^-$ was not required for activation in this condition, and the effects were reversible.

The concentrations of cyclic AMP and IBMX used were supramaximal, as is evident from dose–response curves (Fig. 3) with either CPTcAMP (Fig. 3A,B) or IBMX (Fig. 3C). The EC_{50} values calculated from these experiments were 9–13 μmol l^{-1} for CPTcAMP and 69 μmol l^{-1} for IBMX.

The baseline transepithelial conductance (–30 mV depolarization) and the voltage-activated (+80 mV) Cl$^-$-specific conductance (G_{Cl}) are shown in Fig. 4 for control (tapwater) and other conditions of acclimation. The non-specific conductance (hatched columns) was similar under all acclimation conditions. The voltage-activated (+80 mV) Cl$^-$-dependent conductance G_{Cl} (open columns) varied among the various acclimation groups. The higher the [NaCl] in the acclimation solution, the lower the absolute value of the activated conductance. Note that this conductance was lost from the skins of toads acclimated to 200 mmol l^{-1} NaCl. The high G_{Cl} values of skins from KCl- and distilled-water-acclimated toads did not differ significantly from each other.

The effects of IBMX and CPTcAMP are summarized separately for the deactivated and activated conductances in Fig. 5. The effects in the deactivated (–30 mV) condition in skins from all acclimation groups are shown in Fig. 5A. IBMX (100 μmol l^{-1}) had a very small and usually only transient effect (not significantly different from the control value in all groups), while cyclic AMP (200 μmol l^{-1}) greatly increased the transepithelial conductance in this condition. Similar results were obtained under hyperpolarized (+80 mV) conditions (hatched columns), showing that the conductance is insensitive to the transepithelial potential. Only in the skins from distilled- and tapwater-acclimated toads was the difference between the deactivated and activated conductances in the presence of cyclic AMP significant (P<0.05). Inhibition of the $Na^+/K^+/2Cl^-$ cotransporter by bumetanide (100 μmol l^{-1};

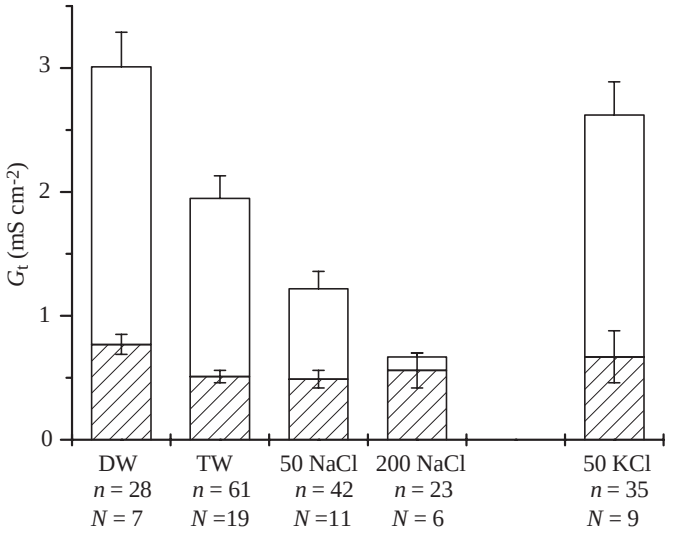

Fig. 4. The effect of ionic acclimation to 50 mmol l^{-1} NaCl or KCl or 200 mmol l^{-1} NaCl on anion conductance (G_t) across toad skin. Hatched columns show the baseline, deactivated conductance (–30 mV). Open columns show the steady-state voltage-activated (+80 mV) conductance, G_{Cl}. The two columns together represent the total maximal conductance in each condition. The apical solution contained amiloride (10^{-5} mol l^{-1}) throughout. Values are means ± S.E.M.; N, number of animals; n, number of skin pieces. DW, distilled water; TW, tapwater.

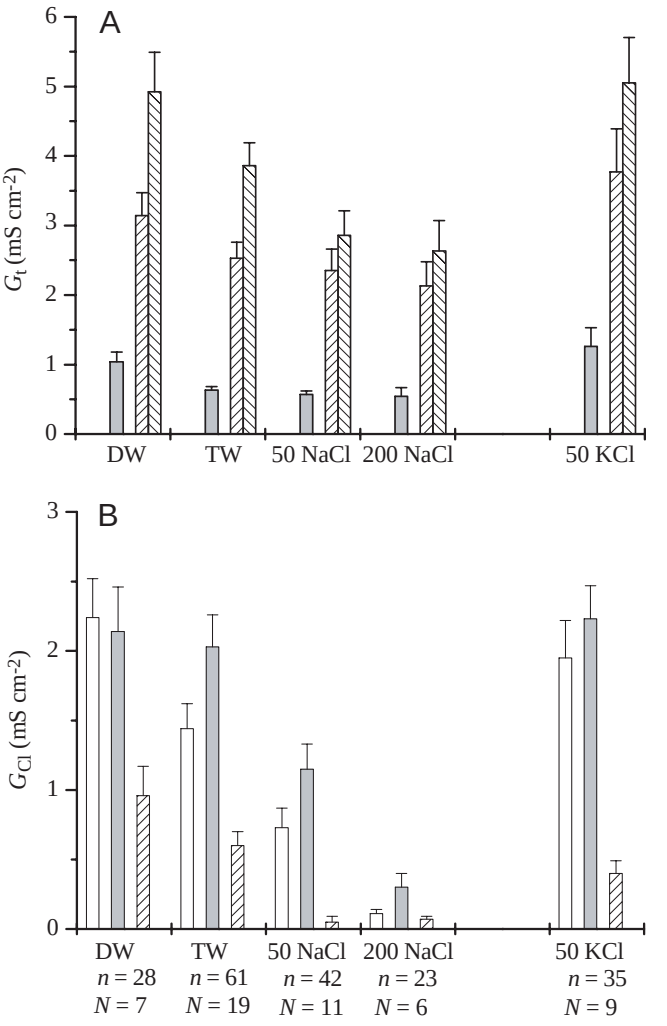

Fig. 5. (A) Effects of CPTcAMP and IBMX on transepithelial conductance (G_t) across toad skin. Stippled columns, steady-state conductance in the deactivated (–30 mV) condition in the presence of IBMX (100 μmol l^{-1}). Hatched columns, effects of CPTcAMP (200 μmol l^{-1}) under depolarized (–30 mV; left-hand hatched columns) and hyperpolarized (+80 mV; right-hand hatched columns) conditions. IBMX did not significantly affect the deactivated conductance in the skin of any of the groups of toads. The increase in total conductance (G_t) in response to cyclic AMP under depolarized and hyperpolarized conditions differed significant only in the distilled-water- and tapwater acclimated toads (P<0.01). (B) Effects of IBMX and CPTcAMP on steady-state voltage-activated (+80 mV) conductance (G_{Cl}) across toad skin. Open columns, control; stippled columns, treated with IBMX (100 μmol l^{-1}); hatched columns, treated with CPTcAMP (200 μmol l^{-1}). Apical solution contained amiloride (10^{-5} mol l^{-1}) throughout. Values are means ± S.E.M.; N, number of animals; n, number of skin pieces. DW, distilled water; TW, tapwater. Toads were also acclimated to 50 or 200 mmol l^{-1} NaCl or 50 mmol l^{-1} KCl.

serosal side) did not affect the response to cyclic AMP (not shown). Fig. 5B shows the voltage-activated time-dependent conductance G_{Cl} in control conditions and following treatment with IBMX (stippled columns) or cyclic AMP (hatched columns). The stimulation of G_{Cl} by IBMX was most pronounced in skins from NaCl- and tapwater-acclimated toads compared with their control. The lower the control G_{Cl}, the greater was the relative effect of IBMX on the activated conductance. Thus, G_{Cl} in skins of distilled-water- and KCl-acclimated toads, which was already at its maximal level, was not affected significantly by IBMX. A completely different response was observed upon application of CPTcAMP. The time-dependent voltage-activated conductance was greatly reduced; in skins from NaCl-acclimated toads, it was abolished. The response became virtually time-independent within the time frame of activation (see Fig. 2).

Fig. 6 relates all the values for the voltage-activated (+80 mV) transepithelial conductance (G_{Cl}) under control condition to the corresponding MR cell density (D_{mrc}). The calculated regression line had a very shallow slope (y=0.0011x+0.93) that was not significantly different from zero. Separate calculations of regression lines are given in Table 2 for skins of all acclimation groups in the two series (winter and summer). Similar values were obtained in all calculations, including those for the tapwater- and distilled-water-acclimated groups calculated separately.

Silver-stained MR cells from the five acclimation groups are shown in Fig. 7. It has already been demonstrated in histological cross sections that the silver deposit is confined to the apical membrane of the cells (Whitear, 1972; Katz et al., 2000). The diameter of the apical silver-stained aperture of the MR cells was largest in the skins of distilled-water- and KCl-acclimated toads (mean diameter 13.8±0.37 μm; N=8). Note

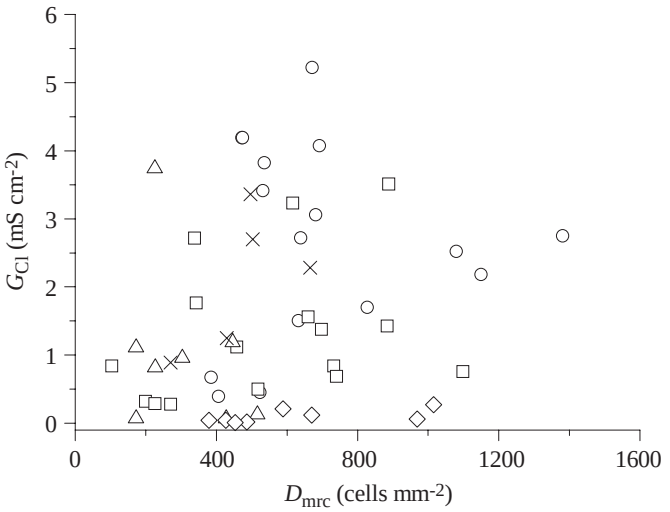

Fig. 6. Relationship between mitochondria-rich cell density (D_{mrc}) and control steady-state voltage-activated (+80 mV) conductance (G_{Cl}) in all experiments. Acclimation conditions: ○, distilled water; □, tapwater; △, 50 mmol l^{-1} NaCl; ◇, 200 mmol l^{-1} NaCl; ×, 50 mmol l^{-1} KCl. The slope of the regression line (not drawn) is y=0.0011x+0.93; P<0.09, N=59, which is not significantly different from zero.

that MR cells with two distinctly different appearances are detected in the skin of KCl-acclimated toads. A smaller cell aperture was measured in the skin of tapwater- and 50 mmol l^{-1} NaCl-acclimated toads (mean diameter 9.4±0.45 μm; N=6). In the 200 mmol l^{-1} NaCl acclimation conditions, skin MR cell density changed little in the first 6–7 days (not typical) and was similar to the value for the 50 mmol l^{-1} acclimation condition. The number and appearance of these cells was subsequently decreased, as shown in Fig. 7E,F. The anion conductance of these skins was reduced similarly.

Discussion

Our data extend previous studies (Katz and Larsen, 1984; Katz and Gabbay, 1995) showing that Cl$^-$ conductance is influenced in the long term by the salinity of the ambient solution. It was also established that the short-term effects of cyclic AMP and IBMX on the anionic conductance across the skin are influenced by the ionic acclimation of the animals.

Table 2. *Regression equations calculated for the relationship between mitochondria-rich cell density and voltage-activated conductance across toad skin*

	N	Equation	r^2	P
First series (winter)	50	y=0.0013x+0.92	0.06	0.11
Second series (summer)	9	y=0.0016x+0.22	0.13	0.33
Series 1+2	59	y=0.0011x+0.93	0.05	0.09
Tapwater (first series)	17	y=0.0014x+0.63	0.14	0.14
Distilled water (first series)	17	y=0.0010x+1.86	0.04	0.44

Tapwater and distilled water acclimation results are for the groups from the first (winter) series.

N, number of skins included in each analysis.

The Cl$^-$ conductance across amphibian skin is controlled by β-adrenergic receptors (Willumsen et al., 1992; Nagel and Katz, 1998) that activate adenylate cyclase, leading to increased cellular cyclic AMP levels and activation of protein kinase. Cyclic AMP and IBMX affect the transepithelial anion conductance in fundamentally different ways (Katz and Nagel, 1995), although cellular cyclic AMP levels must be increased by IBMX because of inhibition of phosphodiesterase (PDE; Ukena et al., 1993). Two steps can be distinguished in the movement of anions across toad skin, activation and conductance (Larsen, 1982; Nagel and Katz, 1997). In this scheme, Cl$^-$-dependent voltage activation is followed by anion conductance (Larsen, 1991; Lacaz-Vieira and Procopio, 1988; Nagel and Katz, 1997). IBMX may have a structural effect, different and separate from its inhibition of PDE that sensitizes voltage activation. Indeed, it was reported recently that xanthine derivatives lacking an inhibitory effect on PDE stimulated the cystic fibrosis transmembrane conductance regulator (CFTR), whereas no such effect was elicited by specific inhibitors of PDE (Chappe et al., 1998). At high concentrations, cyclic AMP exerts a more profound effect, proximal to the voltage sensor, and the pathway becomes insensitive to voltage, keeping the conductive path open to the passage of anions.

The effects of cyclic AMP and IBMX on the anion conductance of the skin were influenced by the conditions of acclimation of the toads. IBMX was effective only on the activated conductance, particularly when the initial value had not attained its maximal level. It had only a small and transient effect on the deactivated conductance, which could be the result of a temporal increase in cellular cyclic AMP levels. The increase in transepithelial anionic conductance in response to a high concentration of cyclic AMP was insensitive to transepithelial potential and became independent of external [Cl$^-$]. Because the total anionic conductances, after voltage activation or in response to cyclic AMP, were similar in magnitude, they may represent the same conductance pathway. Thus, an apparently ‘dormant’ conductance is uncovered by cyclic AMP in skins from NaCl-acclimated toads, in which the activated conductance was greatly reduced. This observation suggests that acclimation to high [NaCl] desensitizes the voltage sensor and that cyclic AMP, at relatively high concentrations, relieves this block, opening the path to the conductive passage of anions.

In earlier studies, it was reported that D_{mrc} is greatly decreased following adaptation to NaCl, in parallel with a reduction in skin Cl$^-$ conductance. This was assumed to result from the long-term exposure of the animals to high ambient [Cl$^-$] (Katz and Larsen, 1984). However, it was discovered subsequently that D_{mrc} is not reduced in response to acclimation to KCl (Ehrenfeld et al., 1989; Katz and Gabbay, 1995), which is shown in the present study to be accompanied by an increase in G_{Cl} that reaches a maximal level similar to that of the conductance of skins from distilled-water-acclimated toads. It has been suggested previously that

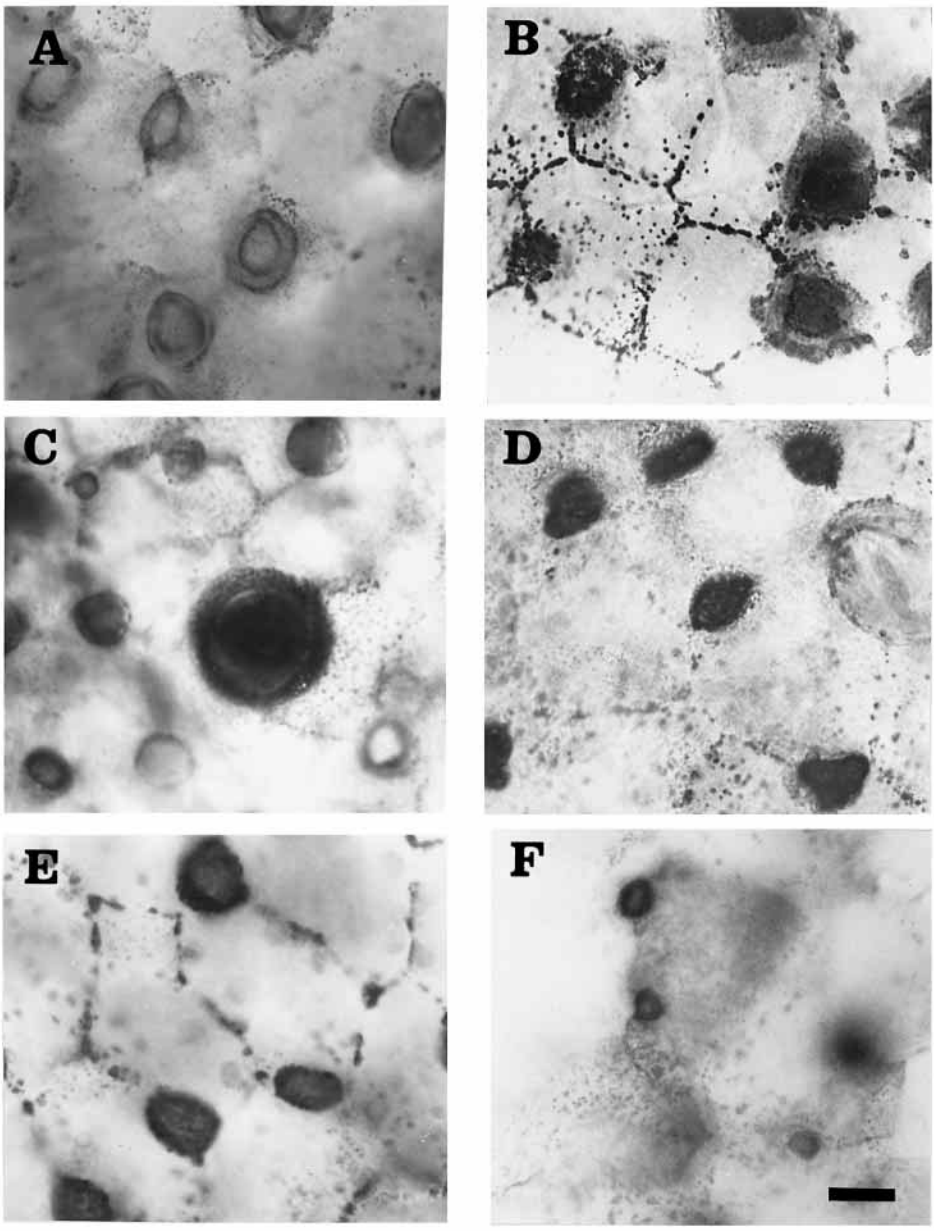

Fig. 7. Silver-stained mitochondria-rich cells in the skin of toads acclimated to distilled water (A), 50 mmol l^{-1} KCl (B), tapwater (C), 50 mmol l^{-1} NaCl (D) and 200 mmol l^{-1} NaCl (E,F). The two micrographs for toads acclimated to 200 mmol l^{-1} NaCl were taken 3 days (E) and 8 days (F) following transfer and represent the typical variation in the appearance of cells acclimated under this condition. Note the gland openings. Scale bar for all micrographs, 10 μm.

acclimation to KCl induces plasma acidosis (Ehrenfeld et al., 1989), which necessitates an increased secretion of protons. Indeed, it was shown that MR cells in frog skin react to anti-H^+-ATPase (Klein et al., 1997), and this has been confirmed recently for *Bufo viridis* (U. Katz and S. Gabbay, unpublished experiments). This supports the assumption that MR cells are involved in proton secretion, a role similar to that of α-type intercalated cells in other tight epithelia (Steinmetz, 1986). The elevated G_{Cl} in KCl-acclimated toads could therefore be explained as a consequence of the maintained D_{mrc}, if MR cells are also endowed with an anion conductance pathway. This remains to be verified more directly.

Our measurements covered a wide range of values of transepithelial conductance and D_{mrc}, but there was no correlation between these two parameters in any of the acclimation conditions. A closer relationship was observed in previous studies (Katz and Gabbay, 1995), and some correlation has been found between Cl^- conductance and D_{mrc} in some studies in other species. Voute and Meier (1978) reported some correlation in frog (*Rana esculenta*) skin, as did Willumsen and Larsen (1985) in toad (*Bufo bufo*) skin. Although no activated conductance can be elicited in skins with no MR cells or only a few MR cells (Katz et al., 2000), Nagel and Dorge (1990) found no correlation between these parameters in frog (*Rana esculenta*) skin. This relationship is therefore unpredictable, and it has not yet been possible to determine the reason for these variable results. It could perhaps be explained if MR cells were functionally heterogeneous (Katz et al., 1997) and if they were not the exclusive route for passive anion conductance. Proton secretion, for example, if carried out by MR cells, could also influence cell density in certain conditions, and Cl^- conductance is not necessarily,

therefore, the sole determinant influencing MR cell density. Maximal skin G_{Cl} differed considerably among the various acclimation conditions, and these two functions, i.e. G_{Cl} and H^+ secretion, which are assumed to be localized in MR cells, may be expressed differently or in different cells according to the physiological demands at a given condition.

The activated conductance was always very low in toads acclimated to high [NaCl], regardless of the density of MR cells, indicating that the conditions of acclimation, rather than D_{mrc} , determine the anion conductance that can exist in different states. We have limited evidence for the existence of different MR cell types. This evidence, at the cellular level, is based on silver staining and immunological methods (Katz et al., 1997).

The primary signal(s) responsible for the acceleration of cell division and/or differentiation is still unknown. The actual functional properties of the cells are yet to be resolved and certainly depend on more than one determinant, external [Cl^-] being only one. This could explain why no correlation between D_{mrc} and G_{Cl} was found, although current results suggest that they must be associated.

In conclusion, we have shown that the transepithelial anionic conductance across *Bufo viridis* skin is influenced differently by long-term acclimation to external NaCl and KCl solutions. These changes could not be corrected by IBMX or by cyclic AMP. No correlation was found between MR cell density and Cl^- conductance across the isolated skin. It is suggested that, as well as being sites of Cl^- conductance across the skin, the MR cells must also be involved in other transport functions that would mask any possible relationship between the density of these cells and Cl^- conductance.

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References

Chappe, V., Mettey, Y., Vierfond, J. M., Hanrahan, J. W., Gola, M., Verrier, B. and Becq, F. (1998). Structural basis for specificity and potency of xanthine derivatives as activators of the CFTR chloride channel. *Br. J. Pharmac.* **123**, 683–693

Ehrenfeld, J., Lacoste, I. and Harvey, B. J. (1989). The key role of the mitochondria-rich cell in Na^+ and H^+ transport across the frog skin epithelium. *Pflügers Arch.* **414**, 59–67.

Katz, U. and Gabbay, S. (1995). Dynamics and density of mitochondria-rich cells in toad skin epithelium. *Biol. Cell* **85**, 185–190.

Katz, U. and Larsen, E. H. (1984). Chloride transport in toad skin (*Bufo viridis*). The effect of salt adaptation. *J. Exp. Biol.* **109**, 353–371.

Katz, U. and Nagel, W. (1994). Biophysics of ion transport across amphibian skin. In *Amphibian Biology*, vol. 1 (ed. H. Heatwole and G. T. Barthalmus), pp. 98–119. Australia: Surrey Beatty & Sons.

Katz, U. and Nagel, W. (1995). Effect of cyclic AMP and theophylline on chloride conductance across toad skin. *J. Physiol., Lond.* **489**, 105–114.

Katz, U., Rozman, A., Zaccone, G., Fasulo, S. and Gabbay, S. (2000). Mitochondria-rich cells in anuran Amphibia: chloride conductance and regional distribution over the body surface. *Comp. Biochem. Physiol*. A **125**, 131–139.

Katz, U., Zaccone, G., Fasulo, S., Mauceri, A. and Gabbay, S. (1997). Lectin binding pattern and band 3 localization in toad skin epithelium and the effect of salt acclimation. *Biol. Cell* **89**, 141–152.

Klein, U., Timme, M., Zeiske, W. and Ehrenfeld, J. (1997). The H^+ pump in frog skin (*Rana esculenta*): identification and localization of V-ATPase. *J. Membr. Biol.* **157**, 117–126.

Lacaz-Vieira, F. and Procopio, J. (1988). Comparative roles of voltage and Cl ions upon activation of a Cl conductive pathway in toad skin. *Pflügers Arch.* **412**, 634–640.

Larsen, E. H. (1982). Chloride current rectification in toad skin epithelium. In *Chloride Transport in Biological Membranes* (ed. J. Zadunaisky), pp. 333–364. London, New York: Academic Press.

Larsen, E. H. (1991). Chloride transport by high-resistance heterocellular epithelia. *Physiol. Rev.* **71**, 235–283.

Nagel, W. and Dorge, A. (1990). Analysis of anion conductance in frog skin. *Pflügers Arch.* **416**, 53–61.

Nagel, W. and Katz, U. (1997). The effect of NEM on the activated Cl^- current conductance in toad skin. *J. Membr. Biol.* **159**, 127–135.

Nagel, W. and Katz, U. (1998). α_1-Adrenoceptors antagonize activated chloride conductance of amphibian skin epithelium. *Pflügers Arch.* **436**, 863–870.

Nagel, W., Somieski, P. and Shipley, A. M. (1998). Mitochondria-rich cells and voltage-activated chloride current in toad skin epithelium. *J. Membr. Biol.* **161**, 131–140.

Steinmetz, P. R. (1986). Cellular organization of urinary acidification. *Am. J. Physiol.* **251**, F173–F187.

Ukena, D., Schudt, C. and Sybrecht, G. (1993). Adenosine receptor blocking xanthines as inhibitors of phosphodiesterase isozymes. *Biochem. Pharmac.* **45**, 847–851.

Voute, C. L. and Meier, W. (1978). The mitochondria-rich cell of frog skin as hormone sensitive 'shunt path'. *J. Membr. Biol.* **40**, 141–165.

Whitear, M. (1972). The location of silver in frog epidermis after treatment by Ranvier's method and possible implication of the flask cells in transport. *Z. Zellforsch.* **133**, 455–461.

Willumsen, N. J. and Larsen, E. H. (1985). Passive Cl^- current in toad skin: potential dependence and relation to mitochondria-rich cell density. In *Transport Processes, Iono- and Osmoreglation* (ed. R. Gilles and M. Gilles-Baillien), pp. 20–30. Berlin, Heidelberg: Springer Verlag.

Willumsen, N. J., Vestergaard, L. and Larsen, E. H. (1992). Cyclic AMP- and β-agonist-activated chloride conductance of a toad skin epithelium. *J. Physiol., Lond.* **449**, 641–653.