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K^+ transport in the mesonephric collecting duct system of the toad *Bufo bufo*: microelectrode recordings from isolated and perfused tubules

Nadja Møbjerg*, Erik Hviid Larsen and Ivana Novak

August Krogh Institute, Department of Zoophysiology, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen Ø, Denmark

*Author for correspondence (e-mail: nmobjerg@aki.ku.dk)

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Summary

We studied the mechanisms of K^+ transport in cells from isolated and perfused collecting tubules and ducts from the mesonephric kidney of the toad *Bufo bufo*. Cells were impaled with microelectrodes across the basal cell membrane. The basolateral membrane potential (V_{bl}) depolarized upon change of bath [K^+] from 3 to 20 mmol l^{-1} demonstrating a large K^+ conductance in this membrane. In collecting tubules and collecting ducts a V_{bl} of –66±2 mV and –74±4 mV depolarized by 30±2 mV and 36±3 mV, respectively (N=23; 15). The K^+ channel inhibitor Ba^{2+} (1 mmol l^{-1}) inhibited the basolateral K^+ conductance and depolarized a V_{bl} of –64±4 mV by 30±6 mV (N=8). Luminal K^+ steps (3 to 20 mmol l^{-1}) demonstrated a K^+ conductance in the apical cell membrane. In collecting tubules and collecting ducts a V_{bl} of –70±3 mV and –73±3 mV depolarized by 11±3 mV and 16±3 mV, respectively (N=11; 11). This conductance could also be inhibited by Ba^{2+}, which depolarized a V_{bl} of –71±5 mV by 9±3 mV (N=5). The pump inhibitor ouabain (1 mmol l^{-1}) depolarized V_{bl}, but addition of furosemide to bath solution did not affect V_{bl}. The [K^+] in urine varied from 1.3 to 22.8 mmol l^{-1}. In conclusion, we propose that the collecting duct system of *B. bufo* secretes K^+ into the urine *via* luminal K^+ channels.

Key words: amphibian, Ba^{2+}, *Bufo bufo*, collecting duct, collecting tubule, K^+ conductance, K^+ secretion, kidney, mesonephros, ouabain, toad.

Introduction

The modern forms of amphibians, the Lissamphibia, include three groups: frogs (Anura), salamanders (Urodela) and caecilians (Gymnophiona). In many respects amphibians span the evolutionary gap between aquatic vertebrates and fully terrestrial tetrapods. Within Amphibia today we find forms that are fully aquatic throughout their life cycle, as well as forms that have adapted to the terrestrial environment. Most amphibians are, however, still dependent on freshwater for reproduction and the development of larvae. The different life modes pose a challenge to the osmoregulatory epithelia of these animals. The mesonephros, the functional kidney of adult amphibians, is one of the organs that participates in regulation of the salt and water balance, as well as the acid–base balance.

The structural and functional unit of the kidney, the nephron, can in the amphibian mesonephros be divided into seven morphological and functional distinct sections: Malpighian corpuscle, neck segment, proximal tubule, intermediate segment, early distal tubule, late distal tubule and, finally, the collecting tubule, which opens into collecting ducts that lead the urine to the ureter (Dantzler, 1992; Dietl and Stanton, 1993; Hentschel and Elger, 1989; Møbjerg et al., 1998). The heterocellular epithelium constituting the collecting tubules and collecting ducts – here referred to as the collecting duct system – takes part in the final adjustment of the urine.

Most studies on amphibian renal tubules have been carried out on aquatic urodeles, because they have a large tubule diameter and large cell size (Richards and Walker, 1937; Stoner, 1977). In particular, the kidney of *Amphiuma* has been used as a model for vertebrate distal tubule transport studies (for a review, see Dietl and Stanton, 1993). Based on data obtained mainly from urodeles, the collecting tubules have been shown to display a lumen-negative voltage, to reabsorb Na^+ and to secrete K^+ (Horisberger et al., 1987; Hunter et al., 1987; Stoner, 1977; Wiederholt et al., 1971). However, little is known about the function of the collecting tubule and especially the collecting ducts of anuran amphibians. The aim of our study was to characterize K^+ transport by the cells within these tubule segments of a terrestrial anuran, the common European toad *Bufo bufo*. For this purpose we isolated and perfused tubules *in vitro* and impaled cells with conventional microelectrodes.

Since the early work by Richards and Walker (1937), who introduced the micropuncture technique in renal tubules from kidneys of amphibians, several investigators have demonstrated the ability of the distal nephron from urodele

amphibians to secrete K^+ (Bott, 1962; Garland et al., 1975; Wiederholt et al., 1971). These data were subsequently confirmed in a study on isolated and perfused tubules from the distal nephron of both urodele and anuran amphibians (Stoner, 1977). In an electrophysiological study on *in vitro* perfused collecting tubules from *Amphiuma* sp., it was subsequently shown that the heterocellular epithelium of the collecting tubule secretes K^+ (Horisberger et al., 1987). These workers, however, could not find a significant K^+ conductance in the luminal cell membrane of the collecting tubule of *Amphiuma*, and therefore argued that K^+ secretion could not occur across the luminal membrane (Horisberger et al., 1987; Hunter et al., 1987). Thus, in the light of these observations it has been postulated that K^+ secretion by the amphibian collecting tubule occurs *via* a passive, paracellular mechanism (Dietl and Stanton, 1993). Therefore, it was believed that K^+ secretion in amphibians differs from secretion by the cortical collecting tubule in mammals (for reviews, see Giebisch, 1998; Palmer, 1999).

Nevertheless, Stoner and Viggiano (1998) recently used patch clamp and characterised maxi K^+ channels from cell-attached patches on the apical membrane of the everted collecting tubule of aquatic-phase tiger salamanders *Ambystoma tigrinum*. K^+ adaptation in the salamanders caused a tenfold increase in the number of detectable maxi K^+ channels. After K^+ adaptation the maxi K^+ channels contributed to the ability of the collecting tubule in *A. tigrinum* to secrete K^+ (Stoner and Viggiano, 1999). Stoner and Viggiano (2000) have also provided evidence for an apical intermediate conductance, amiloride-insensitive, non-specific cation channel in the collecting tubule of the tiger salamander. Since the channel is observed most frequently in K^+-adapted animals it may be important in K^+ secretion.

These contradictory findings on the mechanism by which K^+ is secreted by the collecting tubule led us to explore the means of K^+ transport by the collecting duct system of the mesonephric kidney of *Bufo bufo*, an anuran species that in its adult life is fully adapted to the terrestrial environment.

In the present study on isolated and perfused collecting tubules and collecting ducts, microelectrode recordings demonstrate the presence of a large K^+ conductance in the basolateral cell membrane. Furthermore an apical K^+ conductance is present in both collecting tubules and collecting ducts. Thus, this study provides evidence that is in agreement with a transcellular secretion of K^+ in the collecting duct system of the toad.

Materials and Methods

Specimens of the toad *Bufo bufo* (L.) were kept under terrestrial conditions in the laboratory. The toads were fed meal worms (*Tenebrio molitor*) and had access to tapwater. Urine samples from 21 adult females were collected either from the bladder of decapitated animals or from the cloaca of live specimens. Measurements of urine osmolality were made on a vapor pressure osmometer (Vapro 5520; Wescor Inc., UT, USA). The K^+ levels in the urine samples were measured on a Dionex DX-120 Ion Chromatograph (Dionex Corporation, CA, USA).

Collecting tubules and collecting ducts were dissected from the mesonephros of adult toads. The morphology and ultrastructure of the mesonephric kidney of *Bufo bufo* has been described in a previous study (Møbjerg et al., 1998). Only female toads were used for the present study, as they lack the communication between the mesonephric kidney and gonadal system, which in males is used for sperm transport.

The animals were killed by decapitation and the kidneys were immediately excised and cut into transverse sections approximately 1 mm thick using razor blades. The dissection medium contained (in $mmol\,l^{-1}$): 96.8 Na^+, 3.0 K^+, 1.8 Ca^{2+}, 1.0 Mg^{2+}, 81.6 Cl^-, 20.0 HCO_3^-, 1.0 SO_4^{2-}, 0.8 HPO_4^{2-}, 0.2 $H_2PO_4^-$, 5.5 glucose, 3.3 glycine, 0.4 PVP (polyvinyl-pyrolidone), 5.0 Hepes, titrated to pH 7.8 with NaOH.

Tubules were identified under a stereomicroscope during free-hand dissection with sharpened forceps and needles (Terumo, Neolus 0.4×20 mm) at 6 °C. Collecting ducts were isolated from the dorsal zone of the kidney beneath the connective tissue capsule. Collecting tubules run ventrally from the transition zone with the ducts. The collecting ducts had an outer tubule diameter of approximately 60–70 μm (Fig. 1A,B). The outer diameter of the collecting tubules was approximately 50–60 μm (Fig. 1C,D). The dissected length of the tubules was in the range 300–500 μm.

The isolated tubules were transferred in a small volume of dissection medium to a bath chamber mounted on an inverted microscope equipped with Nomarski optics (Zeiss, Axiovert 135 TV) and perfused *in vitro* at room temperature. The control solution, which was used to perfuse the bath and the lumen of the tubules, consisted of (in $mmol\,l^{-1}$): 101.8 Na^+, 3.0 K^+, 1.8 Ca^{2+}, 1.0 Mg^{2+}, 81.6 Cl^-, 25.0 HCO_3^-, 1.0 SO_4^{2-}, 0.8 HPO_4^{2-}, 0.2 $H_2PO_4^-$, equilibrated with 1.8 % CO_2 in O_2, pH 7.8. In high $[K^+]$ solutions, the K^+ concentration was raised to 20 $mmol\,l^{-1}$ by equimolar substitution with Na^+. In experiments with Ba^{2+}, 1 $mmol\,l^{-1}$ $BaCl_2$ was added to the control solution, or the high $[K^+]$ solution. Ouabain and furosemide were used in control solution at a final concentration of 1 $mmol\,l^{-1}$ and 10^{-5} $mmol\,l^{-1}$, respectively.

In order to hold the concentric pipettes used for tubule perfusion we used the system (Luigs & Neumann, Germany) described by Greger and Hampel (1981) for *in vitro* perfusion of isolated renal tubules, which was modified from the original system described by Burg et al. (1966). The pipette arrangement consisted, on the perfusion side, of a constricted holding pipette and a single-barrelled perfusion pipette inserted into the tubule lumen (Fig. 1A,C). A small glass capillary in the perfusion pipette insured fast fluid exchange (o.d. 0.3 mm, i.d. 0.2 mm, Drummond Scientific Company, PA, USA). The tubule was held by a holding pipette on the fluid-collection side. The dimensions of the pipettes were made to fit the tubules using an SM II/1 Puller from Luigs & Neumann (Germany). Holding pipettes (o.d. 2.1 mm, i.d. 1.6 mm) and perfusion pipettes (o.d. 1.2 mm, i.d. 1.0 mm)

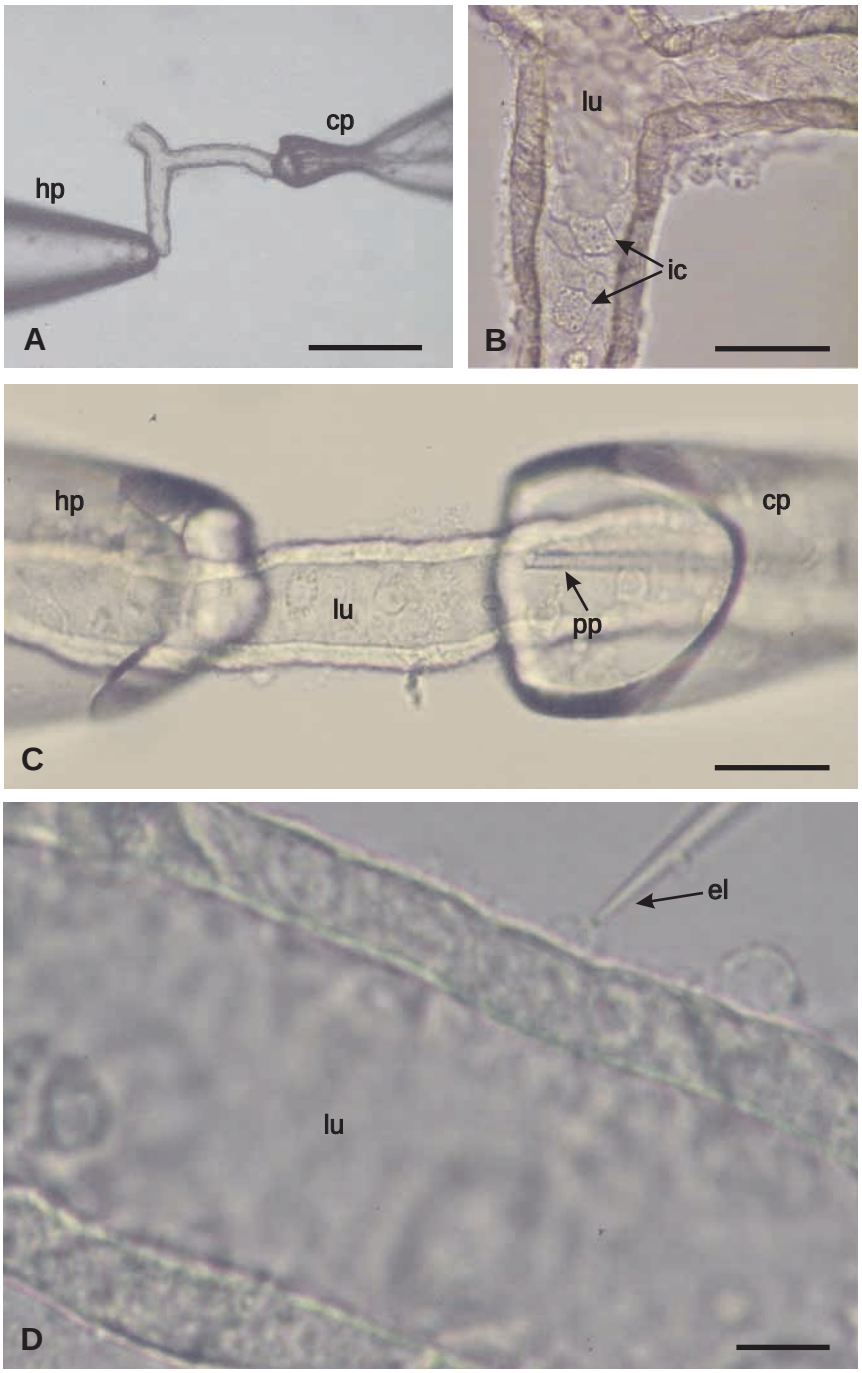

Fig. 1. Photographs of isolated and perfused tubules taken using an inverted Zeiss microscope equipped with Nomarski optics. Tubules were identified according to their position within the nephron. The cellular boundaries in collecting ducts appear sharper under the light microscope when compared to collecting tubules. (A) Isolated and perfused ramification of collecting ducts. The pipette arrangement consists, on the perfusion side (right), of a constricted holding pipette and a single-barrelled perfusion pipette. On the left, a holding pipette holds the tubule. Scale bar, 300 µm. (B) Isolated and perfused collecting duct. Individual cells are clearly recognizable. The apical surface of the intercalated cells possesses microvilli and bulges slightly into the lumen of the tubule. Scale bar, 50 µm. (C) Isolated and perfused collecting tubule. Note the perfusion pipette, which has been advanced into the lumen of tubule. Scale bar, 50 µm. (D) Cellular impalement of a perfused collecting tubule. Cells were impaled across the basal cell membrane with glass microelectrodes. The tip of the microelectrode is below resolution limits. Scale bar, 10 µm. cp, constriction pipette; el, electrode; hp, holding pipette; ic, intercalated cell; lu, lumen; pp, perfusion pipette.

were made from glass tubing from Drummond Scientific Company (PA, USA).

Cells were impaled with microelectrodes mounted on a Leitz micromanipulator (Germany) and the basolateral cell membrane potential (V_{bl}) was recorded with respect to the grounded bath (Fig. 1D). Impalements were achieved by placing the microelectrode tip against the basal surface of the cell and gently tapping the manipulator or the table. The electrodes were pulled from borosilicate glass with filament (Clark Electromedical, UK) on a vertical electrode puller (Narishige, Japan). When filled with 1 mol l^{-1} KCl they had a resistance of 100–150 MΩ. The recording of V_{bl} was accepted if the impalement was achieved by a sudden change in the potential read by the electrode and if it was stable and lasted longer than 30 s. For the intracellular voltage recordings we used a WPI Duo 773 electrometer (World Precision Instruments, FL, USA) interfaced with a PowerLab/4s recording unit using a 16-bit analog-to-digital converter for digitization (ADInstruments, NSW, Australia). The software, Chart, which comes with the PowerLab has a 12-bit resolution. The sampling rate was 40 s^{-1}.

Figures were made in Origin 6.0 (Microcal, MA, USA) and imported to CorelDRAW 9 (Corel Corporation, Canada) for the graphic presentation. The results are presented as original recordings made by the PowerLab/4s recording unit together with the Chart v3.4.11 application program. Values are given as mean ± S.E.M. (standard error of the mean), where N is equal to the number of cells impaled in each experiment. Statistics were performed using two-tailed paired or non-paired t-tests as appropriate and a significance level of 0.05 was applied.

Results

K^+ *urine concentration*

The collecting duct system of the amphibian mesonephric kidney participates in the final stage of urine adjustment before it leaves the kidney and enters the bladder *via* the ureters for

Table 1. *Urine [K^+] and osmolality in 21 toads*

Sample number	[K^+] (mmol l^{-1})	Osmolality (mmol kg^{-1})
1	4.7	81
2	12.1	206
3	15.3	219
4	11.2	102
5	9.1	116
6	4.0	91
7	2.5	74
8	7.2	81
9	6.5	257
10	14.4	237
11	2.3	72
12	2.5	115
13	22.8	237
14	4.9	141
15	4.0	160
16	4.2	205
17	1.3	77
18	7.5	241
19	3.4	68
20	4.2	67
21	13.0	72

further modification and storage. We examined the K^+ concentration of 21 urine samples collected from the bladder or cloaca of 21 toads and compared [K^+] with the total osmolyte concentration in the samples (Table 1). The osmolality of the samples varied from 67 mmol kg^{-1} (sample 20) to 257 mmol kg^{-1} (sample 9). The range of these values for urine osmolality from *Bufo bufo* kept under terrestrial conditions is similar to that of another terrestrial amphibian, the spadefoot toad *Scaphiopus couchi*. In nine foraging spadefoot toads, urine osmolality varied between 65 and 328 mmol l^{-1}, with an average of 187 mmol l^{-1} (McClanahan, 1967). Much lower urine osmolalities are found in aquatic amphibians, e.g. the urodele *Amphiuma* sp., with values <5 mmol l^{-1} (Wiederholt and Hansen, 1980). There was a large variation in K^+ concentration in the urine from *B. bufo*. This is illustrated by comparing two samples with almost equal total osmolyte concentrations. In sample 17, [K^+] was 1.3 mmol l^{-1} and in sample 21 [K^+] was tenfold higher (13.0 mmol l^{-1}). Overall, K^+ concentrations in the urine varied from 1.3 mmol l^{-1} (sample 17) to 22.8 mmol l^{-1} (sample 13). These results indicate that transport mechanisms responsible for reabsorption and secretion in the kidney, or bladder epithelium, modulate urine K^+ concentration. We therefore studied in more detail the pathways for K^+ secretion in the collecting duct system of the toad.

Cell membrane voltage

For the following experiments we dissected collecting tubules and collecting ducts, which both consist of a heterocellular epithelium comprising principal cells and intercalated cells. Fig. 2 is a frequency distribution of V_{bl} in 120 cells from collecting ducts (N=50) and collecting tubules (N=70). Cells from the collecting ducts seemed to fall into two groups; one group was relatively more hyperpolarized, with a V_{bl} averaging –80 to –85 mV and the other being more depolarized, V_{bl} averaging approximately –65 mV (Fig. 2A). The V_{bl} of cells from collecting tubules often fell inbetween the two collecting duct groups, averaging –70 to –75 mV (Fig. 2B). The results presented below are based on 55 impalements made on 37 tubules from 31 toads. It was possible to distinguish between intercalated cells and principal cells at magnifications from 200× using Nomarski optics (Fig. 1B). The apical surface of the intercalated cells possesses microvilli and bulges slightly into the lumen of the tubule, whereas the apical surface of the principal cells appears smooth. However, it was impossible to visualize the tip of the electrode and the impaled cell could therefore not be determined with absolute certainty. So far we have found no functional tests that could distinguish principal cells and intercalated cells in collecting tubules or collecting ducts, as for example reported by Schlatter and Schafer (1987) for the cortical collecting tubule

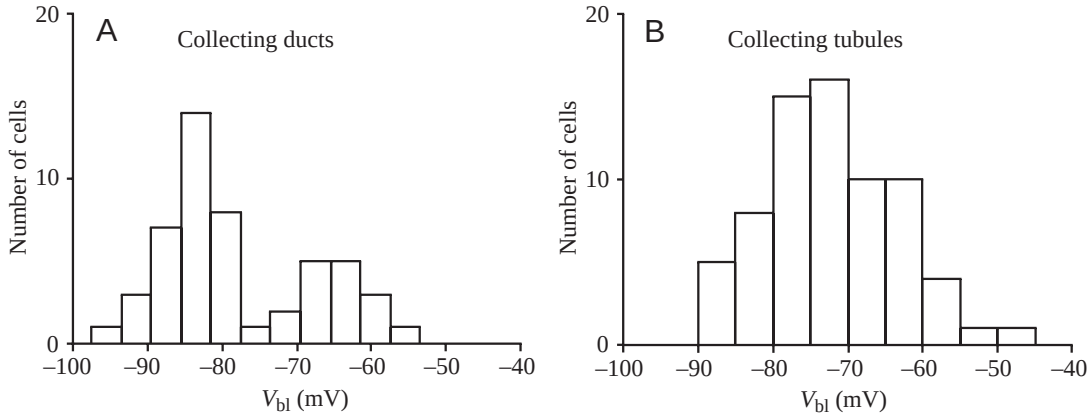

Fig. 2. Frequency distribution of V_{bl} in 120 cells from collecting tubules and collecting ducts. (A) The V_{bl} of collecting duct cells (N=50) fell into two groups, one being more hyperpolarized than the other. (B) V_{bl} of collecting tubule cells (N=70) showed a broader distribution with the mean V_{bl} falling inbetween that of the two collecting duct groups.

of the rat. All cells behaved similarly with respect to K^+ conductance (see below).

Effect of K^+ concentration steps

We examined the presence of basolateral and luminal K^+ conductances in cells from collecting tubules and collecting ducts. Bath and luminal $[K^+]$ were changed from 3 mmol l^{-1} to 20 mmol l^{-1}. The $[K^+]$ changes in both bath and luminal solutions resulted in a depolarization of V_{bl} (Fig. 3A,D).

As illustrated in Fig. 3B,C the basal $[K^+]$ step depolarized V_{bl} in cells from both collecting ducts and collecting tubules. In 15 cells from 12 collecting ducts with a mean V_{bl} of –74±4 mV depolarization was 36±3 mV, and in 23 cells from 15 collecting tubules with a mean V_{bl} of –66±2 mV the depolarization was 30±2 mV.

Notably, V_{bl} depolarized in cells from both collecting tubules and collecting ducts in response to luminal $[K^+]$ steps. Fig. 3E,F summarizes the luminal K^+ substitution experiments from 22 cells. In 11 cells from 9 collecting ducts with a mean V_{bl} of –73±3 mV depolarization was 16±3 mV, and in 11 cells from 7 collecting tubules with a mean V_{bl} of –70±3 mV, V_{bl} depolarized by 11±3 mV. The luminal and basal K^+ substitution experiments indicate that there is a luminal and a basolateral K^+ conductance in cells of both collecting tubules and collecting ducts.

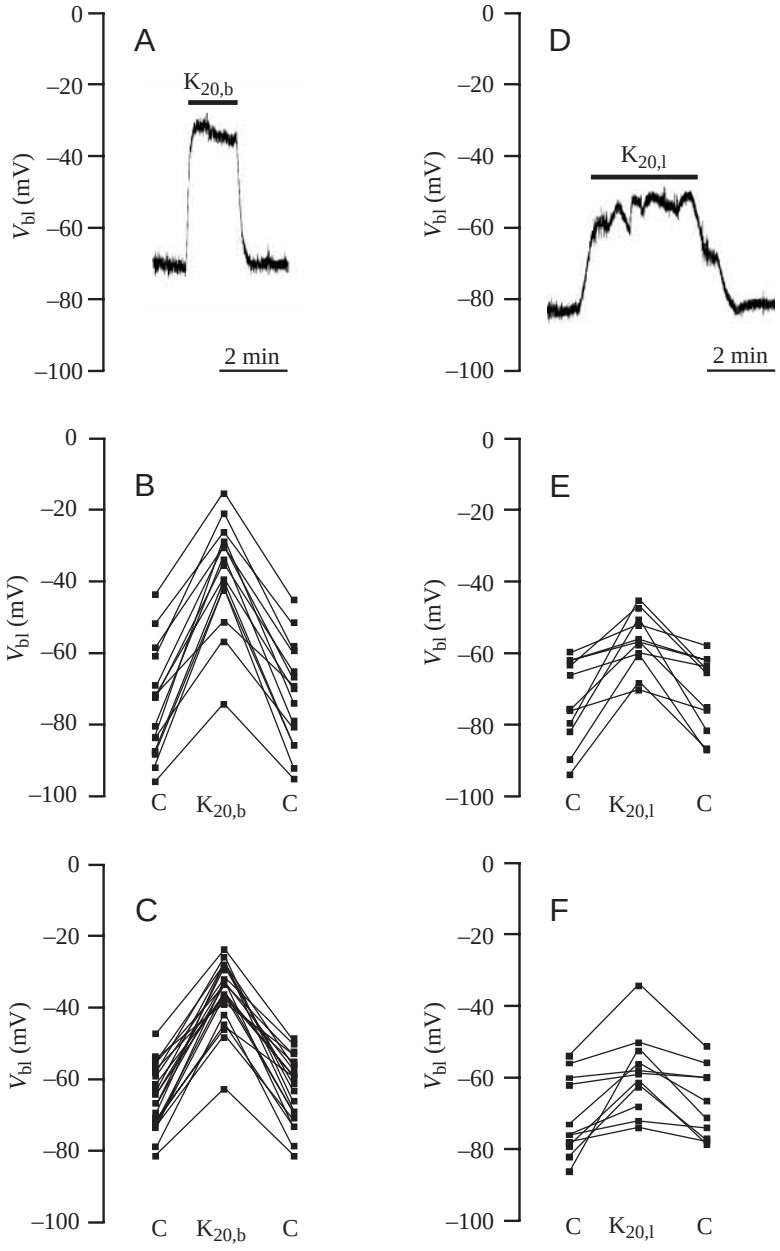

Fig. 3. Effect of bath and luminal $[K^+]$ steps on V_{bl}. (A) Voltage trace from a collecting duct cell. The effect of changing bath $[K^+]$ from 3 to 20 mmol l^{-1}. (B) Summary data illustrating the effect of increasing bath $[K^+]$ from 3 to 20 mmol l^{-1} on V_{bl} from collecting duct cells. (C) Summary data illustrating the effect of the bath $[K^+]$ step on V_{bl} from collecting tubule cells. (D) Voltage trace from a collecting duct cell. Effect of the luminal $[K^+]$ step. (E) Summary data illustrating the effect of increasing luminal $[K^+]$ from 3 to 20 mmol l^{-1} on V_{bl} from collecting duct cells. (F) Summary data illustrating the effect of the luminal $[K^+]$ step on V_{bl} from collecting tubule cells. C, control solution; $K_{20,b}$, high $[K^+]$ in bath; $K_{20,l}$, high $[K^+]$ in lumen.

Effect of Ba^{2+}

In the next series of experiments we tested the effect of Ba^{2+}, an inhibitor of several types of K^+ channels (Greger and Gögelein, 1987). In Fig. 4A a voltage trace from a collecting tubule cell illustrates the effect of 1 mmol l^{-1} $BaCl_2$ in bath control solution and in 20 mmol l^{-1} $[K^+]$ on V_{bl}. A summary of experiments from eight cells originating from four collecting ducts and three collecting tubules with a mean V_{bl} of –64±4 mV is presented in Fig. 4B. Upon addition of Ba^{2+} to the bath control solution, V_{bl} depolarized by 30±6 mV. The depolarizing effect on V_{bl} by the high $[K^+]$ test solution was inhibited by Ba^{2+}.

In Fig. 5A a voltage trace from a collecting tubule cell shows the effect of perfusing the tubule lumen with Ba^{2+} on V_{bl}. In this cell, Ba^{2+} in the control solution had no effect on V_{bl}, although luminal high $[K^+]$ solution elicited a depolarization of V_{bl}. However, the inhibitor abolished the depolarization by the high $[K^+]$ solution. One likely explanation is that, in the most hyperpolarized cells, the K^+ conductance of the basolateral cell membrane dominates the total cell conductance. V_{bl} was therefore not affected by the inhibition of the apical K^+ conductance with Ba^{2+}. The apical K^+ conductance was nevertheless revealed in the presence of a basolateral $[K^+]$ step. Fig. 5B shows the effect of luminal perfusion with Ba^{2+} in the same cell in an experiment where the cell was depolarized by a basally applied high $[K^+]$ solution. The high $[K^+]$ solution depolarized V_{bl} by 32 mV and a further depolarization of 8 mV was observed after the luminal perfusion with Ba^{2+}.

A summary of experiments from four collecting tubule cells and one collecting duct cell originating from five tubules showing the effect of luminal Ba^{2+}

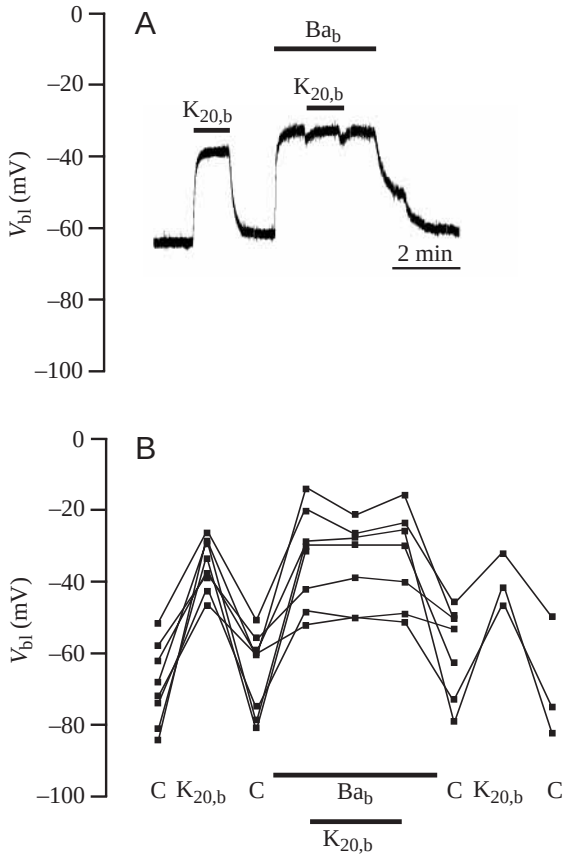

Fig. 4. (A) Voltage trace from a collecting tubule cell illustrating the effect of high $[K^+]$ in bath, and of 1 mmol l^{-1} $BaCl_2$ in bath control solution and in high $[K^+]$ solution, on V_{bl}. Basally applied Ba^{2+} depolarized V_{bl}. In the presence of Ba^{2+} the high $[K^+]$ solution did not depolarize V_{bl} further. (B) Summary data showing the effect of high $[K^+]$ solution, and of 1 mmol l^{-1} $BaCl_2$ in bath control solution and in high $[K^+]$ solution, on V_{bl}. Ba_b, Ba^{2+} in bath; C, control solution; $K_{20,b}$, high $[K^+]$ in bath.

is presented in Fig. 5C. In these five cells Ba^{2+} depolarized a V_{bl} of –71±5 mV by 9±3 mV. Fig. 5D is a summary of three experiments made on two collecting tubule cells and one collecting duct cell, showing the effect on V_{bl} of luminal perfusion with Ba^{2+} in control solution, while depolarizing the cell with a basal high $[K^+]$ solution. Ba^{2+} had an effect if high $[K^+]$ in the bath left the cell relatively hyperpolarized.

Taken together, experiments with the K^+-channel inhibitor Ba^{2+} provide further evidence for the presence of K^+ channels in the both luminal and basolateral cell membranes.

Effect of basolateral inhibitors

In order to test the mechanism by which K^+ enters the cell across the basolateral cell membrane, transport inhibitors were added to the bath solution. A voltage trace illustrating the effect on V_{bl} of 1 mmol l^{-1} ouabain in the bath control solution is shown in Fig. 6A. Addition of ouabain to the bath solution for 2–4 min resulted in a rapid and fully reversible depolarization of V_{bl}, an effect that illustrates the electrogenic contribution of the Na^+-K^+-ATPase to V_{bl}. A summary of experiments from seven cells originating from one collecting duct and six collecting tubules with a mean V_{bl} of –78±4 mV is shown in Fig. 6B. Upon addition of ouabain to the bath control solution, V_{bl} depolarized by 11±2 mV.

In six cells, three from collecting tubules and three from collecting ducts the effect of the loop diuretic furosemide on Na^+-K^+-Cl^- cotransport was tested, and was found to have no significant effect on V_{bl} (Fig. 6C).

These experiments indicate the presence of a Na^+-K^+-ATPase in the basolateral cell membrane. We found no evidence for the presence of a Na^+-K^+-Cl^- cotransporter, hence, K^+ entry into the cell across the basolateral cell membrane may be mediated by the pump.

Discussion

To our knowledge, this study provides the first electrophysiological data on the collecting duct system of an anuran amphibian. We examined the characteristics of K^+ transport by the collecting duct system of the toad *Bufo bufo*. Qualitatively, there seemed to be no difference between K^+ transport in cells from collecting tubules and from collecting ducts. Our data indicate that luminal K^+ channels would provide a pathway for secretion of K^+ from the cell into the lumen.

The collecting duct system of amphibians is a heterocellular epithelium composed of principal and intercalated cells (Møbjerg et al., 1998). In this context it should be noted that the V_{bl} of collecting duct cells fell into two groups, one being more hyperpolarized than the other. We do not know if this distribution (Fig. 2A) reflects two populations of cells in different functional states, or cells with different transport characteristics, e.g. principal and intercalated cells. In the present study it was not possible to distinguish between principal and intercalated cells at a functional level, as K^+ conductances were similar in all cells. In an ultrastructural investigation of the distal nephron, Stanton et al. (1984) reported that K^+ adaptation in *Amphiuma means* led to an increase in the basolateral membrane area of principal cells in collecting tubules. A similar increase was not observed in the intercalated cells. These data indicate that K^+ secretion is limited to the principal cells. It has been proposed that in the mammalian cortical collecting tubule an apical H^+-K^+-ATPase in the luminal membrane of intercalated cells enables this minority cell type to reabsorb K^+ (for a review, see Giebisch, 1998). In this context it is interesting that H^+-K^+-ATPase activity has been demonstrated in the late distal tubule and collecting tubule from *Necturus maculosus* and from *Rana ridibunda* (see Planelles et al., 1991).

We found that the basolateral cell membrane of cells from both collecting tubules and collecting ducts possessed a large K^+ conductance. Our evidence for the K^+ conductance is relatively straightforward and consistent with previous published studies on the collecting tubule of *Amphiuma* sp. (see Horisberger et al., 1987; Hunter et al., 1987). Raising the concentration of K^+ in the bath solution resulted in a reversible

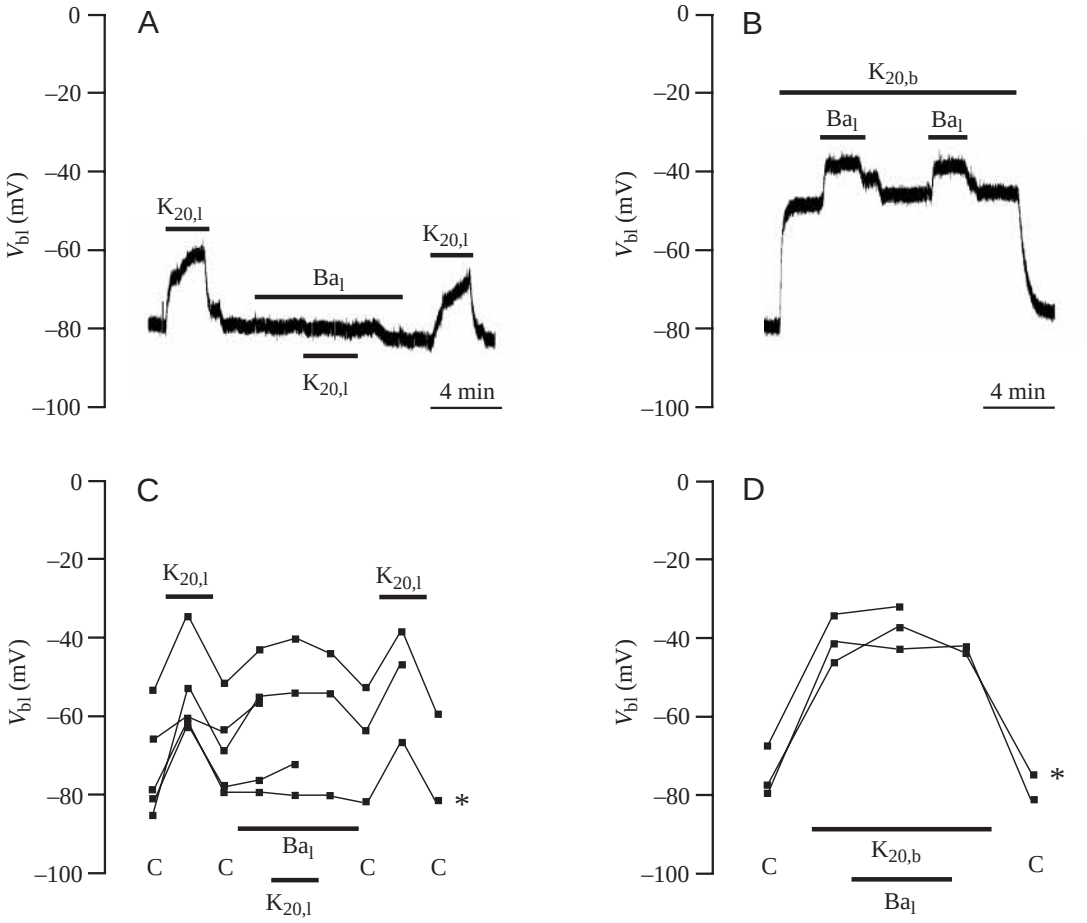

Fig. 5. Effect of luminal Ba^{2+}. (A) Effect on V_{bl} of luminal $[K^+]$ step (3–20 mmol l^{-1}) and of 1 mmol l^{-1} $BaCl_2$ in luminal control solution and in high $[K^+]$ solution. In this cell, Ba^{2+} in the control solution had no effect on V_{bl}. Depolarization by the high $[K^+]$ solution was abolished by Ba^{2+}. (B) Effect of luminal perfusion with Ba^{2+} on V_{bl} in the same cell as in A in an experiment where the cell was depolarized by high bath $[K^+]$. (C) Summary data showing the effect of a luminal $[K^+]$ step (3–20 mmol l^{-1}) and of 1 mmol l^{-1} $BaCl_2$ in luminal control solution and in high $[K^+]$ solution on V_{bl}. The asterisk marks the cell shown in A and B. (D) Summary of experiments showing the effect on V_{bl} of luminal perfusion with Ba^{2+} in the control solution, while depolarizing the cell with a high basal $[K^+]$ solution. The asterisk marks the cell shown in A and B. Ba_b, Ba^{2+} in bath; Ba_l, Ba^{2+} in lumen; C, control solution; $K_{20,b}$, high $[K^+]$ in bath; $K_{20,l}$, high $[K^+]$ in lumen.

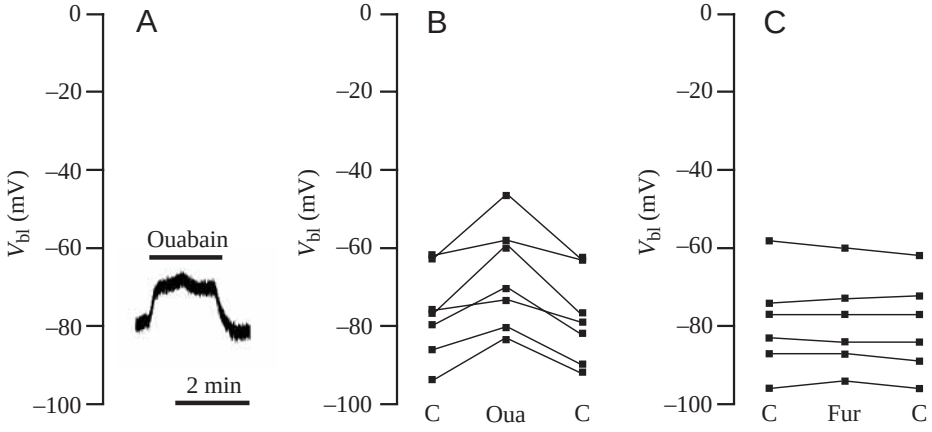

Fig. 6. (A) Voltage trace from a collecting tubule cell illustrating the effect on V_{bl} of 1 mmol l^{-1} ouabain in bath control solution. (B) Effect of 1 mmol l^{-1} ouabain on V_{bl}. (C) Effect of 10^{-5} mol l^{-1} furosemide on V_{bl}. Inhibitors were applied to the bath for 2–4 min. C, control solution; Fur, furosemide; Oua, ouabain.

depolarization of V_{bl}. The addition of millimolar concentrations of the K^+ channel inhibitor Ba^{2+} to the bath solution inhibited the basolateral K^+ conductance. Assuming that the intracellular K^+ activity of the cells in the collecting duct system of *Bufo bufo* is in the same range (56 mmol l^{-1}) as the one measured with ion-sensitive microelectrodes in the collecting tubule of *Amphiuma* by Horisberger and Giebisch (1988), the equilibrium potential (given by the Nernst equation), when using the ion

activities for K^+ in control solution and in high [K^+] solution, would be approximately –83 mV and –35 mV, respectively. These values are in agreement with the results presented for the most hyperpolarized cells in Fig. 3B and indicate that K^+ is the main contributor to the total cell conductance.

This study provides the first evidence for K^+ conductance in the apical cell membrane of cells from the collecting duct system of an amphibian. Our experiments on the toad show that V_{bl} depolarized rapidly in cells from both collecting tubules and collecting ducts in response to luminal perfusion with a high [K^+] solution (Fig. 3D, Fig. 5A). Changing the luminal K^+ concentration had a less pronounced effect on V_{bl}, indicating that the contribution of the apical K^+ conductance to total cell conductance was smaller than that of the basolateral conductance. Ba^{2+} inhibited the depolarizing effect of the high [K^+] solution in the luminal fluid, which confirms the presence of a K^+-conducting pathway in the apical cell membrane. The influence of luminal Ba^{2+} seemed to be determined by the magnitude of the basolateral K^+ conductance to V_{bl}.

A low-conductance K^+-selective channel, such as the one described by Frindt and Palmer (1989) for the apical cell membrane of the rat cortical collecting tubule and considered to play an essential role in K^+ secretion in this mammalian species (Palmer, 1999), has not been found in amphibians. Nevertheless, in recent studies Stoner and Viggiano (1998, 1999, 2000) used the patch-clamp technique and described single K^+ channel currents in the apical cell membrane of the collecting tubule of larval *Ambystoma tigrinum*. Maxi K^+ channels and intermediate-conductance, non-specific cation channels were active under hyperkalemic conditions, and may therefore be involved in K^+ secretion in K^+-adapted salamanders. Whether these channels contribute to K^+ secretion under normal physiological conditions is unknown. It is also unknown whether the channels underlie K^+ entry into the lumen of the collecting duct system in *Bufo bufo*.

In the urine of *Bufo bufo* we found a large variation in K^+ concentration, consistent with the presence of transport mechanisms responsible for K^+ reabsorption and secretion in the kidney or bladder epithelium. We propose that under normal physiological conditions, cells of the collecting duct system of *B. bufo* secrete K^+ into the urine in two steps. K^+ is actively taken into the cell over the basolateral cell membrane by the Na^+-K^+-ATPase and a large K^+ conductance in the basolateral cell membrane recycles K^+ for the pump. Lack of an effect of basally applied furosemide indicates that transport by a Na^+-K^+-Cl^- cotransporter is not significant. K^+ channels in the apical cell membrane provide a path by which K^+ can diffuse down its electrochemical gradient into the lumen of the tubule.

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