Basic Properties and Potential Regulators of the Apical K^+ Channel in Macula Densa Cells

A. M. HURST, J.-Y. LAPOINTE, A. LAAMARTI, and P. D. BELL*

From the Groupe de Recherche en Transport Membranaire, Université de Montréal, Montréal, Québec HC3J7; and *Nephrology Research and Training Center, Departments of Medicine and Physiology, University of Alabama at Birmingham, Birmingham, Alabama, 35294

ABSTRACT These studies examine the properties of an apical potassium (K^+) channel in macula densa cells, a specialized group of cells involved in tubuloglomerular feedback signal transmission. To this end, individual glomeruli with thick ascending limbs (TAL) and macula densa cells were dissected from rabbit kidney and the TAL covering macula densa cells was removed. Using patch clamp techniques, we found a high density (up to 54 channels per patch) of K^+ channels in the apical membrane of macula densa cells. An inward conductance of 41.1 ± 4.8 pS was obtained in cell-attached patches (patch pipette, 140 mM K^+). In inside-out patches (patch pipette, 140 mM; bath, 5 mM K^+), inward currents of 1.1 ± 0.1 pA (n = 11) were observed at 0 mV and single channel current reversed at a pipette potential of −84 mV giving a permeability ratio (P_K/P_{Na}) of over 100. In cell-attached patches, mean channel open probability (*N.Po*, where N is number of channels in the patch and *Po* is single channel open probability) was unaffected by bumetanide, but was reduced from 11.3 ± 2.7 to 1.6 ± 1.3 (n = 5, $p < 0.02$) by removal of bath sodium (Na^+). Simultaneous removal of bath Na^+ and calcium (Ca^{2+}) prevented the Na^+-induced decrease in *N.Po* indicating that the effect of Na^+ removal on *N.Po* was probably mediated by stimulation of Ca^{2+} entry. This interpretation was supported by studies where ionomycin, which directly increases intracellular Ca^{2+}, produced a fall in *N.Po* from 17.8 ± 4.0 to 5.9 ± 4.1 (n = 7, $p < 0.02$). In inside-out patches, the apical K^+ channel was not sensitive to ATP but was directly blocked by 2 mM Ca^{2+} and by lowering bath pH from 7.4 to 6.8. These studies constitute the first single channel observations on macula densa cells and establish some of the characteristics and regulators of this apical K^+ channel. This channel is likely to be involved in macula densa transepithelial Cl^- transport and perhaps in the tubuloglomerular feedback signaling process.

INTRODUCTION

Within each cortical thick ascending limb (TAL) glomerulus complex, specialized cells, called macula densa cells, reside in the TAL on the side of the tubule that is

Address correspondence to Dr. P. D. Bell, Departments of Medicine and Physiology, 865 Sparks Center, UAB Station, University of Alabama at Birmingham, Birmingham, AL 35294.

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closest to the glomerulus (Schnermann and Briggs, 1985). This close association between macula densa cells and vascular components of the juxtaglomerular apparatus forms the anatomical basis for the physiological response observed during flow rate changes in the loop of Henle (Barajas, 1981); i.e., increases in TAL tubular fluid flow rate result in elevations in luminal [NaCl] at the macula densa and decreases in glomerular filtration rate (Schnermann, Ploth, and Hermle, 1976). Macula densa cells are thought to sense a change in composition of tubular fluid and to initiate signals that alter vascular resistance. This response is known as tubuloglomerular feedback (Briggs, 1981; Wright, 1981; Bell, Franco-Guevera, Abrahamson, Lapointe, and Cardinal, 1988; Schnermann and Briggs, 1985).

Experimental evidence regarding the nature of the tubuloglomerular feedback mechanism has come largely from in vivo micropuncture studies (Briggs and Schnermann, 1987; Schnermann and Briggs, 1985). It was not until 1985 that the macula densa cells could be directly visualized during microperfusion of an individually dissected TAL with associated glomerulus (Kirk, Bell, Barfuss, and Ribadeneira, 1985; Skott and Briggs, 1987). In 1988, electrophysiological techniques utilizing microelectrodes were combined with the isolated perfused macula densa preparation to examine transport properties of macula densa cells (Bell et al., 1988). This work, as well as other microelectrode studies, (Bell, Lapointe, and Cardinal, 1989; Schlatter, Salmonsson, Persson, and Greger, 1989; Lapointe, Bell, and Cardinal, 1990; Lapointe, Bell, Hurst, and Cardinal, 1991) provided evidence for an electroneutral $Na^+/K^+/2Cl^-$ cotransporter at the apical membrane of macula densa cells, a large basolateral conductance for Cl^- and a much smaller basolateral conductance for K^+.

The scheme for ion transport in macula densa cells is similar in many respects to that proposed for TAL cells. There does, however, appear to be differences between the two cell types. With the addition of furosemide, a faster membrane potential response time is observed in TAL compared to macula densa cells (Schlatter, Salmonsson, Persson, and Greger, 1989; and unpublished observation, Lapointe, Cardinal and Bell). In addition, there may be differences in the affinity of the apical cotransporter for Na^+ and Cl^- between macula densa and TAL cells (Lapointe et al., 1990). In spite of these differences, the proposal has been made that macula densa cells transport Na^+, Cl^- and K^+ in a manner which is similar to cells of the TAL. This has, in part, lead to the suggestion that macula densa cells possess an apical K^+ conductance since this channel is present in the apical membrane of TAL cells. The existence of such an apical K^+ conductance, however, has not been determined experimentally in macula densa cells. One reason for this is the inherent difficulty of interpreting changes in basolateral membrane potential with alterations in luminal $[K^+]$ because changes in luminal K^+ may influence membrane potential through the K^+ conductance and/or indirectly through the cotransporter and intracellular $[Cl^-]$. Therefore, a more direct approach was required in order to determine if an apical K^+ conductance existed in macula densa cells. Using the isolated macula densa-glomerulus-TAL unit, we found that it was possible to remove the TAL which covers the macula densa plaque thus exposing the apical membrane of macula densa cells. This allowed us to use patch clamp techniques to investigate directly the conductive properties of the apical membrane of macula densa cells. Our results provide evidence for the existence of a high density of K^+ channels in the apical membrane of

macula densa cells. We report the basic characteristics of this channel; conductance, selectivity, voltage dependence, and the effects of different potential regulators of channel activity. This knowledge allowed us to compare the properties and regulation of this K^+ channel with what is known for the apical K^+ channel of cortical TAL and provided some insight into the issue of similarities in transport characteristics between macula densa and TAL cells. In addition, an understanding of the characteristics and regulation of this apical K^+ channel may help in elucidating the mechanisms involved in the generation of tubuloglomerular feedback signals.

METHODS

Tubule Preparation

Studies were performed using kidneys obtained from New Zealand white rabbits. The renal artery was cannulated and the left kidney perfused with 30 ml of chilled preservation fluid (Na_2HPO_4, 56 mM; NaH_2PO_4, 13 mM; sucrose, 140 mM) (Pirie and Potts, 1985). Transverse

TABLE I

Composition of Solutions Used for Cell-Attached and Inside-out Experiments

	Pipette	Ringer	0Na	0Na/0Ca	0Ca EGTA	0Ca
NaCl	0	135	0	0	135	135
KCl	140	5	5	5	5	5
NMDG	0	0	135	135	0	0
$CaCl_2$	2	2	2	0	0	0
$MgCl_2$	1	1	1	1	1	1
HEPES	5	5	5	5	5	5
EGTA	0	0	0	0	1.5	0
glucose	5	5	5	5	5	5
mannitol	0	0	0	2	0	0

The pH of each solution was adjusted to pH 7.4 by titration of HEPES with either KOH for the pipette solution or NaOH for all the other solutions.

slices were cut and placed in chilled preservation fluid. Mid-cortical TAL's with attached glomeruli were isolated by dissection at a magnification of 80 with sharpened forceps. That portion of the TAL covering the macula densa plaque was carefully removed by dissection to expose the apical membrane of macula densa cells (Fig. 1). An individual macula densa-glomerulus-TAL unit was then transferred to a chamber which was mounted on the stage of an inverted microscope. The glomerulus was stabilized with a glass holding pipette in such a position that the apical membrane of macula densa cells was clearly visible and accessible to a patch pipette. The bathing solution exchange time was on the order of 30 s. All experiments were performed at room temperature and the composition of the solutions used are shown in Table I.

Patch Clamping

A Narishige PP-83 two stage patch pipette puller was used to fabricate patch pipettes from hematocrit capillary tubes (Fisher Scientific Co., Pittsburgh, PA). Standard patch clamp techniques (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981) were used to record channel currents which were amplified with a List (LM-EPC7, FRG) patch clamp amplifier and recorded

onto video tape, following pulse code modulation (Neurodata DR-384), for later analysis. Unless stated otherwise, all experiments were performed at a pipette potential of 0 mV.

Data Analysis

Data analysis was performed using PClamp software (version 5.5.1, Axon Instruments, Inc., Foster City, CA). Channel currents were low pass filtered at 500 Hz (Frequency Devices) and digitized at 1 kHz via a TL-1 DMA Labmaster interface (Axon Instruments, Inc.) using an 386 IBM compatible PC.

Mean channel open probability (*N.Po*) was calculated from the following equation:

$$N.Po = \langle I \rangle / i$$

where N is the number of channels in the patch, Po is the single channel open probability, $\langle I \rangle$ is the mean current and i is the single channel current. In patches with a high level of channel activity, recording times in the order of 4 s were sufficient to determine *N.Po* whereas longer recording times (up to 60 s) were needed whenever channel activity was low. This calculation makes the assumption that the leak current is minimal at a pipette potential of 0 mV. This assumption is supported by the finding that, in 10 patches where channel activity decreased to zero during an experimental maneuver, the current measured at a pipette potential of 0 mV was only -0.27 ± 0.07 pA (inward current). In experiments where *N.Po* was measured during changes in the applied potential, we assumed that leak current was linearly related to voltage. All acceptable recordings had a minimum seal resistance of 10 giga Ohms as estimated from the current measured at the reversal potential of the channel. Under these conditions, the contribution of leak current to the determination of *N.Po* at, for example, +20 mV (cell-attached patches), was $<10\%$. Channel conductance was estimated from linear regression analysis of single channel current-voltage curves. Voltage applied to the pipette (Vp) is referenced to the bath potential ($Vp - V_{bath}$). In cell-attached patches, potential across the patch is equal to the cell membrane potential minus Vp whereas in inside-out patches, the potential across the patch is simply equal to minus Vp.

Solutions

In all experiments, a high K^+ solution was used in the patch pipette (Table I). The composition of the bathing solutions are also shown in Table I. In some experiments, the following drugs were added to the Ringer solution: 5 μM bumetanide, 5 μM ionomycin, 0.1 mM dibutyryl cyclic AMP + 1 μM forskolin and 2 mM ATP. In some inside-out patch experiments, pH of the 0 Ca^{2+} solution (without EGTA) was adjusted to pH 6.8 with HCl. Care was taken to adjust the pH of the final solution to pH 7.4 by addition of small amounts of NaOH, KOH (pipette solution) or HCl (0 Ca^{2+} solution without EGTA). All chemicals were purchased from Sigma Chemical Co., (St. Louis).

Statistics

Data are presented as mean ± SEM. Because control and experimental values were always obtained in the same patch, statistical analysis was performed using the paired t test. Data in Fig. 8 were analyzed using the Wilkinson Rank Test.

RESULTS

Fig. 1 is a photograph of a macula densa preparation used for patch clamp experiments. As shown in the example, macula densa cells are clearly visible and the apical membrane is directly exposed to the bathing solution because the TAL which

covered the plaque has been pulled away. The preparation was steadied at the bottom of the chamber by gently applying pressure to the glomerulus with a glass pipette. Using this arrangement, the success rate of seal formation with a seal resistance higher than 10 GΩ (assuming a voltage-independent open probability, see below) was 38% in 363 attempts.

Conductance, Selectivity and Voltage Dependence

A high density of a single type of K^+ channels was observed on the apical membrane of macula densa cells. A single patch could contain up to 54 simultaneously open channels. There was, however, a considerable variation in *N.Po* values obtained in

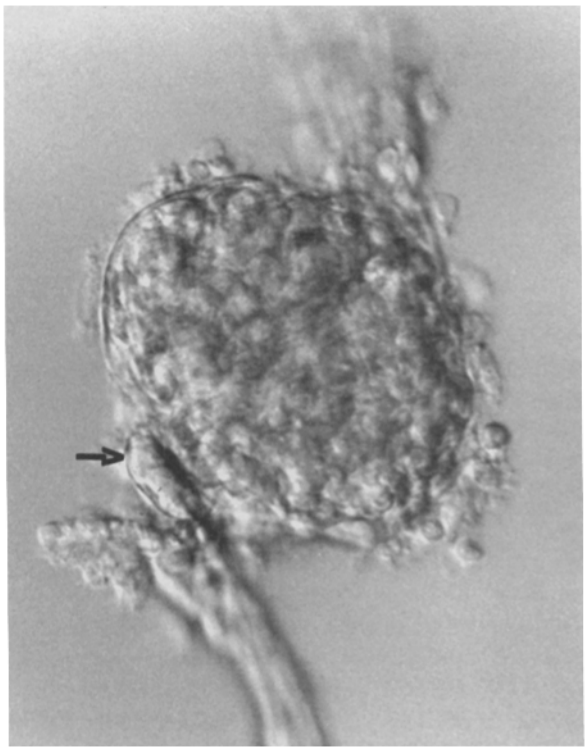

FIGURE 1. Photograph of exposed macula densa plaque (at *arrow*), thick ascending limb and attached glomerulus. The diameter of the macula densa cells is ~20 μM.

these studies. The most likely explanation for this finding is that the density of K channels may vary from cell to cell or that there may be uneven distribution of channels within the apical membrane. Fig. 2 shows the current-voltage relationship for the channel we observed in cell-attached (Fig. 2 *A*) and inside-out patches (Fig. 2 *B*), in the presence of a high K^+ pipette solution and the control Ringer's bathing solution. In cell-attached patches, inward currents of 2.8 ± 0.3 pA (n = 6) were observed at 0 mV pipette potential. Average inward conductance of the channel was 41.1 ± 4.8 pS (n = 6) and, as estimated by extrapolation, the single channel current would reverse at a pipette potential of −86 mV. This is consistent with a high intracellular [K^+] and the presence of a K^+ selective channel.

Measurements of channel selectivity were performed in inside-out patches with a K^+ gradient of 140 mM to 5 mM (pipette to bath). Patches were excised into a Ca^{2+} free Ringer solution containing EGTA (see section entitled; Modulators of K^+ channel activity in inside-out patches) and single channel inward currents of 1.1 ± 0.1 pA (n = 11) were observed at a Vp of 0 mV and the reversal potential estimated by extrapolation was −84 mV. From the reversal potential, a permeability ratio (P_K/P_{Na}) of greater than 100 can be estimated, indicating a highly K^+ selective channel. However, conductance of the channel in inside-out patches was only 17.9 ± 2.4 pS (n = 8), a value which was approximately half of that obtained in cell-attached patches. A part of this decrease in channel conductance could be due to the dependence of the conductance for inward currents on cytosolic [K^+] as predicted by the constant field equation. However, this effect is greatest at the reversal potential

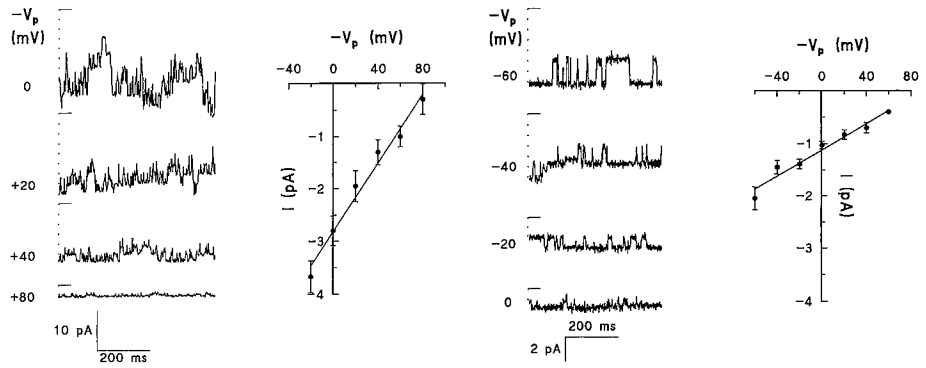

FIGURE 2. (*A*) On the left is an example of the current recordings at different pipette potentials obtained from cell-attached patches with high K^+ in the pipette and the bath containing normal Ringer solution. The graph on the right is the *I-V* relationship and the solid line is a linear regression fit of the data. Downward deflection and negative currents represent inward currents (pipette to cell) and the horizontal lines represent the zero current level from which channel currents (and some leak current) have been measured. (*B*) On the left is an example of the current-voltage relationship from inside-out patches with high K^+ in the pipette and 0 Ca^{2+} Ringer + EGTA in the bath. On the right is the *I-V* relationship and the solid line is a linear regression fit of the data.

and should be small at potentials where the channel conductance was measured. It is also possible that the decrease in conductance in inside-out patches, could be due to the absence of important cytosolic regulators of channel permeability in the inside-out configuration. Finally, there is the possibility that the channels seen in excised patches are different from the channels in cell-attached configuration. We believe that this is a rather unlikely explanation as it requires a peculiar combination of rapid activation and deactivation of the channels upon excision. In addition, the Ca sensitivity (see below) seen in both configurations argues against this possibility.

The macula densa apical K^+ channel does not appear to be very voltage dependent in either cell-attached or inside-out configuration (Fig. 3). To reduce the error associated with leak current, paired measurements of *N.Po* were performed over a relatively narrow voltage range. In cell-attached patches (left side of Fig. 3), *N.Po* was

11.5 ± 2.0 in the absence of an applied potential and did not change significantly *N.Po* = 12.2 ± 2.9 ($n = 6$, $p > 0.6$) over a 40 mV depolarization. *N.Po* was consistently less in inside-out patches (right side of Fig. 3) compared to cell-attached patches, further suggesting that the inside-out configuration resulted in a modification of the channel or a loss of cytosolic regulators. In the absence of an applied potential *N.Po* was 5.3 ± 1.0, and did not change significantly (5.1 ± 1.0 [$n = 3$, $p > 0.7$]) with the application of a 20 mV depolarization.

Effects of Bumetanide on K^+ Channel Activity

Previous work on apical K^+ channels in the frog diluting segment (Hurst and Hunter, 1992), a preparation which has been used as a model for the mammalian TAL, found that loop diuretics such as furosemide can alter K^+ channel activity indicating some form of indirect coupling between K^+ channel and $Na^+/K^+/2Cl^-$ cotransporter. Because the apical membrane of macula densa cells also contains both K^+ channel and $Na^+/K^+/2Cl^-$ cotransporter (Bell et al., 1989; Schlatter et al., 1989; Lapointe et al., 1990) it was of interest to determine if bumetanide altered the activity of K^+ channels in macula densa cells. This $Na^+/K^+/2Cl^-$ transporter in macula densa cells is sensitive to loop diuretics and it has been shown that furosemide inhibits feedback responses (Wright and Schnermann, 1974).

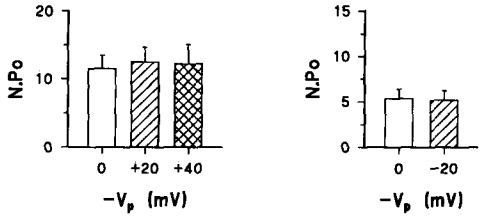

FIGURE 3. (*Left*) Dependence of channel open probability (*N.Po*) on voltage in cell-attached patches. (*Right*) Dependence of channel open probability (*N.Po*) on voltage in inside-out patches.

In cell-attached patches, 5 μM of the loop diuretic bumetanide was added to the Ringer solution. In six experiments, addition of bumetanide did not significantly alter *N.Po*. Under control conditions, *N.Po* was 15.2 ± 5.3 and 14.4 ± 6.8 ($p > 0.2$) in the presence of bumetanide.

Effect of Na^+ and Ca^{2+} on K^+ Channel Activity

In other cell-attached patch experiments, the effects of replacement of bath Na^+ with *n*-methyl-D-glucamine (see Table I, $0Na^+$) on macula densa K^+ channel activity was assessed. (It should be noted that pH of the $0Na^+$ solution was adjusted to pH 7.4 with NaOH, therefore the $0Na^+$ solution contained ~3 mM Na^+) Fig. 4 shows a typical record from a cell-attached patch illustrating the decline in channel activity during removal of bath Na^+. As summarized on the left side of Fig. 5, *N.Po* fell significantly from 11.3 ± 2.7 to 1.6 ± 1.3 ($n = 5$, $p < 0.02$) during removal of bath Na^+.

Removal of Na^+ from the bathing solution may have multiple effects on macula densa cells including alterations in intracellular concentrations of other electrolytes. We hypothesized that one effect of Na^+ removal might be an increase in macula

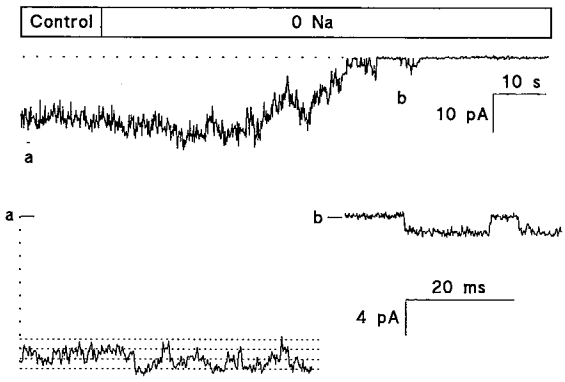

FIGURE 4. Representative recording showing the effect of elimination of bath Na^+ on channel activity (*top record*). The lower records are on an expanded time base and were taken from the places indicated by *a* and *b* in the upper record. In *a*, the solid line is the closed channel current level; in *b* there is only one channel open. Downward deflections are channel opening events and represent current flow from pipette to cell.

densa intracellular [Ca^{2+}] perhaps through Ca^{2+} entry via a Na^+:Ca^{2+} exchanger. The increase in cytosolic [Ca^{2+}] would, in turn, be responsible for the decline in K^+ channel activity. If so, then inhibition of channel activity during Na^+ removal would be dependent upon the presence of external Ca^{2+}. The right side of Fig. 5 shows that K^+ channel activity did not significantly change during simultaneous removal of Na^+ and Ca^{2+} from the bath solution. In control experiments *N.Po* was 21.9 ± 5.5, whereas in 0 Na^+/0 Ca^{2+} *N.Po* was 21.1 ± 7.2 ($n = 5$, $p > 0.8$). This result clearly shows that the decrease in K^+ channel activity during Na^+ removal is dependent upon the presence of external Ca^{2+}.

To characterize further the effect of intracellular Ca^{2+} on K^+ channel activity, experiments were performed in which intracellular [Ca^{2+}] was elevated using the Ca^{2+} ionophore, ionomycin. Fig. 6 shows that ionomycin (5 μM) addition in the presence of 2 mM Ca^{2+} in the bathing solution, produced substantial deceases in channel activity from cell-attached patches. Mean *N.Po* in the absence of ionomycin was 17.8 ± 4.0 and decreased significantly to 5.9 ± 4.1 ($n = 7$, $p < 0.02$) in the presence of ionomycin.

The Effect of Cyclic AMP on Macula Densa K^+ Channels

A close association often exists between intracellular Ca^{2+} and cyclic AMP signaling systems such that cyclic AMP can modulate Ca^{2+}-mediated events (Rasmussen and

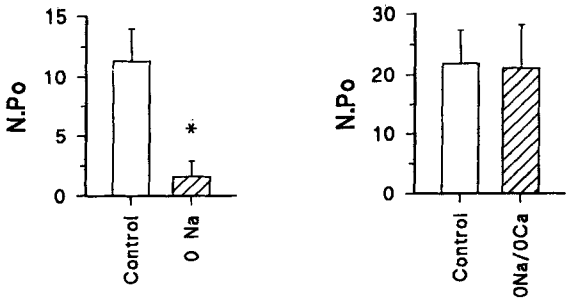

FIGURE 5. (*Left*) The effect of Na^+ removal on average *N.Po* in cell-attached patches. *Indicates significance at the 5% level. (*Right*) The effect of simultaneous removal of Na^+ and Ca^{2+} from the bath on average *N.Po* in cell-attached patches.

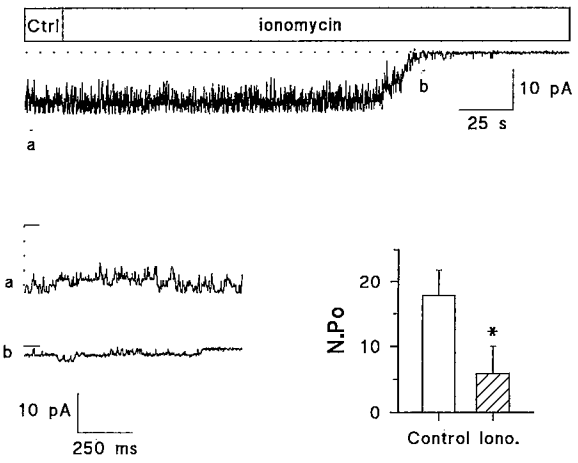

FIGURE 6. Representative tracing showing inhibition of K^+ channel activity with 5 μM ionomycin. (*Left*) The lower traces (*a* and *b*) are expanded time base records taken from the upper trace at the positions marked. The solid line under trace *a* is the closed channel current level and in *b*, there is only one channel open. Downward deflections are channel opening events and represent current flow from the pipette to the cell. (*Right*) Presents average data obtained in these experiments. *Indicates significance at the 5% level.

Barrett, 1984). Also, agents that increase cell cyclic AMP levels previously have been shown to significantly inhibit the transmission of tubuloglomerular feedback signals (Bell, 1985, 1987). In cell-attached patches, macula densa cell cyclic AMP content was increased by addition of dibutyryl cyclic AMP (0.1 mM) and forskolin (1 μM), an activator of adenylate cyclase. In three experiments, addition of dibutyrl cyclic AMP plus forskolin had no effect on K^+ channels; control *N.Po* was 29.4 ± 6.9 compared to 25.1 ± 8.0 ($p > 0.2$) in the presence of dibutyryl cyclic AMP + forskolin.

Modulators of K^+ Channel Activity in Inside-out Patches

To elucidate further the regulation of macula densa K^+ channel, experiments were performed using inside-out patches. Because channel activity may be inhibited by Ca^{2+}, patches were initially excised into a Ca^{2+}-free Ringer solution containing

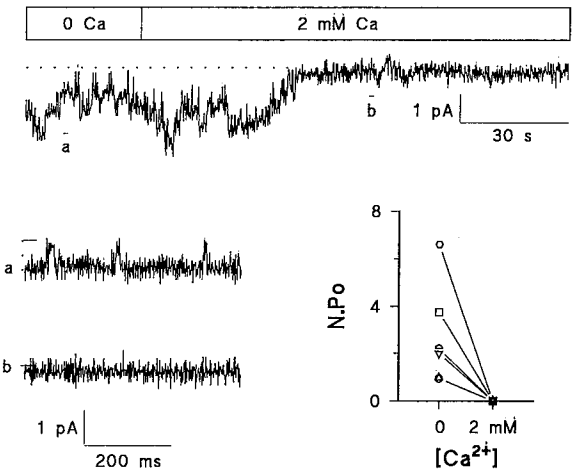

FIGURE 7. Representative tracing showing the effects of bath Ca^{2+} on channel activity in an inside-out patch. (*Left*) Traces *a* and *b* are expanded time base records of the upper trace taken from the place indicated. The solid line in *a* is the closed channel current level and in *b*, all channels are closed. Downward deflections are channel opening events and represent current flow from pipette to bath. (*Right*) The effect of 2 mM Ca^{2+} on *N.Po* in six experiments.

EGTA. As previously discussed, inside-out patches exhibited lower channel activity compared to that obtained in cell-attached patches but this lower channel activity was routinely observed to be stable for a few minutes (2–4 min). This length of time was sufficient to discriminate between rapid effects (10–20 s), responses to experimental manipulations and channel rundown. Fig. 7 shows an example and summarizes K^+ channel activity during addition of Ca^{2+} (2 mM) to the bath solution. In six experiments, *N.Po* under control conditions averaged 2.7 ± 0.9 and decreased to zero ($p < 0.03$) in each experiment with addition of Ca^{2+}. Reactivation of channel activity by removal of bath Ca^{2+} was unsuccessful at least over a 4-min recovery period.

Recent work has indicated that there may be physiological changes in macula densa cell pH during alterations in luminal [NaCl] (Fowler, Lapointe, and Bell, 1991). In addition, other studies have shown that certain renal K^+ channels are

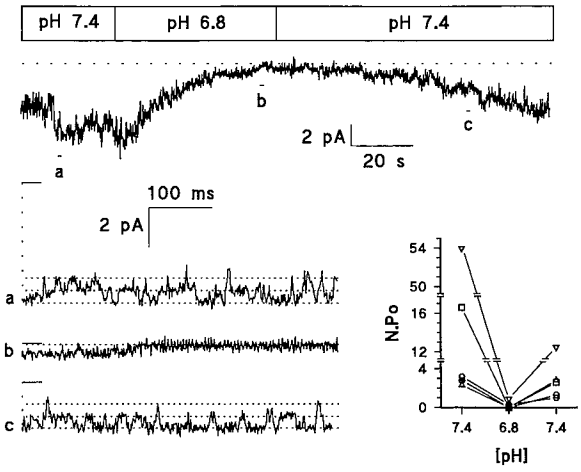

FIGURE 8. Representative recording depiciting the effect of bath acidification on channel activity in inside-out patches is shown in the upper portion of this figure. (*Left*) Expanded time-base records taken from the place indicated: (*a*) during control (pH 7.4); (*b*) at pH 6.8; and (*c*) after return to pH 7.4. Solid lines above the traces (*a* and *c*) are the closed channel current levels and in *b* there is no channel activity. Downward deflections are channel opening events and represent current flow from pipette to cell. (*Right*) Individual data obtained in inside-out patches during changes in bath pH.

sensitive to changes in pH (Hurst and Hunter, 1989; Beck, Hurst, Lapointe, and Laprade, 1993; Bleich et al., 1990). Therefore, studies were performed in inside-out patches to determine if the macula densa apical K^+ channel was sensitive to pH. As shown in the tracing (Fig. 8), there was a fall in channel activity with a reduction in bath pH from 7.4 to 6.8 that was partially reversible upon return of bath pH to 7.4. Data from individual experiments are shown in the lower right hand corner of Fig. 8. In this group, there was a large variability in initial *N.Po* values. Nevertheless, in each experiment lowering bath pH resulted in a substantial fall in *N.Po* to values which were at or near zero. Because of the initial variability in *N.Po,* these data were analyzed with a Wilkinson Rank Test which revealed a highly significant decrease in *N.Po* upon reducing bath pH ($p < 0.001$).

A number of recent studies have shown that certain K^+ channels are sensitive to ATP including the K^+ channel found on the apical membrane of TAL cells (Wang et

al., 1990; Bleich et al., 1990). In inside-out patches we tested for ATP sensitivity of the macula densa K^+ channel. Control *N.Po* was 1.9 ± 0.7 and addition of 2 mM disodium ATP to the 0 Ca^{2+} (no EGTA) bathing solution did not produce a significant change in *N.Po*, 1.6 ± 0.6 ($n = 3$, $p > 0.2$).

DISCUSSION

In the present study we report that the apical membrane of macula densa cells contains a high density of K^+ selective channels. Careful removal of the TAL covering the macula densa cells enabled us to perform patch clamp experiments on this previously inaccessible region of the nephron. A similar approach, but using collagenase, was recently used to measure cell potential in whole cell recordings (Schlatter, 1993). The abundance of K^+ channels and high rate of successful seal formation allowed us to document certain biophysical properties of the channel and to determine the effect of some potential regulators. In addition, careful examination of the recordings revealed the presence of another smaller channel (single-channel

TABLE II

Comparison of K^+ Channels in Macula Densa and TAL Cells

	Macula densa	Thick ascending limb		
	Apical Rabbit*	Apical Rabbit‡	BLM Rabbit§	Apical Rat‖
G (pS) room temperature	41	22	35	—
G (pS)37°C	—	27	—	60
Vdep	No	No	Yes	No
Ca^2	Yes	No	No	Yes
pH	Yes	No	N/D	Yes
ATP	No	Yes	N/D	Yes

Abbreviations: BLM, basolateral membrane; G, conductance; Vdep, voltage dependence; N/D, not determined.

*Present study; ‡Wang, White, Geibel, and Giebisch, 1990; §Hurst, Duplain, and Lapointe, 1992; ‖Bleich, Schlatter, and Greger, 1990.

current of 0.7 pA at zero mV pipette potential in cell attached patches) with an unknown specificity. It was present in less than 10% of the patches and was most evident under conditions where *N.Po* of the K^+ channel was reduced to zero. Additional studies will be necessary to identify and characterize this other apical channel. Nevertheless, the results with the macula densa apical K^+ channel allowed us to compare the characteristics of this channel with the properties reported for K^+ channels in the TAL, to speculate on the involvement of an apical K^+ channel in transepithelial transport by macula densa cells and to discuss the possible role of this K^+ channel in the tubuloglomerular feedback signal transmission.

Comparison of the Macula Densa and TAL K^+ Channels

As shown in Table II, the apical K^+ channel of macula densa cells exhibits characteristics which are different from those already identified for the K^+ channel on either the apical membrane of rat (Bleich et al., 1990) or rabbit TAL (Wang et al., 1990) or even on the basolateral membrane of rabbit TAL (Hurst et al., 1992). One

difference between macula densa and TAL K^+ channels is the higher incidence and abundance of channels observed in macula densa compared with TAL cells. The density of K^+ channels in the TAL appears to be extremely low (Bleich et al., 1990; Wang et al., 1990; Hurst et al., 1992) as indicated by a very low success rate for recording channel activity from giga Ohm patches. Whereas in macula densa cells, gigaohm seal formation was successful 38% of the time and of these, 75% exhibited channel activity with nearly all recordings exhibiting multiple channels.

There also seems to be differences in the conductance of various renal K^+ channels. In the cell-attached configuration with a high K^+ solution in the patch pipette, the macula densa cell K^+ channel has an inward conductance of 41 pS (room temperature) compared to 60 pS (37°C) in rat (Bleich et al., 1990) and 22 pS (room temperature) in rabbit (Wang et al., 1990). Voltage sensitivity of the renal K^+ channel has only been demonstrated in cell-attached patches of rabbit TAL basolateral membrane where this K channel was found to open with membrane depolarization. The apical membrane channels of rat and rabbit TAL as well as the apical K^+ channel of macula densa cells are voltage insensitive (Wang et al., 1990; Bleich et al., 1990) in cell attached patches while in the rat, the apical K^+ channel is apparently sensitive to voltage in inside-out patches (Bleich et al., 1990). In macula densa cells, the K^+ channel remains voltage insensitive (at least over the range of voltages tested) after the membrane patch has been pulled away from the cell and an inside-out patch is formed.

The regulation of the macula densa cell K^+ channel is also different from that of the K^+ channels in TAL. In rabbit, both apical (Wang et al., 1990) and basolateral (Hurst et al., 1992) K^+ channels are insensitive to changes in cell $[Ca^{2+}]$, however, the apical K^+ channel in rat TAL (Bleich et al., 1990) is inhibited by millimolar $[Ca^{2+}]$. In addition to its direct inhibitory effect, the presence of Ca^{2+} renders the channel less sensitive to ATP by a factor of ~ 10. In macula densa cells, the K^+ channel appears to be sensitive to Ca^{2+} but not to ATP. The pH sensitivity of the K^+ channel varies with species; in rabbit the apical K^+ channel is not sensitive to pH (Wang et al., 1990), whereas in rat, the channel activity was reduced with a decrease in pH (Bleich et al., 1990). In macula densa cells, the channel is almost completely inhibited by acidification to a pH of 6.8.

In summary, the macula densa apical K^+ channel presents a distinct set of characteristics in terms of pH, Ca^{2+}, ATP and voltage sensitivity with respect to either rat or rabbit apical TAL channels. The fact that single channel conductances in cell-attached configuration are not the same further suggests that the K channels in these three tissues are different.

Macula Densa Apical K^+ Channels

In the TAL, an apparent low density of apical K^+ channels recycles K^+ across the luminal membrane (Greger, 1985). This provides for a continuous source of luminal fluid K^+ which permits sustained flux through the $Na^+/K^+/2Cl^-$ and generation of a lumen positive potential that drives paracellular reabsorption. Therefore, apical K^+ channels play an essential role in transepithelial transport in TAL. In macula densa cells, it is not clear whether the abundance of apical K^+ channels serves the same function. Under normal conditions, NaCl transport by the TAL has substantially

reduced luminal [NaCl] before the macula densa. Therefore, the magnitude of transepithelial NaCl transport by macula densa cells is probably small. Also, because of the small size of the macula densa plaque, relative to the TAL, luminal [K^+] is most likely "set" by the upstream TAL. These considerations suggest that luminal K^+ recycling may not the primary role of the apical K^+ channel in macula densa cells.

In recent studies we have found that vectorial transport by macula densa cells is reabsorptive when luminal NaCl is in the range of 20–150 mM (luminal K^+ = 10 mM) (Lapointe, J. Y., A. Laamarti, A. M. Hurst, B. C. Fowler, and P. D. Bell, manuscript in preparation). One criteria for continued reabsorption at the lower range of luminal [NaCl]'s is the ability of these cells to reduce intracellular chloride ($[Cl^-]_i$) to low levels when NaCl transport is reduced. Using the fluorescent probe SPQ, previous work (Salomonsson, Gonzalez, Westerlund, and Persson, 1991*b*; Salomonsson et al., 1993) estimated macula densa $[Cl^-]_i$ at 6–28 mM in the presence of luminal furosemide; indicating that macula densa cells are capable of achieving a low $[Cl^-]_i$. In previous studies, we reported that macula densa cells have a single conductive pathway for Cl^- which is located at the basolateral membrane (Lapointe et al., 1991). Cl^- exit through this conductive pathway is driven by cell membrane potential since there is a large chemical gradient favoring Cl^- entry. Because basolateral K^+ conductance is small (Lapointe et al., 1991), it is likely that the apical K^+ channels play a major role in maintaining a cell negative membrane potential which controls the rate of Cl^- exit. This interpretation is indeed supported by the experimental observation that addition of 1 mM barium to a 20 mM NaCl luminal solution depolarized basolateral membrane potential by 15 to 30 mV ($n = 4$; unpublished observations). Thus, by controlling cell membrane potential, the apical K^+ channel would be responsible for "setting" macula densa steady state $[Cl]_i$ which, in turn, would influence NaCl transport since ionic flux through the cotransporter is inversely related to $[Cl]_i$. Because tubuloglomerular feedback responses are inhibited by furosemide (Wright and Schnermann, 1974), ionic flux through the Na^+:K^+:$2Cl^-$ cotransport may be involved in the initiation of the tubuloglomerular feedback signaling process. Macula densa apical K^+ channels could play an important role in feedback signal transmission process by indirectly modulating this flux.

Because $[Ca^{2+}]_i$ and pH appear to regulate channel activity it is interesting to consider how these two systems could modulate channel activity during tubuloglomerular feedback responses. Observations reported in the present study suggest the presence of a basolateral Na^+:Ca^{2+} exchanger because replacing Na^+ in the bathing solution resulted in inhibition of the apical K channel only in the presence of external Ca^{2+}. If a Na^+:Ca^{2+} exchanger exists at the basolateral membrane of macula densa cells then increases in Na^+:K^+:$2Cl^-$ would increase $[Ca^{2+}]_i$ through both an increase in $[Na]_i$ and cellular depolarization (Schlatter et al., 1989; and Lapointe et al., 1990). However, the direction and magnitude of changes in macula densa $[Ca^{2+}]_i$ with alterations in luminal [NaCl] and osmolality is not clear. This is, in part, due to technical issues involved in the measurement of Fura 2 fluorescence during changes in cell volume (Bell et al., 1988; Salomonsson 1991*a*). In addition, the sensitivity of the macula densa apical K^+ channel to $[Ca^{2+}]_i$ in situ is not known. In three preliminary experiments using Fura 2 to measure $[Ca^{2+}]_i$, we found that removing extracellular Na increased macula densa $[Ca^{2+}]_i$ by only 70 to 150 nM. This same

maneuver resulted in a decrease in N.Po from 11.3 to 1.6 (see Fig. 5) suggesting that the channel may be sensitive to changes in $[Ca^{2+}]_i$ within the physiological range. Additional studies will be needed to establish the relationship between cytosolic calcium concentration, K channel activity and the tubuloglomerular feedback mechanism.

Recent studies have provided evidence for a Na^+:H^+ exchanger located on the apical membrane of macula densa cells. Using the fluorescent probe BCECF to monitor changes in macula densa pH, an amiloride-sensitive alkalinization was obtained with increases in luminal [Na^+] or [NaCl] (Fowler et al., 1991). When luminal [NaCl] was increased from 25 to 150 mM, pH in macula densa cells increased from 7.15 to 7.30. In the present study, we found that reductions in bath pH from 7.4 to 6.8 completely inhibited the macula densa K^+ channel using inside-out patches. Further studies are needed to determine if increases in cell pH from a baseline of 7.15 to 7.30 would result in activation of the K^+ conductance. However, there is some doubt that changes in pH within this range will alter channel activity. The addition of furosemide or bumetanide to the lumen alkalinize macula densa cells presumably by reducing $[Na]_i$ and increasing Na^+:H^+ exchange. However, in the present studies, addition of bumetanide did not alter K^+ channel activity. If this observation is confirmed, then it would suggest that variations in luminal fluid [NaCl] between 20 and 150 mM would not alter K^+ channel activity, at least through changes in cell pH. This would then allow $[Cl]_i$ to change as a function of prevailing luminal fluid [NaCl] which may be important in providing a constant tubuloglomerular feedback signal.

In summary, we report the first single channel observations on a renal cell type involved in the regulation of single nephron glomerular filtration rate. A high density of Ca^{2+} and pH sensitive K^+ channels have been found in the apical membrane of macula densa cells. This channel, which appears to be different from K^+ channels in other renal epithelia, is likely to be involved in the control of membrane potential, basolateral Cl^- flux and influx through the Na^+:K^+:$2Cl^-$. By controlling these processes, the apical K^+ channel may serve an important modulator role in the transmission of tubuloglomerular feedback signals.

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REFERENCES

Barajas, L. 1981. The juxtaglomerular apparatus: anatomical considerations in feedback control of glomerular filtration rate. *Federation Proceedings.* 40:76–86.

Beck, J. S., A. M. Hurst, J. Y. Lapointe, and R. Lapade. 1993. Regulation of basolateral K^+ channel in proximal tubule studied during continuous microperfusion. *American Journal of Physiology.* 264: F494–F501.

Bell, P. D. 1985. Cyclic AMP-calcium interaction in the transmission of tubuloglomerular feedback signals. *Kidney International.* 28:728–732.

Bell, P. D. 1987. Calcium antagonists and intrarenal regulation of glomerular filtration rate. *American Journal of Nephrology.* 7(Suppl. 1):24–31.

Bell, P. D., M. Franco-Guevera, D. R. Abrahamson, J. Y. Lapointe, and J. Cardinal. 1988. Cellular mechanisms for tubuloglomerular feedback signalling. *In* The Juxtaglomerular apparatus. A. E. G. Persson, and U. Boberg, editors. Elsevier Science Publishers, New York. 63–77.

Bell, P. D., J. Y. Lapointe, and J. Cardinal. 1989. Direct measurement of basolateral membrane potentials from cells of the macula densa. *American Journal of Physiology.* 257:F463–F468.

Bleich, M., E. Schlatter, and R. Greger. 1990. The luminal potassium channel of the thick ascending limb of Henle's loop. *Pfluegers Archives.* 415:449–460.

Briggs, J. 1981. The macula densa sensing mechanism for tubuloglomerular feedback. *Federation Proceedings.* 40:99–103.

Briggs, J. P., and J. Schnermann. 1987. The tubuloglomerular feedback mechanism: functional and biochemical aspects. *Annual Review of Physiology.* 49:251–273.

Fowler, B. C., J-Y. Lapointe, and P. D. Bell. 1991. Influence of luminal NaCl transport on macula densa intracellular pH. *Journal of the American Society of Nephrology.* 2:736. (Abstr.)

Greger, R. 1985. Ion transport mechanisms in thick ascending limb of Henle's loop of mammalian nephron. *Physiological Reviews.* 65:760–797.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch clamp techniques for high resolution current recording from cells and cell free membrane patches. *Pfluegers Archives.* 391:85–100.

Hurst, A. M., M. Duplain, and J-Y. Lapointe. 1992. Basolateral membrane potassium channels in rabbit cortical thick ascending limb (CTAL). *American Journal of Physiology.* 263:F262–F267.

Hurst, A. M., and M. Hunter. 1992. Apical membrane potassium channels in frog diluting segment stimulation by furosemide. *American Journal of Physiology.* 262:F606–614.

Hurst, A. M., and M. Hunter. 1989. Apical K channels of frog diluting segment: inhibition by acidification. *Pflügers Archiv.* 415:115–117.

Kirk, K. L., P. D. Bell, D. W. Barfuss, and M. Ribadeneira. 1985. Direct visualisation of the isolated and perfused macula densa. *American Journal of Physiology.* 248:F890–F894.

Lapointe, J. Y., P. D. Bell, A. M. Hurst, and J. Cardinal. 1991. Basolateral ionic permeabilities of macula densa. *American Journal of Physiology.* 260:F856–F860.

Lapointe, J. Y., P. D. Bell, and J. Cardinal. 1990. Direct evidence for apical Na^+:$2Cl^-$:K^+ cotransport in macula densa cells. *American Journal of Physiology.* 258:F1466–F1469.

Pirie, S. C., and D. J. Potts. 1985. Application of cold flush preservation to in vitro microperfusion studies of kidney tubules. *Kidney International.* 28:982–984.

Rasmussen, H., and P. Q. Barrett. 1984. Calcium messenger system: an integrated view. *Physiological Reviews.* 64:938–984.

Salomonsson, M., E. Gonzalez, M. Kornfield, and A. E. G. Persson. 1993. The cytosolic chloride concentration in macula densa and cortical thick ascending limbs. *Acta Physiologica Scandinavica.* 147:305–313.

Salomonsson, M., E. Gonzalez, P. Westerlund, and A. E. G. Persson. 1991*a*. Intracellular cytosolic free calcium concentration in the macula densa and in ascending limb cells at different luminal concentrations of sodium chloride and with added furosemide. *Acta Physiologica Scandinavica.* 142:283–290.

Salomonsson, M., E. Gonzalez, P. Westerlund, and A. E. G. Persson. 1991*b*. Chloride concentration in macula densa and cortical thick ascending limb cells. *Kidney International Supplement.* 32:S51–S54.

Schlatter, E., M. Salomonsson, A. E. G. Persson, and R. Greger. 1989. Macula densa cells sense luminal NaCl concentration via furosemide sensitive Na^+,K^+,$2Cl^-$ cotranport. *Pfluegers Archives.* 414:286–290.

Schlatter, E. 1993. Effect of various diuretics on membrane voltage of macula densa cells. Whole-cell patch-clamp experiments. *Pflugers Archives.* 423:74–77.

Schnermann, J., and J. Briggs. 1985. Function of the juxtaglomerular apparatus: local control of glomerular hemodynamics. *In* The Kidney: Physiology and Pathophysiology. Seldin, D. W., and G. Giebisch, editors. Raven Press, New York. 669–697.

Schnermann, J., D. W. Ploth, and M. Hermle. 1976. Activation of tubuloglomerular feedback by chloride transport. *Pfluegers Archives.* 362:229–240.

Skott, O., and J. P. Briggs. 1987. Direct demonstration of macula densa-mediated renin secretion. *Science.* 237:1618–1620.

Wang, W., S. White, J. Geibel, and G. Giebisch. 1990. A potassium channel in the apical membrane of rabbit thick ascending limb of Henle's loop. *American Journal of Physiology.* 258:F244–F253.

Wright, F. S., and J. Schnermann. 1974. Interference with feedback control of glomerular filtration rate by furosemide, triflocin and cyanide. *Journal of Clinical Investigation.* 53:1695–1708.

Wright, F. S. 1981. Characteristics of feedback control of glomerular filtration rate. *Federation Proceedings.* 40:87–92.