Two Inwardly Rectifying Potassium Channels, \textit{Irk1} and \textit{Irk2}, Play Redundant Roles in \textit{Drosophila} Renal Tubule Function

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**RUNNING HEAD**

Inwardly rectifying K\textsuperscript{+} channels in \textit{Drosophila} renal function

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Inwardly rectifying potassium channels play essential roles in renal physiology across phyla. Barium-sensitive K⁺ conductances are found on the basolateral membrane of a variety of insect Malpighian (renal) tubules, including *Drosophila melanogaster*. We found that barium decreases the lumen-positive transepithelial potential difference in isolated perfused *Drosophila* tubules, and decreases fluid secretion and transepithelial K⁺ flux. In those insect species in which it has been studied, transcripts from multiple genes encoding inwardly rectifying K⁺ channels are expressed in the renal (Malpighian) tubule. In *Drosophila melanogaster*, this includes transcripts of the *Irk1*, *Irk2* and *Irk3* genes. The role of each of these gene products in renal tubule function is unknown. We found that simultaneous knockdown of *Irk1* and *Irk2* in the principal cell of the fly tubule decreases transepithelial K⁺ flux, with no additive effect of *Irk3* knockdown, and decreases barium sensitivity of transepithelial K⁺ flux by approximately 50%. Knockdown of any of the three inwardly rectifying K⁺ channels individually has no effect, nor does knocking down *Irk3* simultaneously with *Irk1* or *Irk2*. *Irk1/Irk2* principal cell double knockdown tubules remain sensitive to the kaliuretic effect of cAMP. Inhibition of the Na⁺/K⁺-ATPase with ouabain and *Irk1/Irk2* double knockdown have additive effects on K⁺ flux, and 75% of transepithelial K⁺ transport is due to *Irk1/Irk2* or ouabain-sensitive pathways. In conclusion, *Irk1* and *Irk2* play redundant roles in transepithelial ion transport in the *Drosophila melanogaster* renal tubule, and are additive to Na⁺/K⁺-ATPase-dependent pathways.
INTRODUCTION

Inwardly rectifying potassium channels play key roles in vertebrate and invertebrate renal physiology and epithelial ion transport (13, 27, 28, 31, 32, 36-38, 43, 44, 47). Mutations in KCNJ1/Kir1.1 (also known as renal outer medullary K⁺ channel, or ROMK) and KCNJ10/Kir4.1 result in human diseases characterized by salt-losing tubulopathies and electrolyte disturbances (1, 40, 42). Inwardly rectifying K⁺ channels also play important roles in insect iono- and osmoregulation. For example, in mosquitoes, pharmacological inhibition of inwardly rectifying K⁺ channels decreases renal tubule fluid secretion, and alters both transepithelial ion fluxes in the tubule as well as whole-body ion clearances and urine excretion (28, 31, 32, 36, 38, 41). This results in decreased viability and flight capacity (31, 32, 38). As such, inwardly rectifying K⁺ channels are potential targets for insecticidal agents.

*Drosophila melanogaster* is an insect model organism that can lend insights into the biology of mammals as well as other insects, such as disease vector-carrying insects or insect pests. The *Drosophila* genome encodes three inwardly rectifying K⁺ channels, *Irk1* (also called *Ir, Dir, or DrKir1*), *Irk2* (also called *DrKir2*), and *Irk3* (also called *dKirIII or DrKir3*) (7, 20, 23). Roles for *Irk* genes have been described in wing development (4) and cardiotropic viral
infections (10). In addition, all three \textit{Irk} genes are expressed in the principal cell of the Malpighian tubule, and pharmacological experiments using sulphonylureas have suggested a role for \textit{Ir}k channels in tubule function (11). Here, we used pharmacological and genetic approaches to examine the role of \textit{Ir}k channels on the physiological function of adult \textit{Drosophila melanogaster} renal tubules. Our data are consistent with two of the \textit{Ir}k channels, \textit{Irk1} and \textit{Irk2}, playing a role in transepithelial ion transport in the fly tubule.

\textbf{MATERIALS AND METHODS}

\textit{Chemicals and reagents}

All chemicals and reagents were from Sigma (St. Louis, MO) or Fisher (Pittsburgh, PA) unless otherwise specified.

\textit{Fly stocks and genetics}

The following \textit{Drosophila melanogaster} strains were used: \textit{wBerlin} (wild-type), obtained from Dr. Adrian Rothenfluh (University of Texas Southwestern Medical Center, Dallas, TX); \textit{w; c42-GAL4}, expressing GAL4 in the principal cells of the main and lower segments, as well as bar-shaped cells in the initial and transitional segments (35), obtained from Dr. Julian Dow (University of Glasgow, Glasgow, UK) and outcrossed for five generations to \textit{wBerlin}. \textit{w;UAS-Irk2\textsuperscript{RNAi}}, an RNA interference (RNAi) line targeting \textit{Irk2}, and \textit{w;UAS-Irk1\textsuperscript{RNAi}}, targeting \textit{Irk1}, were obtained from the Vienna Drosophila Resource Center (Vienna, Austria; lines 108140 and 28430) (6) and were outcrossed for five generations to \textit{wBerlin}. 
yw;UAS-Irk1RNAi (y¹v¹; P{TRiP.JF01841}attP2) and yw;UAS-Irk3RNAi (y¹v¹; P{TRiP.JF02262}attP2) were obtained from the Bloomington Stock Center (Bloomington, IN) and outcrossed for five generations to ywBerlin.

Flies were reared at 28°C unless otherwise noted on cornmeal/yeast/molasses food prepared in a central kitchen at UT Southwestern. Female flies were collected within 48 hours of eclosion and kept on standard food for 3-5 days before tubule dissection.

**Quantitative RT-PCR**

Irk1, Irk2, and Irk3 mRNA levels were quantified in knockdown vs. control tubules. In each case, w; c42-GAL4 served as the control. Four sets of tubules, with 50 tubules/genotype in each set, were compared for each Irk gene. RNA was prepared from tubules dissected in *Drosophila* saline and transferred to 600 μl ZR RNA buffer from the Zymo Quick-RNA microprep kit (Zymo Research, Irvine, CA). Tubules were homogenized by 10-20 passes with a 27G needle. RNA was isolated according to the Zymo protocol and eluted in 6 μl DNase/RNase-free H₂O. RNA amounts were quantified using a NanoDrop 2000c (Thermo Scientific). 150 ng of total RNA was used for reverse transcription using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) and random hexamer primers according to manufacturer’s instructions. cDNA from 10 ng total RNA was used per reaction of qPCR. qPCR was performed using the CFX Connect Real-Time PCR detecting system (BioRad, Hercules, CA) with the iTaq™ Universal Probes Supermix (BioRad, Hercules, CA). The TaqMan
primer/probe sets for *Ir* (Dm02143602_g1), *Irk2* (Dm02143724_m1), *Irk3* (Dm01796588_g1) and endogenous control *RpL32* (Dm02151827_g1) were ordered from Invitrogen (Carlsbad, CA). The PCR cycle was 95°C x 3min, 95°C x 10 sec, 60°C x 30 sec (repeated x40). Results were analyzed with Bio-Rad CFX Manager.

Perfusion of isolated tubules and measurement of transepithelial potential difference

Anterior Malpighian tubules were dissected free hand at room temperature from adult *Drosophila* females in *Drosophila* saline, consisting of (in mM): NaCl 117.5, KCl 20, CaCl₂ 2, MgCl₂ 8.5, NaHCO₃ 10.2, NaH₂PO₄ 4.3, HEPES 15, and glucose 20, pH 7.0. Tubules were then transferred to a bath chamber of an inverted microscope. The tubule was cannulated with a concentric glass pipette. The lumen was perfused with *Drosophila* saline. Transepithelial potential difference was measured using the perfusion pipette as the bridge into the tubular lumen. The reference electrode was in the bathing solution that was also composed of *Drosophila* saline. The potential difference was measured using a Keithley Electrometer, model no. 6517B (Keithley, Cleveland, OH). After obtaining a stable transepithelial potential, the bathing solution was changed to *Drosophila* saline containing 2 mM BaCl₂ and the potential recorded after the potential difference stabilized. The barium-containing bath was then exchanged with *Drosophila* saline and potential recorded until a stable potential was again achieved.
Ramsay assay and ion-specific electrodes

The Ramsay assay and ion-specific electrodes were used to measure fluid secretion and K⁺ concentration from isolated adult female Drosophila tubules as previously described (34, 46). Fluid secretion rates and K⁺ concentration were measured after 2 hours of fluid secretion unless otherwise specified. Potassium ionophore I cocktail B was used for the K⁺ ionophore. The K⁺ flux of each tubule was calculated by multiplying K⁺ concentration by the secretion rate for each tubule. Unless otherwise indicated, the tubules were bathed in standard bathing medium consisting of a 1:1 mixture of Drosophila saline and Schneider’s medium (Invitrogen, Carlsbad, CA). The composition of Schneider’s medium is (in mM):

- glycine, 3.33
- L-arginine, 2.3
- L-aspartic acid, 3.01
- L-cysteine, 0.496
- L-cystine, 0.417
- L-glutamic acid, 5.44
- L-glutamine, 12.33
- L-histidine, 2.58
- L-isoleucine, 1.15
- L-leucine, 1.15
- L-lysine hydrochloride, 9.02
- L-methionine, 5.37
- L-phenylalanine, 0.909
- L-proline, 14.78
- L-serine, 2.38
- L-threonine, 2.94
- L-tryptophan, 0.49
- L-tyrosine, 2.76
- L-valine, 2.56
- β-alanine, 5.62
- CaCl₂, 5.41
- MgSO₄, 15.06
- KCl, 21.33
- KH₂PO₄, 3.31
- NaHCO₃, 4.76
- NaCl, 36.21
- Na₂HPO₄, 4.94
- α-ketoglutaric acid, 1.37
- D-glucose, 11.11
- fumaric acid, 0.862
- malic acid, 0.746
- succinic acid, 0.847
- trehalose, 5.85
- and yeastolate, 2000 mg/L.

In experiments testing the effect of barium, large amounts of barium phosphate precipitation occurred when using standard bathing medium, due to the higher concentrations of phosphate in this solution compared to Drosophila saline. Tubules were therefore incubated in amino acid-replete solution, which
consists of *Drosophila* saline supplemented with amino acids, and supports more fluid secretion in the Ramsay assay than *Drosophila* saline (19). Amino acid-replete solution consists of (in mM) (19): NaCl 117.5, KCl 20, CaCl₂ 2, Mg Cl₂ 8.5, NaHCO₃ 10.2, HEPES 15, glucose 20, NaH₂PO₄ 4.3, L-glycine 1.7, L-proline 7, L-glutamine 6.16, L-histidine 0.95, L-leucine 0.55, L-lysine 4.5, L-valine 1.3, pH 7.0. Tubules were incubated for 1 hour in the amino acid-replete solution. Fluid secretion and K⁺ activity were measured. 2 mM BaCl₂ was then added to the bathing solution. The tubules were incubated for an additional hour and fluid secretion and K⁺ activity determined.

In experiments to assess the effects of cAMP, 1 mM dibutyryl cAMP was added to standard bathing medium. In other experiments, 100 μM ouabain was added to standard bathing medium to inhibit the Na⁺/K⁺-ATPase. Tubules were bathed in the drug-containing standard bathing medium for the entirety of the experiment (two hours). Ouabain stocks were prepared fresh each day.

**Statistics**

Paired t-test (two comparisons) or one-way repeated measures analysis of variance (ANOVA, three comparisons) were used for experiments in which the same tubule was analyzed over time under different conditions. In experiments with separate groups of tubules, unpaired t-test was used in experiments with two groups, or one-way ANOVA in experiments with three or more groups. In those experiments in which there were three or more groups with more than one variable (for example, genotype and drug), two-way ANOVA was used.
Significance level was set at $p < 0.05$. Post-hoc comparisons were performed using Bonferroni’s correction. All statistical analyses were performed using GraphPad Prism, version 5.0 or 6.0 (GraphPad Software, La Jolla, CA).

RESULTS

Urine generation by the main segment of the *Drosophila* renal tubule occurs through the secretion of KCl-rich fluid. In the principal cell, the apical vacuolar H$^+$-ATPase generates a lumen-positive transepithelial potential difference that drives cation secretion – primarily K$^+$ and to a lesser extent Na$^+$ – across the apical membrane by driving H$^+$/cation exchange (5, 8, 19, 25, 33). Cl$^-$ secretion occurs in parallel through the transcellular flux of Cl$^-$ through the stellate cell (3, 25, 26). Previous studies have demonstrated an effect of barium, an inhibitor of inwardly rectifying K$^+$ channels, on the basolateral membrane potential and K$^+$ conductance in the *Drosophila melanogaster* Malpighian tubule (15, 25).

To determine whether barium also affects transepithelial ion flux, we measured transepithelial potential difference in isolated perfused tubules in the presence or absence of 2 mM peritubular barium. Barium reversibly decreased the lumen-positive transepithelial potential difference by 21 mV, from 30.5±5.2 mV before barium, to 9.5±1.9 mV during barium treatment, to 30.5±4.0 mV after washout, mean±SEM (Figure 1A). Since the transepithelial potential difference drives transepithelial fluid secretion and cation flux (8, 25), we tested the effect of barium on transepithelial fluid secretion and K$^+$ flux in the Ramsay assay, which
measures fluid and $K^+$ secretion by the main segment of the tubule (8). Barium
decreased transepithelial $K^+$ flux when added to the bathing solution in the
Ramsay assay (Table 1 and Figure 1B). These results suggest that basolateral
barium-sensitive $K^+$ channels play a role in transepithelial $K^+$ secretion across the
main segment of the tubule.

Transcripts for all three *Drosophila* inwardly rectifying $K^+$ channel genes
are expressed in the Malpighian tubule principal cell (11). In addition, there are
four annotated alternatively spliced products of *Irk2*, of which RA, RB and RC are
expressed in the tubule, with greatest expression of *Irk2-RA* (11). In order to test
a role for each *Irk* gene, we used the GAL4-UAS system (2) and RNA
interference (RNAi) to knock down each of the *Irk* genes, individually and in
combination, in the principal cells of the tubule. *c42-GAL4* was used to drive
expression of hairpin RNAs in the tubule principal cells (35). The RNAi constructs
used to knock down *Irk2* targets the four annotated *Irk2* transcripts. Using
quantitative RT-PCR, we demonstrated that each of the three *Irk* genes was
efficiently knocked down (Figures 2B, 3B and 4B); the degree of transcript
knockdown is likely underestimated since the genes are only being knocked
down in a subset of the tubule cells examined. Despite the observed decreases
in transcript abundance, knocking down *Irk1*, *Irk2*, or *Irk3* individually did not
result in a change in transepithelial $K^+$ flux, as measured in the Ramsay assay
(Table 2 and Figures 2A, 3A and 4A). Similarly, knocking down *Irk1* and *Irk3* in
combination, or *Irk2* and *Irk3*, also did not result in a change in $K^+$ flux (Table 2
and Figure 5). However, knocking down *Irk1* and *Irk2* simultaneously resulted in
decreased transepithelial $K^+$ flux (Table 2 and Figure 6A), while $lrk1/lrk2/lrk3$
triple knockdown did not further decrease $K^+$ flux (Table 2 and Figure 6B).
Together, these results suggest that $lrk1$ and $lrk2$ play redundant roles in
transepithelial $K^+$ flux, as a phenotype was only revealed when both genes were
knocked down simultaneously, while no role for $lrk3$ was apparent in this
process.

Having determined that $lrk1$ and $lrk2$ appear to function redundantly in
transepithelial $K^+$ flux, we next wanted to determine how much of the barium-
sensitive component of $K^+$ flux seen in wild-type tubules (Figure 1) can be
explained by $lrk1$ and $lrk2$. We therefore measured $K^+$ flux before and after
addition of 2 mM barium in control tubules, and in tubules in which $lrk1$, $lrk2$ or
both $lrk1$ and $lrk2$ were knocked down in the principal cell under the control of the
c42-GAL4 driver (Table 1 and Figure 7). Similar to the results shown in Figure 1,
~80% of $K^+$ flux was abolished by the addition of barium in the control tubules, as
well as in tubules in which $lrk1$ and $lrk2$ were individually knocked down. In
contrast, only 44% of the $K^+$ flux in the $lrk1/lrk2$ double knockdown tubules was
barium-sensitive. This suggests that approximately half of the barium-sensitive
$K^+$ flux in control tubules is mediated by Irk1 and Irk2. These data are also
consistent with the idea that either Irk1 and Irk2 compensate for each other when
either channel is knocked down individually, or that other barium-sensitive
channels are upregulated.

Previous studies have demonstrated an increase in fluid secretion (8) and
$K^+$ flux (19) after stimulation of the Drosophila tubule with cAMP. We previously
observed that tubules carrying a homozygous null mutation in the fly sodium-potassium-2-chloride cotransporter (NKCC) remain sensitive to the stimulatory effect of cAMP (34). To determine whether the inwardly rectifying K\(^+\) channels play a role in the cAMP response, we stimulated control tubules or \(Irk1/Irk2\) principal cell double knockdown tubules with cAMP. Increased K\(^+\) flux was seen with cAMP treatment in both genotypes (Table 2 and Figure 8A). Whether this is due to stimulation of other transport pathways in the principal cells, or the stimulation of separate pathways in the stellate cell, which are also cAMP-responsive (9), is presently unknown.

We previously demonstrated that ouabain, an inhibitor of the Na\(^+\)/K\(^+\)-ATPase, decreases K\(^+\) flux in control tubules (34). No effect of ouabain was seen in NKCC null mutant tubules (34), indicating that the primary role of the Na\(^+\)/K\(^+\)-ATPase in transepithelial K\(^+\) flux is to generate a favorable gradient for NKCC activity. A possible role of inwardly rectifying K\(^+\) channels is to recycle the K\(^+\) entering through the Na\(^+\)/K\(^+\)-ATPase. If this is the case for \(Irk1\) and \(Irk2\), addition of ouabain to the \(Irk1/Irk2\) double knockdown tubules would not be expected to further decrease K\(^+\) flux. Instead, a decrease in K\(^+\) flux was observed in both control and \(Irk1/Irk2\) double knockdown tubules (Table 2 and Figure 8B). This suggests that inwardly rectifying K\(^+\) channels have roles independent of the Na\(^+\)/K\(^+\)-ATPase. Additionally, in this experiment \(~75\%\) of transepithelial K\(^+\) flux could be attributed to the combination of the Na\(^+\)/K\(^+\)-ATPase, which as described above drives the activity of the NKCC, and the inwardly rectifying K\(^+\) channels \(Irk1\) and \(Irk2\).
DISCUSSION

Inwardly-rectifying K⁺ channels play important roles in invertebrate and vertebrate renal physiology. In insects, basolateral membrane barium-sensitive K⁺ conductances have been demonstrated in a variety of insect renal tubules. Examples include the yellow fever mosquito *Aedes aegypti* (22, 41), the forest ant *Formica polyctena* (17), the Chagas vector *Rhodnius prolixus* (12), the agricultural pest *Locusta migratoria* (14), and the mealworm *Tenebrio molitor* (45). In addition, transcripts for inwardly-rectifying K⁺ channels are expressed in the Malpighian tubules of *Aedes aegypti*, the vector for yellow fever, dengue and chikungunya (29); *Anopheles gambiae*, the malaria vector (30); and the bed bug *Cimex lectularius* (21). Drugs targeting renal tubule inwardly-rectifying K⁺ channels are currently being developed as mosquitocidal insecticides in *Aedes aegypti* (31, 32, 38). These channels may represent targets for the control of other insect pests as well, while killing of benign or beneficial insects will need to be avoided. Our study extends the understanding of the role of these channels in insect renal tubule function.

Barium has been extensively used to probe the function of inwardly-rectifying K⁺ channels in insects. Here, we replicated previous findings that barium decreases fluid secretion in the fly renal tubule (25) and showed that it decreases transepithelial K⁺ flux. We also observed a decrease (lumen less positive) in the transepithelial potential difference of 21 mV. This is consistent with prior reports of hyperpolarization of the basolateral membrane potential of
similar magnitude (15, 25), likely explaining the effect on the transepithelial potential difference. Both Irk1 and Irk2 are sensitive to barium when expressed in heterologous cells (7), and barium blocks the basolateral K⁺ conductance of the Drosophila tubule (15). Barium may also have additional effects on other, undefined ion channels on the basolateral membrane of the fly tubule (15). Indeed, Irk1 and Irk2 appear to account for only half of the barium sensitivity we observed for transepithelial K⁺ flux. Genetic studies, in which specific channels can be manipulated, therefore provide complementary information to pharmacological studies. This is particularly important given the number of genes encoding inwardly-rectifying K⁺ channels. The genomes of the mosquito species Aedes aegypti, Anopheles gambiae, and Culex quinquefasciatus encode Irk1 and Irk3 homologs, while the Irk2/Kir2 gene has undergone a duplication event, resulting in Kir2A and Kir2B genes. Additional duplication events have resulted in Kir2A and Kir2A’ genes in Anopheles and Culex, and Kir2B and Kir2B’ genes in Aedes. In Culex and Anopheles, the Kir3 gene has also duplicated to result in Kir3A and Kir3B genes (23, 29, 30).

Here, we observed that Irk1 and Irk2, but not Irk3, are important for transepithelial fluid secretion and K⁺ flux. Interestingly, no functional channel activity was observed with attempts at expression of Irk3 in Xenopus oocytes or Drosophila S2 cultured cells, whereas both Irk1 and Irk2 possessed inwardly-rectifying K⁺ channel activity (7). Similarly, no channel activity was observed in Xenopus oocytes expressing the Aedes aegypti Irk3 homolog, AeKir3, although it is highly expressed in the mosquito tubule (29). In addition, recent
immunolocalization data indicates that AeKir3 is expressed in intracellular punctae in the mosquito tubule (28). In bed bugs, the Irk3 homolog ClKir3 transcript is expressed at very high levels in the Malpighian tubules, yet whole organism silencing using RNA interference had no effect on bed bug viability (21). The functional role of Irk3 and its homologs in other insects in the renal tubule is therefore unclear.

In contrast, compounds which inhibit AeKir1 and AeKir2B have inhibitory effects on whole-mosquito urine excretion (31, 32, 38) and on transepithelial fluid secretion and K+ flux in the Ramsay assay (28, 36). This is broadly consistent with our results that both Irk1 and Irk2 play roles in the fly tubule, although there are interesting differences: in Aedes, AeKir1 is located in the stellate cell, whereas AeKir2B is in the principal cell, and inhibition of both AeKir1 and AeKir2 has additive effects on K+ flux. However, this may also reflect the effects of acutely inhibiting the channels pharmacologically, as opposed to the longer-term genetic knockdown used in this study. Additional open questions remain. For example, why is expression of AeKir2B enriched in the mosquito tubule, rather than the fly Irk2 homolog, AeKir2A? Does AeKir2B play a specific role in fluid secretion and ion flux after a blood meal, a situation not faced by Drosophila? Do specific splice isoforms of Irk2/Kir2A, demonstrated in Drosophila, Aedes aegypti and Anopheles gambiae (11, 37), have specific functional roles in the tubule? Given the ease of genetic manipulation and transgenesis in Drosophila, the fly renal tubule could serve as a platform to explore not only the role of the Drosophila channels, but potentially also the physiological roles and/or the drug
sensitivities of various inwardly-rectifying K⁺ channels of other insects, aiding in
the development of pharmacological agents to control insect disease vectors or
insect pests.

Irk1 and Irk2 both have K⁺ channel activity as homomeric channels when
heterologously expressed (7). It is possible that they could also function as
heteromeric channels, as is the case for some other inwardly rectifying K⁺
channels (13). However, the fact that Irk1 and Irk2 must simultaneously be
knocked down in order to see a change in transepithelial flux suggests that even
if the two channels do have heteromeric interactions, these are not absolutely
required for their function in the tubule.

What roles are Irk1 and Irk2 playing in transepithelial ion flux? One
possibility is that Irk1 and Irk2 constitute all or part of the basolateral K⁺
conductance and are important for maintaining the basolateral membrane
potential. Could they also serve as a conductive pathway for K⁺ entry from the
hemolymph into the principal cell? In an analysis by Ianowski and O'Donnell, E_K
(-52 mV) was close to the basolateral membrane potential (-43 mV), with a net
outward driving force for K⁺ movement from cell to bath (15). Since E_K and the
basolateral membrane potential were close to one another, relatively modest
changes in conditions could affect the direction of the driving force. Indeed, the
bathing solution for our Ramsay assay studies of genetically modified tubules
differed from those used by Ianowski and O'Donnell (15). In the Formica tubule,
E_K was also close to the basolateral membrane potential, and depending on the
measurement technique as well as the bath K⁺ concentration, driving forces were
observed that were either inward, outward or zero (18). A subsequent study proposed that at high hemolymph K$^+$ concentrations, K$^+$ uptake occurs through basolateral K$^+$ channels (17). Similarly, studies in the deoxycorticosterone-treated rabbit cortical collecting duct demonstrated an inward (bath to cell) driving force for potassium across the basolateral membrane (16, 39). In this preparation, transepithelial K$^+$ flux from bath to lumen increased when bath K$^+$ concentration increased, and this increase was attenuated by the basolateral application of barium, indicating that basolateral K$^+$ channels allow K$^+$ uptake into the cortical collecting duct principal cell (24). Similarly, application of an AeKir1/AeKir2B inhibitor to the basolateral membrane of the Aedes aegypti tubule depolarized the basolateral membrane potential and decreased input conductance under high bath K$^+$ (34 mM) conditions (28).

We found in a previous study that the Na$^+/K^+$-ATPase generates the driving force for NKCC activity in the fly renal tubule (34). Another potential role for K$^+$ channels could be to recycle the K$^+$ entering through the Na$^+/K^+$-ATPase. However, we observed additive effects of the Na$^+/K^+$-ATPase inhibitor ouabain and knockdown of Irk1 and Irk2, indicating that Irk1 and Irk2 have functions beyond recycling the K$^+$ entering through the Na$^+/K^+$-ATPase. Indeed, about 75% of transepithelial K$^+$ was mediated by Irk1, Irk2 and ouabain-sensitive pathways, which could include the Na$^+/K^+$-ATPase itself as well as secondary active K$^+$ uptake by the NKCC.

PERSPECTIVES AND SIGNIFICANCE
Our genetic and pharmacological results are most consistent with a role for the inwardly-rectifying K$^+$ channels, Irk1 and Irk2, on the basolateral membrane of the *Drosophila melanogaster* main segment principal cell. Possible roles of Irk1 and Irk2 are the maintenance of the basolateral membrane potential, or to allow the movement of K$^+$ from the hemolymph into the principal cell during transepithelial K$^+$ flux. Since flies eat a K$^+$-rich diet, the existence of multiple mechanisms to allow principal cell K$^+$ uptake – Irk1 and Irk2, as well as the Na$^+$/K$^+$-ATPase and NKCC – builds redundancy into the system for renal K$^+$ excretion.
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Stimulates Potassium Flux through the WNK-SPAK/OSR1 Kinase Cascade and
the Ncc69 Sodium-Potassium-2-Chloride Cotransporter in the Drosophila Renal

Lifton RP, and Wang WH. KCNJ10 determines the expression of the apical Na-
Cl cotransporter (NCC) in the early distal convoluted tubule (DCT1). *Proc Natl
FIGURE CAPTIONS

Figure 1. Barium applied to the basolateral membrane of the *Drosophila melanogaster* renal tubule decreases transepithelial potential difference and transepithelial $K^+$ flux. A) Transepithelial potential difference was measured in isolated perfused wild-type tubules (*wBerlin*, $n=4$) at baseline, after the application of peritubular 2 mM barium, and after washout. Transepithelial potential difference was reversibly decreased after barium administration. The time to initial effect after changing the bath to the barium-containing bath was 4±1 min. The time to maximal effect after changing the bath to the barium-containing bath was 11±2 min. There was no significant difference between "before" and "recovery" transepithelial potentials. B) Transepithelial $K^+$ flux (pmol/min/tubule) was measured in wild-type (*wBerlin*) tubules using the Ramsay assay and ion-specific electrodes. $K^+$ flux was measured for one hour prior to drug addition ("Baseline"), and in the same tubules for one hour after the addition of 2 mM barium or vehicle ($n=8$ tubules/condition). There was an increase in $K^+$ flux after vehicle addition, while the addition of barium resulted in a decrease in $K^+$ flux. All values shown in this and subsequent figures are mean ± standard error of the mean (SEM). *, $p<0.05$, ***, $p<0.001$.

Figure 2. Knocking down *Irk1* in the principal cell of the tubule decreases transcript levels but has no effect on $K^+$ flux.
The GAL4-UAS system, using the principal cell GAL4 driver c42-GAL4, was used to knock down Irk genes in the principal cells of the tubule. A) Transepithelial K⁺ flux (pmol/min/tubule) was measured in tubules from control flies (w;c42-GAL4/+ and w/yw;UAS-Irk1RNAi/+), or from flies in which Irk1 was knocked down in the principal cells under the control of c42-GAL4 (w/yw;c42-GAL4/UAS-Irk1RNAi). K⁺ flux was unchanged in the Irk1 knockdown tubules. n=13-14 tubules/genotype. One-way ANOVA p-value=0.2537. B) Irk1 transcript levels were measured using quantitative RT-PCR from control tubules (w;c42-GAL4/+) or from tubules in which Irk1 was knocked down in the principal cells (w/yw;c42-GAL4/UAS-Irk1RNAi). Transcript levels were decreased in the knockdown tubules. n =4 pairs of 50 tubules/genotype. **, p<0.01.

Figure 3. Knocking down Irk2 in the principal cell of the tubule decreases transcript levels but has no effect on K⁺ flux.

A) Transepithelial K⁺ flux (pmol/min/tubule) was measured in tubules from control flies (w;c42-GAL4/+ and w;UAS-Irk2RNAi/+), or from flies in which Irk2 was knocked down in the principal cells under the control of c42-GAL4 (w;c42-GAL4/UAS-Irk2RNAi). K⁺ flux was unchanged in the Irk2 knockdown tubules. This experiment was performed simultaneously with the experiment shown in Figure
2, therefore the value for \textit{w;c42-GAL4/} is the same. \textit{n}=12-14 tubules/genotype.

One-way ANOVA p-value=0.4608. B) \textit{Irk2} transcript levels were measured using quantitative RT-PCR from control tubules (\textit{w;c42-GAL4/+}) or from tubules in which \textit{Irk2} was knocked down in the principal cells (\textit{w;c42-GAL4/UAS-\textit{Irk2}RNAi}). Transcript levels were decreased in the knockdown tubules. \textit{n}=4 pairs of 50 tubules/genotype. ****, \textit{p}<0.0001.

Figure 4. Knocking down \textit{Irk3} in the principal cell of the tubule decreases transcript levels but has no effect on \textit{K}⁺ flux.

A) Transepithelial \textit{K}⁺ flux (pmol/min/tubule) was measured in tubules from control flies (\textit{w;c42-GAL4/+} and \textit{w/yw;UAS-\textit{Irk3}RNAi/+}), or from flies in which \textit{Irk3} was knocked down in the principal cells under the control of \textit{c42-GAL4} (\textit{w/yw;c42-GAL4/UAS-\textit{Irk3}RNAi}). \textit{K}⁺ flux was unchanged in the \textit{Irk3} knockdown tubules. \textit{n}=14-15 tubules/genotype. One-way ANOVA p-value=0.6862. B) \textit{Irk3} transcript levels were measured using quantitative RT-PCR from control tubules (\textit{w;c42-GAL4/+}) or from tubules in which \textit{Irk3} was knocked down in the principal cells (\textit{w/yw;c42-GAL4/UAS-\textit{Irk3}RNAi}). Transcript levels were decreased in the knockdown tubules. \textit{n}=4 pairs of 50 tubules/genotype. ***, \textit{p}<0.001.
Figure 5. Knocking down \textit{Irk1} and \textit{Irk3}, or \textit{Irk2} and \textit{Irk3}, in the principal cell of the tubule has no effect on K$^+$ flux.

A) Transepithelial K$^+$ flux (pmol/min/tubule) was measured in tubules from control flies \((w; UAS-Irk1^{RNAi}/+; UAS-Irk3^{RNAi}/+)\), or from flies in which \textit{Irk1} and \textit{Irk3} were knocked down in the principal cells under the control of \textit{c42-GAL4} \((w; UAS-Irk1^{RNAi}/+; c42-GAL4/UAS-Irk3^{RNAi})\). K$^+$ flux was unchanged in the \textit{Irk1/Irk3} double knockdown tubules. \(n=10-13\) tubules/genotype. Unpaired t-test p-value=0.2371.

B) Transepithelial K$^+$ flux (pmol/min/tubule) was measured in tubules from control flies \((w;c42-GAL4/+\) and \(w; UAS-Irk2^{RNAi}/+; UAS-Irk3^{RNAi}/+)\), or from flies in which \textit{Irk2} and \textit{Irk3} were knocked down in the principal cells under the control of \textit{c42-GAL4} \((w; UAS-Irk2^{RNAi}/+; c42-GAL4/UAS-Irk3^{RNAi})\). K$^+$ flux was unchanged in the \textit{Irk1/Irk3} double knockdown tubules. \(n=7-10\) tubules/genotype. One-way ANOVA p-value=0.6862.

Figure 6. Knockdown of \textit{Irk1} and \textit{Irk2} together reveals a K$^+$ secretory defect, with no additional effect of \textit{Irk3} knockdown.

A) Transepithelial K$^+$ flux (pmol/min/tubule) was measured in tubules from control flies \((w;c42-GAL4/+\) and \(w; UAS-Irk2^{RNAi}/+; UAS-Irk1^{RNAi}/+)\), or from flies in which \textit{Irk1} and \textit{Irk2} were simultaneously knocked down in the principal cells under the
control of c42-GAL4 (w;UAS-Irk2RNAi/+;c42-GAL4/UAS-Irk1RNAi). K⁺ flux was
decreased in the Irk1/Irk2 double knockdown tubules. Flies in this experiment
were reared at room temperature, ~22-23°C. n=41-42 tubules/genotype. *,
p<0.05; ***, p<0.001. B) Transepithelial K⁺ flux (pmol/min/tubule) was measured
in tubules from control flies (w;UAS-Irk2RNAi/+;UAS-Irk1RNAi/+), or from flies in
which Irk1 and Irk2 were simultaneously knocked down in the principal cells
under the control of c42-GAL4 (w;UAS-Irk2RNAi/+;c42-GAL4/UAS-Irk1RNAi), or
from flies in which Irk1, Irk2 and Irk3 were simultaneously knocked down
(w;UAS-Irk2RNAi/+;c42-GAL4 UAS-Irk3RNAi/UAS-Irk1RNAi). K⁺ flux was decreased in
the Irk1/Irk2 double knockdown tubules, but was not further decreased in the
Irk1/Irk2/Irk3 triple knockdown tubules. Flies in this experiment were reared at
room temperature, ~22-23°C. n=20-22 tubules/genotype. **, p<0.01.

Figure 7. The barium-sensitive component of K⁺ flux is decreased in
Irk1/Irk2 double knockdown tubules.

A) Transepithelial K⁺ flux (pmol/min/tubule) was measured in tubules from control
flies (w;c42-GAL4/+), flies from which Irk1 or Irk2 were individually knocked down
in the principal cells under the control of c42-GAL4 (w/yw;UAS-Irk1RNAi/c42 and
w; UAS-Irk2RNAi/+;c42-GAL4/+), or flies in which Irk1 and Irk2 were
simultaneously knocked down in the principal cells (w;UAS-Irk2RNAi/+;c42-GAL4/UAS-Irk1RNAi). K⁺ flux was measured for one hour prior to drug addition (“Baseline”), and in the same tubules for one hour after the addition of 2 mM barium (n=9-11 tubules/genotype). B) The barium-sensitive K⁺ flux was calculated from the data shown in A. Barium-sensitive K⁺ flux was similar in control tubules and the Irk1 and Irk2 knockdown tubules, but decreased in the Irk1/Irk2 double knockdown tubules. NS, not significant; **, p<0.01.

Figure 8. Tubule response to cAMP and ouabain is unchanged in Irk1/Irk2 double knockdown tubules.

A) Transepithelial K⁺ flux (pmol/min/tubule) was measured in tubules from control flies (w;c42-GAL4/+), or from flies in which Irk1 and Irk2 were simultaneously knocked down in the principal cells under the control of c42-GAL4 (w;UAS-Irk2RNAi/+;c42-GAL4/UAS-Irk1RNAi), in the presence of 1 mM cAMP or vehicle control. cAMP stimulated K⁺ flux in both genotypes. N=18-19 tubules/group. *, p<0.05. B) Transepithelial K⁺ flux (pmol/min/tubule) was measured in tubules from control flies (w;c42-GAL4/+), or from flies in which Irk1 and Irk2 were simultaneously knocked down in the principal cells under the control of c42-GAL4 (w;UAS-Irk2RNAi/+;c42-GAL4/UAS-Irk1RNAi), in the presence of 100 μM ouabain, a
Na\textsuperscript{+}/K\textsuperscript{+}-ATPase inhibitor, or vehicle control. K\textsuperscript{+} flux was decreased by ouabain treatment in both genotypes. Flies in this experiment were reared at room temperature, ~22-23°C. N=12-18 tubules/group. *, p<0.05; **, p<0.01.
TABLE 1. Effect of barium on secretion rate, [K$^+$/] and K$^+$ flux

All values shown are mean ± SEM. Note that K$^+$ flux was calculated separately for each tubule analyzed, and therefore the value of mean secretion rate x mean [K$^+$] may differ slightly from measured K$^+$ flux, due to rounding.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Secretion rate nl/min/tubule</th>
<th>[K$^+$] mM</th>
<th>K$^+$ flux pmol/min/tubule</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effect of 2 mM barium</strong></td>
<td></td>
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</tr>
<tr>
<td><em>wBerlin</em></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>0.29 ± 0.048</td>
<td>139 ± 7.8</td>
<td>41 ± 7.5</td>
<td>7</td>
</tr>
<tr>
<td>After vehicle</td>
<td>0.37 ± 0.057</td>
<td>157 ± 8.3</td>
<td>68 ± 16.7</td>
<td>7</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.31 ± 0.060</td>
<td>150 ± 21.9</td>
<td>43 ± 9.0</td>
<td>8</td>
</tr>
<tr>
<td>After barium</td>
<td>0.01 ± 0.010</td>
<td>156 ± 19.1</td>
<td>9 ± 7.0</td>
<td>8</td>
</tr>
<tr>
<td><strong>GAL4 control: w;c42-GAL4/+</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.21 ± 0.028</td>
<td>117 ± 6.4</td>
<td>25 ± 3.6</td>
<td>10</td>
</tr>
<tr>
<td>After barium</td>
<td>0.02 ± 0.028</td>
<td>116 ± 11.0</td>
<td>4 ± 1.6</td>
<td>10</td>
</tr>
<tr>
<td><strong>Irk1 knockdown: w/yw;UAS-Irk1RNAi/c42</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.23 ± 0.028</td>
<td>116 ± 10.3</td>
<td>28 ± 4.6</td>
<td>9</td>
</tr>
<tr>
<td>After barium</td>
<td>0.05 ± 0.029</td>
<td>121 ± 10.2</td>
<td>8 ± 3.5</td>
<td>9</td>
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<tr>
<td><strong>Irk2 knockdown: w;UAS-Irk2RNAi/+;c42-GAL4/+</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.24 ± 0.024</td>
<td>129 ± 11.0</td>
<td>32 ± 4.6</td>
<td>11</td>
</tr>
<tr>
<td>After barium</td>
<td>0.03 ± 0.010</td>
<td>131 ± 10.8</td>
<td>6 ± 1.7</td>
<td>11</td>
</tr>
<tr>
<td><strong>Irk1/Irk2 double knockdown: w;UAS-Irk2RNAi/+;c42-GAL4/UAS-Irk1RNAi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.18 ± 0.032</td>
<td>105 ± 10.6</td>
<td>20 ± 4.4</td>
<td>10</td>
</tr>
<tr>
<td>After barium</td>
<td>0.08 ± 0.022</td>
<td>123 ± 12.5</td>
<td>13 ± 4.1</td>
<td>10</td>
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</tbody>
</table>
### TABLE 2. Secretion rate, [K⁺] and K⁺ flux

All values shown are mean ± SEM. Note that K⁺ flux was calculated separately for each tubule analyzed, and therefore the value of mean secretion rate x mean [K⁺] may differ slightly from measured K⁺ flux, due to rounding.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Secretion rate nl/min/tubule</th>
<th>[K⁺] mM</th>
<th>K⁺ flux pmol/min/tubule</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Irk1 and Irk2 knockdown</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w;c42-GAL4/+</td>
<td>0.47 ± 0.032</td>
<td>150 ± 9.1</td>
<td>71 ± 6.5</td>
<td>13</td>
</tr>
<tr>
<td>w/yw;c42-GAL4/UAS-Irk1\textsuperscript{ONAI}</td>
<td>0.37 ± 0.027</td>
<td>158 ± 5.9</td>
<td>58 ± 4.8</td>
<td>14</td>
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<tr>
<td>w/yw;UAS-Irk1\textsuperscript{ONAI}/+</td>
<td>0.38 ± 0.040</td>
<td>148 ± 11.3</td>
<td>57 ± 7.9</td>
<td>13</td>
</tr>
<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;c42-GAL4/+</td>
<td>0.47 ± 0.070</td>
<td>147 ± 5.0</td>
<td>70 ± 11.4</td>
<td>14</td>
</tr>
<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+</td>
<td>0.59 ± 0.090</td>
<td>150 ± 8.1</td>
<td>86 ± 10.5</td>
<td>12</td>
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<tr>
<td><strong>Irk3 knockdown</strong></td>
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<td></td>
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</tr>
<tr>
<td>w;c42-GAL4/+</td>
<td>0.31 ± 0.035</td>
<td>147 ± 8.7</td>
<td>47 ± 6.5</td>
<td>14</td>
</tr>
<tr>
<td>w/yw;c42-GAL4/UAS-Irk3\textsuperscript{ONAI}</td>
<td>0.32 ± 0.035</td>
<td>157 ± 8.0</td>
<td>52 ± 6.5</td>
<td>15</td>
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<tr>
<td>w/yw;UAS-Irk3\textsuperscript{ONAI}/+</td>
<td>0.33 ± 0.052</td>
<td>166 ± 6.6</td>
<td>56 ± 9.1</td>
<td>15</td>
</tr>
<tr>
<td><strong>Irk1/Irk3 double knockdown</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>w:UAS-Irk1\textsuperscript{ONAI}/+,UAS-Irk3\textsuperscript{ONAI}/+</td>
<td>0.40 ± 0.042</td>
<td>164 ± 8.9</td>
<td>66 ± 7.0</td>
<td>13</td>
</tr>
<tr>
<td>w:UAS-Irk1\textsuperscript{ONAI}/+;c42-GAL4/UAS-Irk3\textsuperscript{ONAI}</td>
<td>0.34 ± 0.047</td>
<td>150 ± 12.0</td>
<td>52 ± 9.1</td>
<td>10</td>
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<tr>
<td><strong>Irk2/Irk3 double knockdown</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>w;c42-GAL4/+</td>
<td>0.36 ± 0.052</td>
<td>145 ± 10.3</td>
<td>54 ± 9.3</td>
<td>10</td>
</tr>
<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;c42-GAL4/UAS-Irk3\textsuperscript{ONAI}</td>
<td>0.48 ± 0.053</td>
<td>144 ± 8.8</td>
<td>70 ± 8.7</td>
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<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;UAS-Irk3\textsuperscript{ONAI}/+</td>
<td>0.41 ± 0.046</td>
<td>160 ± 12.9</td>
<td>63 ± 6.3</td>
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<tr>
<td><strong>Irk1/Irk2 double knockdown</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>w;c42-GAL4/+</td>
<td>0.45 ± 0.024</td>
<td>154 ± 6.7</td>
<td>78 ± 4.0</td>
<td>42</td>
</tr>
<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;c42-GAL4/UAS-Irk3\textsuperscript{ONAI}</td>
<td>0.37 ± 0.018</td>
<td>136 ± 5.6</td>
<td>53 ± 4.7</td>
<td>41</td>
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<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;UAS-Irk3\textsuperscript{ONAI}/+</td>
<td>0.49 ± 0.030</td>
<td>158 ± 3.9</td>
<td>77 ± 5.0</td>
<td>41</td>
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<tr>
<td><strong>Irk1/Irk2/Irk3 triple knockdown</strong></td>
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<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;UAS-Irk3\textsuperscript{ONAI}/+;UAS-Irk1\textsuperscript{ONAI}/+</td>
<td>0.64 ± 0.051</td>
<td>166 ± 6.8</td>
<td>109 ± 9.7</td>
<td>22</td>
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<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;c42-GAL4/UAS-Irk1\textsuperscript{ONAI}</td>
<td>0.38 ± 0.049</td>
<td>135 ± 14.7</td>
<td>60 ± 11.5</td>
<td>21</td>
</tr>
<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;c42-GAL4/UAS-Irk3\textsuperscript{ONAI}/+;UAS-Irk1\textsuperscript{ONAI}</td>
<td>0.47 ± 0.058</td>
<td>147 ± 11.2</td>
<td>77 ± 12.0</td>
<td>20</td>
</tr>
<tr>
<td><strong>Effect of cAMP</strong></td>
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</tr>
<tr>
<td>w;c42-GAL4/+ , vehicle</td>
<td>0.33 ± 0.051</td>
<td>98 ± 6.5</td>
<td>35 ± 6.2</td>
<td>19</td>
</tr>
<tr>
<td>w;c42-GAL4/+ , cAMP</td>
<td>0.55 ± 0.067</td>
<td>110 ± 6.5</td>
<td>64 ± 9.6</td>
<td>19</td>
</tr>
<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;c42-GAL4/UAS-Irk1\textsuperscript{ONAI} , vehicle</td>
<td>0.16 ± 0.037</td>
<td>62 ± 6.2</td>
<td>11 ± 3.1</td>
<td>18</td>
</tr>
<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;c42-GAL4/UAS-Irk1\textsuperscript{ONAI} , cAMP</td>
<td>0.38 ± 0.070</td>
<td>84 ± 6.7</td>
<td>37 ± 8.6</td>
<td>19</td>
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<tr>
<td><strong>Effect of ouabain</strong></td>
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</tr>
<tr>
<td>w;c42-GAL4/+ , vehicle</td>
<td>0.37 ± 0.050</td>
<td>140 ± 8.9</td>
<td>56 ± 8.2</td>
<td>16</td>
</tr>
<tr>
<td>w;c42-GAL4/+ , ouabain</td>
<td>0.27 ± 0.050</td>
<td>81 ± 7.0</td>
<td>25 ± 6.1</td>
<td>18</td>
</tr>
<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;c42-GAL4/UAS-Irk1\textsuperscript{ONAI} , vehicle</td>
<td>0.29 ± 0.051</td>
<td>132 ± 12.8</td>
<td>43 ± 9.0</td>
<td>12</td>
</tr>
<tr>
<td>w:UAS-Irk2\textsuperscript{ONAI}/+;c42-GAL4/UAS-Irk1\textsuperscript{ONAI} , ouabain</td>
<td>0.16 ± 0.021</td>
<td>94 ± 6.6</td>
<td>15 ± 2.6</td>
<td>13</td>
</tr>
</tbody>
</table>
Figure 1

A

Transepithelial potential (mV)

Before Barium Recovery

Treatment

B

K⁺ flux (pmol/min)

Baseline Vehicle Baseline Barium
Figure 2

A

![Graph showing K flux (pmol/min) with Tubule Gal4 and UAS-Irk1RNAi conditions.](image)

B

![Graph showing transcript abundance (a.u.) with Tubule Gal4 and UAS-Irk1RNAi conditions.](image)
Figure 3

A

K⁺ flux (pmol/min)

Tubule Gal4 | UAS-Irk2RNAi
---|---
+ | +
- | -

B

Transcript abundance (a.u.)

Tubule Gal4 | UAS-Irk2RNAi
---|---
+ | +
Figure 4

A

K⁺ flux (pmol/min)

Tubule Gal4

UAS-Irk3RNAi

+ + −

− + +

NS

B

Transcript abundance (a.u.)

Tubule Gal4

UAS-Irk3RNAi

+ +

− +
Figure 5

A

B
Figure 6

A

Tubule Gal4
UAS-Irk1RNAi
UAS-Irk2RNAi

B

Tubule Gal4
UAS-Irk1RNAi
UAS-Irk2RNAi
UAS-Irk3RNAi
Figure 7

A

Barium-sensitive K⁺ flux (%)

Barium
- + - + - + - +

Control Irk1RNAi Irk2RNAi Irk1RNAi Irk2RNAi

B

Barium-sensitive K⁺ flux (%)

Tubule GAL4 + + + +

UAS-Irk1RNAi - + - - +

UAS-Irk2RNAi - - + - +
Figure 8

A

B

K⁺ flux (pmol/min)

K⁺ flux (pmol/min)

CAMP

ouabain

Control

Irk1/Irk2 knockdown

Control

Irk1/Irk2 knockdown