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K^+ transport in Malpighian tubules of *Tenebrio molitor* L.: is a K_{ATP} channel involved?

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Summary

The presence of ATP-regulated K^+ (K_{ATP}) channels in *Tenebrio molitor* Malpighian tubules was investigated by examining the effect of glibenclamide on both fluid secretion and basolateral membrane potentials (V_{bl}). Glibenclamide, a K_{ATP} channel blocker, slowed fluid secretion of *Tenebrio* tubules. In low bath K^+ concentration (5 mmol l^{-1}), glibenclamide either hyperpolarized or depolarized V_{bl}, resembling the effect seen with Ba^{2+}. Subsequent addition of 6 mmol l^{-1} Ba^{2+} caused a further hyper- or depolarization of V_{bl}. In control Ringer (50 mmol l^{-1} KCl, 90 mmol l^{-1} NaCl), glibenclamide had no visible effect on V_{bl}. The effect of ouabain was investigated in low bath [K^+] in the presence of Ba^{2+}. V_{bl} responded by a small but significant hyperpolarization from –51±4 mV to –56±4 mV (n=16, P<0.001) in response to 1 mmol l^{-1} ouabain. Repeating the experiments in the presence of both glibenclamide and Ba^{2+} resulted in a depolarization of V_{bl} when ouabain was added. In low bath [K^+] (high Na^+), the Na^+/K^+-ATPase is expected to function at a high rate. In the presence of Ba^{2+}, replacing Na^+ by K^+ rapidly depolarized V_{bl}, but this was followed by a repolarization. Repeating the experiments in the presence of glibenclamide markedly reduced the depolarizing effect and abolished the repolarization, with a gradual decrease in the sensitivity of V_{bl} to the surrounding [K^+]. These results suggest the presence of K_{ATP} channels in the basolateral membrane. Glibenclamide had no visible effect on V_{bl} in high K^+ or in the absence of Ba^{2+}, indicating that other highly conductive K^+ channels may mask the effect on K_{ATP} channels. This is the first demonstration of the presence of K_{ATP} channels in an insect epithelium.

Key words: K^+ transport, K_{ATP} channel, Malpighian tubules, *Tenebrio molitor*, glibenclamide, basolateral membrane potential, fluid secretion rate.

Introduction

Insect Malpighian tubules play a pivotal role in maintaining ion and water homeostasis in the face of extreme and variable conditions. Electrophysiological studies indicate that in Malpighian tubules of *Tenebrio molitor* (Wiehart et al., 2003), in common with other insect species (for reviews see Pannabecker, 1995; Van Kerkhove, 1994), the prime mover of primary urine production is active K^+ transport across the epithelium. Basolateral entry of K^+ occurs mainly *via* Ba^{2+}-sensitive K^+ channels (Nicolson and Isaacson, 1990; Leyssens et al., 1993) and the $Na^+/K^+/2Cl^-$ and K^+/Cl^- cotransporters. However, active K^+ transport *via* a basolaterally located Na^+/K^+-ATPase has been suggested for a number of insect species (Anstee and Bowler, 1979; Maddrell and Overton, 1988; Caruso-Neves and Lopes, 2000; Linton and O'Donnell, 1999).

In transporting epithelia of vertebrates, the activity of the basolateral Na^+/K^+-ATPase is directly linked to the basolateral K^+ conductance (Grasset et al., 1983; Matsumura et al., 1984). Inhibition of the Na^+/K^+-ATPase by ouabain increases the intracellular ATP concentration, which in turn reduces the open probability of ATP-regulated K^+ (K_{ATP}) channels (Balaban et al., 1980; Hurst et al., 1993; Urbach et al., 1996).

In Na^+-reabsorbing epithelia, transport of Na^+ is facilitated by passive entry mechanisms in the apical membrane and an active Na^+-translocation step, the basolateral Na^+/K^+-ATPase. K_{ATP} channels recycle the obligatory influx of K^+ *via* the Na^+/K^+-ATPase (Mauerer et al., 1998; Wang et al., 1990). This recycling process prevents intracellular K^+ accumulation and maintains a favourable electrical gradient for Na^+ transport across the apical membrane (Hurst et al., 1993). Far less is known about the presence of K_{ATP} channels in K^+-secreting epithelia. Wang et al. (1990), however, have documented the presence of a low-conductance K_{ATP} channel in the K^+-secreting principal cells of the rat cortical collecting tubule.

A role for K_{ATP} channels in insects is expected to be different. Secretion of K^+ from cell to lumen in insect Malpighian tubules is generally thought (see Nicolson, 1993) to occur *via* an apical cation/nH^+ antiporter. A vacuolar-type H^+-ATPase actively extrudes H^+ across the apical membrane, and this (1) energizes the antiporter, enabling exchange of

protons for K^+ (or Na^+), and (2) keeps the cell at a negative potential, beyond the Nernst potential for K^+, thereby creating an inward electrochemical gradient for K^+ across the basolateral membrane (Leyssens et al., 1993; Wiehart et al., 2003). The possible function of K_{ATP} channels, if present, in Malpighian tubule cells may be to contribute to K^+ uptake in certain conditions, in parallel with the Na^+/K^+-ATPase and other K^+ uptake mechanisms.

K_{ATP} channels were first discovered in cardiac myocytes (Noma, 1983) and were later found in many other tissues (Ashcroft and Ashcroft, 1990). The properties of K_{ATP} channels have been described (for reviews, see Ashcroft and Ashcroft, 1990; Seino, 1999; Wang et al., 1992). Depending on location, these channels exhibit differences in function and therefore differ somewhat in their properties; however, all K_{ATP} channels are highly selective for K^+ ions, displaying inward rectification with inward conductances in the range of 20–300 pS. They are regulated by the intracellular ATP concentration and blocked by the highly specific sulfonylureas, of which glibenclamide and tolbutamide are best described (Ashcroft and Ashcroft, 1990).

The present study investigates the possible presence of K_{ATP} channels in the tubule epithelium of *Tenebrio* by testing the effect of glibenclamide on Malpighian tubule secretion rates and basolateral membrane potentials. We investigate the possibility of a functional link between the activity of the basolateral Na^+/K^+-ATPase and K^+ conductance *via* the proposed K_{ATP} channels by first stimulating this pump with an increase in Na^+ concentration and then inhibiting it by means of ouabain. Finally, we examine the basolateral membrane sensitivity to the bath K^+ in the presence and absence of glibenclamide. To our knowledge, this is the first study that investigates the presence of K_{ATP} channels in the Malpighian tubules of an insect.

Materials and methods

Animals

Tenebrio molitor L. larvae were kept under crowded conditions at room temperature (20–23°C) and fed on a diet of bran and apple. Care was taken in selecting mealworms of similar size for all experiments.

Artificial salines

The composition of the control bathing solution was as follows (Nicolson, 1992): 90 mmol l^{-1} NaCl, 50 mmol l^{-1} KCl, 5 mmol l^{-1} $MgCl_2$, 2 mmol l^{-1} $CaCl_2$, 6 mmol l^{-1} $NaHCO_3$, 4 mmol l^{-1} NaH_2PO_4, 10 mmol l^{-1} glycine, 10 mmol l^{-1} proline, 10 mmol l^{-1} serine, 10 mmol l^{-1} histidine, 10 mmol l^{-1} glutamine and 50 mmol l^{-1} glucose. The pH was adjusted to 7.0 with HCl and the osmolality was kept at 390 mosmol kg^{-1}. Low $[K^+]$ solutions were obtained by replacing KCl with NaCl, and low $[Na^+]$ solutions by replacing NaCl with KCl (low-Na^+ solutions contained 6 mmol l^{-1} Na^+). Solutions were freshly prepared each week, filtered through 0.22 μm Millipore filters and kept at 2°C until used. The pH was measured daily before use. In low $[Na^+]$ experiments and experiments containing Ba^{2+}, NaH_2PO_4 was omitted from all salines to maintain constant osmolality and prevent precipitation. Control experiments in which NaH_2PO_4 was omitted showed no change in secretion rate or electrical profile.

The following pharmacological substances were tested on Malpighian tubule preparations: barium chloride (Sigma, Bornen, Belgium), ouabain (Fluka, Buchs, Switzerland), glibenclamide (Sigma) and cyclic AMP (cAMP; Sigma).

Fluid secretion experiments

The technique of measuring fluid secretion rates was described previously (Wiehart et al., 2002). Secretion was measured in control Ringer containing 1 mmol l^{-1} cAMP (control) and subsequently in control Ringer containing cAMP and glibenclamide. Rates of secretion were expressed as a percentage of the third control rate reading. 6–10 replicates were done for each experiment.

Electrical potential difference measurements

This method was described in detail previously (Wiehart et al., 2003). In short, a portion of a Malpighian tubule (3–5 mm) was suspended in a Ringer bath. Intracellular [basolateral membrane potential (V_{bl})] measurements were performed with 3 mol l^{-1} KCl-filled microelectrodes. Cell impalement was accepted if a sudden drop in potential occurred, if the potential was stable for at least a few minutes and if the electrode potential differed by not more than 3 mV from the baseline after withdrawal.

Statistics

Results are presented as means ± S.E.M., with the number of tubules (*N*) or number of measurements (*n*) in parentheses. The statistical significance of differences in fluid secretion or electrode potentials was evaluated by paired or unpaired Student's *t*-tests (two-tailed). A value of $P<0.05$ was accepted as statistically significant.

Results

The effects of glibenclamide on fluid secretion

Glibenclamide, a sulfonylurea derivative known to block K_{ATP} channels, was tested on *Tenebrio* tubules. Application of either 0.1 mmol l^{-1} or 0.5 mmol l^{-1} glibenclamide inhibited the fluid secretion rates by 34.2±5.9% (n=8) and 42.2±6.6% (n=6), respectively, after 15 min (Fig. 1). The inhibitory effect of glibenclamide was not reversible after washout. Subsequent addition of the endogenous diuretic peptide Tenmo-DH_{37} (100 nmol l^{-1}), however, increased fluid secretion rates, indicating that tubules were still viable.

The effect of glibencamide on V_{bl}

In a low bath $[K^+]$ (5 mmol l^{-1}), the addition of 0.5 mmol l^{-1} glibenclamide elicited a similar change in V_{bl} to that previously seen in the presence of Ba^{2+} (Wiehart et al., 2003), although to a lesser degree. V_{bl} responded to glibenclamide by either a

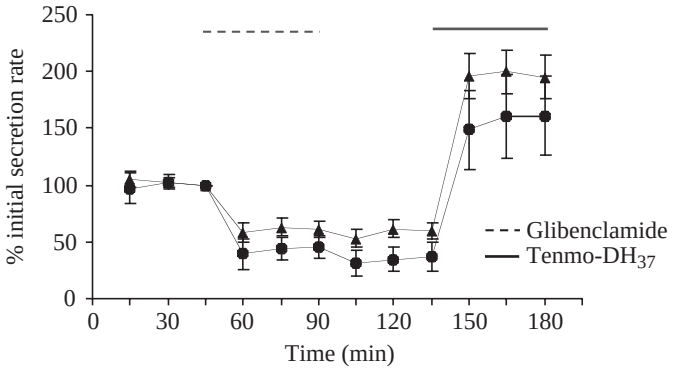

Fig. 1. Effect of glibenclamide on fluid secretion by tubules of *Tenebrio*. Glibenclamide was tested at 0.1 mmol l^{-1} (triangles) and 0.5 mmol l^{-1} (circles) in control Ringer (50 mmol l^{-1} K^+). Secretion rates recovered after stimulation with Tenmo-DH_{37} (100 nmol l^{-1}). The horizontal bars indicate the time of exposure to glibenclamide (broken bar) and to Tenmo-DH_{37} (solid bar). Data are presented as means ± 1 S.E.M. for 7–8 tubules.

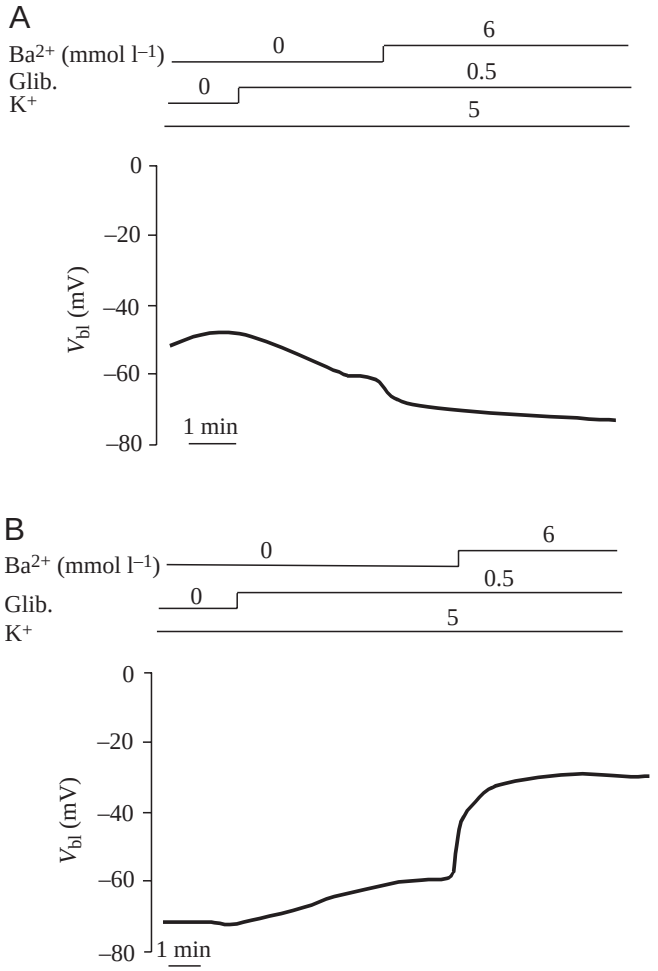

Fig. 2. Response of basolateral membrane potential (V_{bl}) to glibenclamide (Glib.). In a low [K^+] bath (5 mmol l^{-1} K^+) V_{bl} responded to glibenclamide by either (A) a small but significant hyperpolarization of 3.6±1.2 mV (P=0.01, n=8) or (B) a significant depolarization of 9±1.5 mV (P=0.008, n=4). Addition of 6 mmol l^{-1} Ba^{2+} reinforced the initial response of glibenclamide.

small but significant hyperpolarization from –56.6±3.3 mV to –59.7±3.3 mV (Fig. 2A; P=0.01, n=8; in one experiment, there was a marked hyperpolarization of 12 mV) or a significant depolarization from –68.3±3.8 mV to –52.3±1.5 mV (Fig. 2B; P=0.008, n=4).

Subsequent addition of Ba^{2+} reinforced either the hyperpolarization or the depolarization initiated by glibenclamide (both responses are shown in Fig. 2).

The experimental protocol was reversed to determine whether glibenclamide had an effect on V_{bl} in the presence of Ba^{2+}. Again, glibenclamide caused a further hyperpolarization of V_{bl} from –51.8±5.6 mV to –54.7±5.8 mV (n=5, P<0.006) or depolarization from –45 mV to –41 mV (n=1) (results not shown), following the response initiated by Ba^{2+}. The addition of glibenclamide to control Ringer (50 mmol l^{-1} K^+) had no visible effect on V_{bl} (n=10).

V_{bl} *in the presence of ouabain*

Previously, we found that ouabain (1 mmol l^{-1}) added to control Ringer (50 mmol l^{-1} K^+) significantly reduced fluid secretion but had no visible effect on V_{bl} (Wiehart et al., 2003). Blocking of the outward electrogenic current of the Na^+/K^+ pump by ouabain is expected to cause, if anything, a depolarization of the membrane. The absence of a visible effect could be due to the high conductance (mainly due to K^+) of the basolateral membrane. Ouabain had a variable effect on V_{bl} in low bath [K^+] (5 mmol l^{-1}), the tubule cells responding either by a small hyperpolarization of 3 mV (n=1) or a depolarization of 3–6 mV (n=3; results not shown). This variable result was further investigated in the presence of 6 mmol l^{-1} Ba^{2+} to reduce the impact of highly conductive K^+ channels. Two of 16 experiments showed a slight depolarization of V_{bl} in the presence of ouabain. Surprisingly, in all other experiments, V_{bl} responded by a small but significant hyperpolarization. Fig. 3A shows the result of an experiment in which V_{bl} hyperpolarized from –64 mV to –73 mV. The observed hyperpolarization occurred gradually over a period of 3–5 min and dropped back to pre-ouabain-treated potentials within 1 min of washout. Fig. 3B summarises the results of all 16 experiments. Ouabain had no detectable effect on V_{bl} in control Ringer (50 mmol l^{-1} K^+) in the presence of Ba^{2+} (n=4).

The effect of ouabain on V_{bl} *in the presence of glibenclamide*

The effect of ouabain was tested again but this time in the presence of glibenclamide (and Ba^{2+}). The experiments in Fig. 4 illustrate the result. After the addition of glibenclamide and Ba^{2+}, which either caused a hyperpolarization (Fig. 4A) or depolarization (Fig. 4B) of V_{bl}, the addition of 1 mmol l^{-1} ouabain always resulted in a depolarization of V_{bl}, averaging 8.5±1.4 mV (n=8, P=0.001).

Further indications of K_{ATP} *channels in the basolateral membrane*

In the presence of Ba^{2+}, although a loss of K^+ sensitivity is

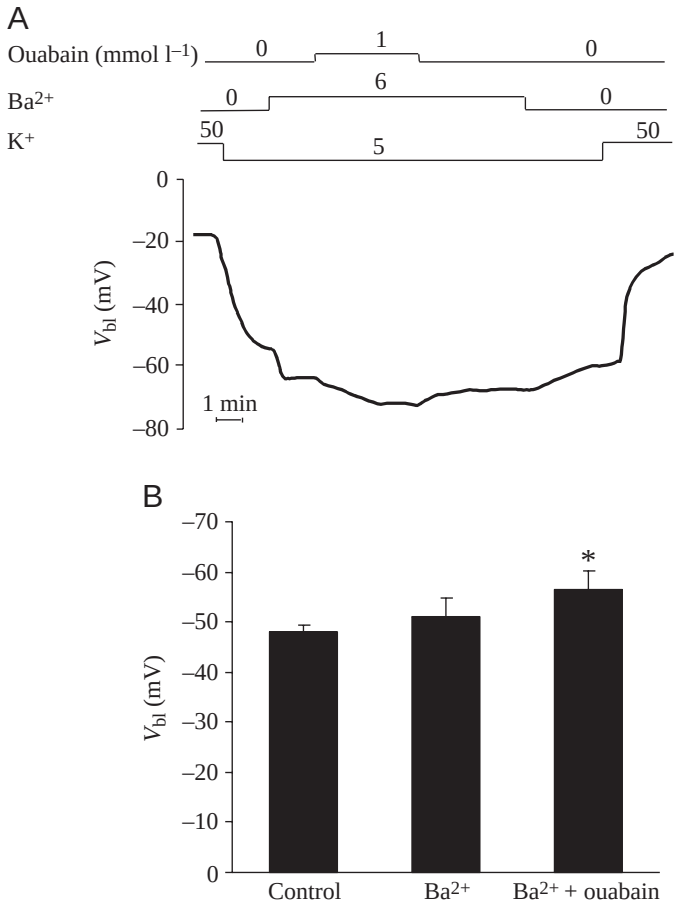

Fig. 3. (A) Effect of ouabain on basolateral membrane potential (V_{bl}) in low bath $[K^+]$ (5 mmol l^{-1}) measured in the presence of Ba^{2+}. (B) Summary of the response of V_{bl} to ouabain. Data are presented as means ± 1 S.E.M. (n=16, P<0.001).

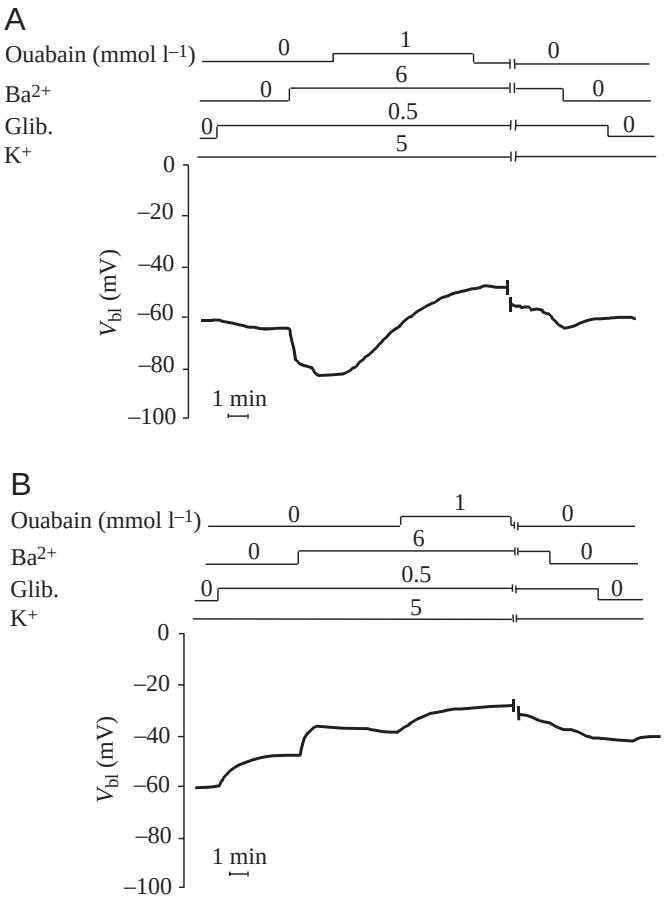

Fig. 4. Typical experiments showing the effect of ouabain on basolateral membrane potential (V_{bl}) in the presence of glibenclamide (Glib.) and Ba^{2+}. The hyperpolarization (A) or depolarization (B) of glibenclamide and Ba^{2+} is followed by a depolarization of V_{bl} with the addition of 1 mmol l^{-1} ouabain. The experiments were carried out in low bath $[K^+]$ (5 mmol l^{-1}).

expected, a change in the bath $[K^+]$ from 5 mmol l^{-1} K^+ to 140 mmol l^{-1} K^+ caused a depolarization of V_{bl} from –88.8±2.7 mV to –13.7±1.9 mV, followed by a repolarization to –51.8±7.0 mV beginning after 3–8 min (n=6). A typical experiment is shown in Fig. 5A, and Fig. 5B summarises the results of six experiments. Such a repolarization was never seen after a 20 min period in a high $[K^+]$ in the absence of Ba^{2+} (result not shown).

Fig. 6 shows the result of a similar experiment but in the presence of 0.5 mmol l^{-1} glibenclamide. Although V_{bl} still depolarized by 43.1±5.7 mV (n=6) when the bath $[K^+]$ was changed from 5 mmol l^{-1} K^+ to 140 mmol l^{-1} K^+, this was significantly less than the depolarization of 75 mV previously seen in the absence of glibenclamide. Furthermore, no subsequent repolarization of V_{bl} was seen in any of the experiments even after 30 min of high $[K^+]$. The rate of response of the basolateral membrane to either the high or low $[K^+]$ was noticeably affected in the presence of glibenclamide. With both Ba^{2+} and glibenclamide present, V_{bl} hyperpolarized over a mean time of 15 min (n=6) in response to low bath $[K^+]$ compared with 8 min (n=7) in the presence of Ba^{2+} alone. Likewise, V_{bl} depolarized over a mean period of 12 min (n=6) in response to a high $[K^+]$ compared with 3 min when only Ba^{2+} was present. During these experiments, the basolateral membrane became increasingly less sensitive to the bath $[K^+]$ with time. Reintroduction of a low bath $[K^+]$ hyperpolarized V_{bl} to –57±3.8 mV compared with the previous –66±6.2 mV (n=6, P=0.002).

Discussion

The effect of glibenclamide on fluid secretion rates

The existence of a functional link between the activity of the basolateral Na^+/K^+-ATPase and the basolateral K^+ conductance *via* K_{ATP} channels is well established in vertebrate epithelial cells (Grasset et al., 1983; Matsumura et al., 1984; Smith and Frizzell, 1984; Tsuchiya et al., 1992). At concentrations of 0.1 mmol l^{-1} and 0.5 mmol l^{-1}, glibenclamide, a specific K_{ATP} channel blocker, decreases fluid secretion rates of stimulated *Tenebrio* tubules by 34% and

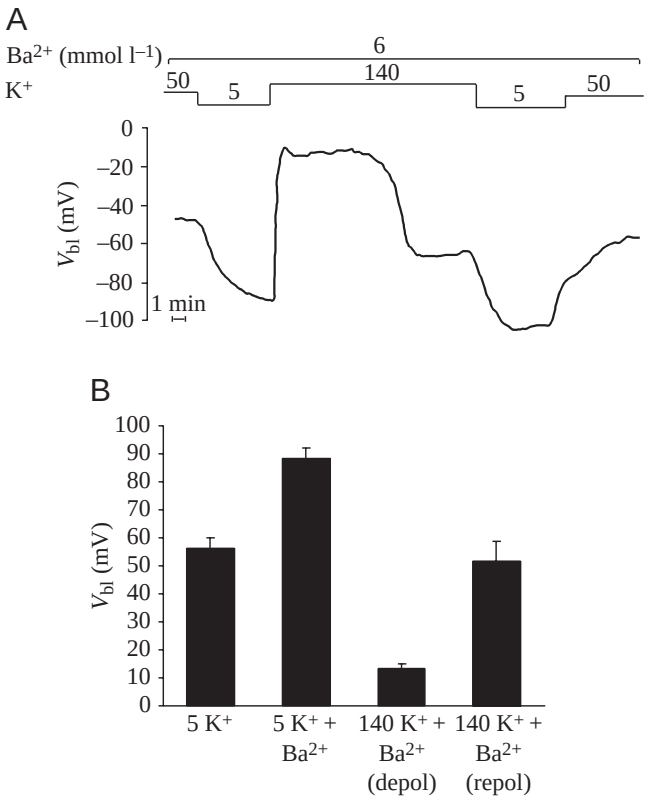

Fig. 5. (A) Response of basolateral membrane potential (V_{bl}) to different bathing K^+ concentrations in the presence of Ba^{2+}. The depolarization of V_{bl} when changing the $[K^+]$ from 5 mmol l^{-1} to 140 mmol l^{-1} is followed by a repolarization after 3–8 min. (B) Summary of the effect of Ba^{2+} in various $[K^+]$. Data are presented as means ± 1 S.E.M. (n=6).

42%, respectively (Fig. 1). The drug concentrations used are relatively high compared with those used to inhibit K_{ATP} channels of pancreatic β-islet cells but are comparable with doses used in the renal proximal tubule (Tsuchiya et al., 1992). The reason for the apparent differences in sensitivity of K_{ATP} channels to glibenclamide is not clear but appears to depend on the association of different sulfonylurea receptors with the K_{ATP} channel unit (Benz and Kohlhardt, 1994). This is in accordance with the existence of a large family of K_{ATP} channels, which have, among other properties, different sensitivities to sulfonylureas (Ashcroft and Ashcroft, 1990).

The effect of glibenclamide on the basolateral membrane potential

The involvement of K_{ATP} channels in control Ringer (50 mmol l^{-1} K^+) seems to be indicated by the inhibition of fluid secretion by glibenclamide, although the substance had no visible effect on V_{bl} in control Ringer. The lack of response might be due to the masking of K_{ATP} channel activity by other highly conductive K^+ channels present in the basolateral membrane of insect tubules.

In low bath $[K^+]$ (5 mmol l^{-1}), glibenclamide had a detectable effect on V_{bl} similar to that previously observed with the K^+ channel blocker Ba^{2+} (Wiehart et al., 2003). Depending on the putative electrochemical gradient for K^+, glibenclamide either caused a small but significant hyperpolarization of 3.6±1.2 mV (Fig. 2A) or a significant depolarization of 9±1.5 mV (Fig. 2B) of V_{bl}, indicating the inhibition of either inward (hyperpolarization) or outward (depolarization) K^+ movement through glibenclamide-sensitive K^+ channels. The open probability of the K_{ATP} channels at bath concentrations of 135 mmol l^{-1} NaCl and 5 mmol l^{-1} KCl must therefore be relatively high. This is supported by a patch-clamp study on rat cortical collecting tubules in which the authors found a bath concentration of 5 mmol l^{-1} KCl and 135 mmol l^{-1} NaCl to be optimal for K_{ATP} channels to be in an open state (Wang et al., 1990).

The addition of 6 mmol l^{-1} Ba^{2+} complements the initial response observed with glibenclamide by a further hyperpolarization or depolarization of V_{bl}, demonstrating the inward and outward electrochemical gradient for K^+, respectively (Fig. 2). The sensitivity of this large family of K_{ATP} channels to Ba^{2+} is not clear. Tsuchiya et al. (1992) determined that K_{ATP} channels are almost exclusively responsible for the K^+ conductance in the renal proximal tubule. Blocking the conductive K^+ channels with glibenclamide caused a 95% inhibition in the basolateral membrane K^+ conductance compared with 84% when blocking with Ba^{2+}. This difference indicated that the K_{ATP} channels were less sensitive to Ba^{2+}. In line with this study, the K_{ATP} channels present in *Tenebrio* Malpighian tubule cells appear less sensitive to Ba^{2+}. The additional hyperpolarization from –51.8±5.6 mV to –54.7±5.8 mV (n=5) or depolarization

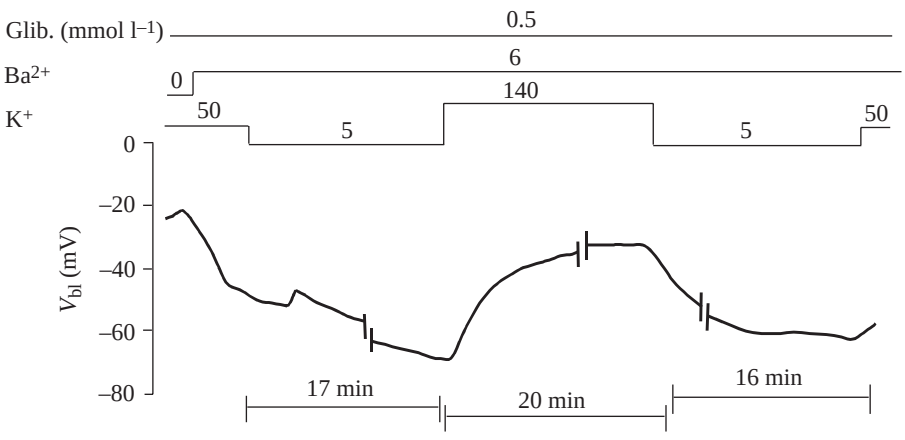

Fig. 6. Slow response of basolateral membrane potential (V_{bl}) to different bathing K^+ concentrations in the presence of glibenclamide (Glib.) and Ba^{2+}. The depolarization of V_{bl} when changing the $[K^+]$ from 5 mmol l^{-1} to 140 mmol l^{-1} is no longer followed by a repolarization (n=6).

from –45 mV to –41 mV (n=1) caused by glibenclamide in the presence of Ba^{2+} substantiates this.

However, caution must be exercised when interpreting results with glibenclamide, as this sulphonylurea compound has been shown to inhibit the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel (Sheppard and Welsh, 1992; Schultz et al., 1996), which is present in most secreting epithelia of vertebrates. Although this CFTR channel has not been characterized in insects, we cannot rule out its existence.

The effect of ouabain and glibenclamide on V_{bl}

Fluid secretion is inhibited by 1 mmol l^{-1} ouabain (Wiehart et al., 2003). However, ouabain has no detectable effect on V_{bl} in control conditions (50 mmol l^{-1}). Possibly, the presence of high conductance K^+ channels masked an effect on any other electrogenic process in the basolateral membrane. The effect expected when the outward electrogenic pump current is blocked is a depolarization of the membrane (Messner et al., 1985; Horisberger and Giebisch, 1988). This has been observed in unstimulated salivary glands of *Calliphora* (Berridge and Schlue, 1978) as well as in Malpighian tubule cells of *Drosophila* (Linton and O'Donnell, 1999). In low K^+, in the presence of Ba^{2+}, V_{bl} was affected: in 14 out of 16 cells, the membrane hyperpolarized in the presence of ouabain. Involvement of K_{ATP} channels was confirmed by applying ouabain after pretreatment with glibenclamide (in the presence of Ba^{2+}): the effect was reversed and in all experiments (n=8) ouabain depolarized V_{bl} (Fig. 4).

Glibenclamide changes the sensitivity of V_{bl} *to the external* $[K^+]$

In contrast to findings for the forest ant *Formica polyctena* (Weltens et al., 1992), the basolateral membrane of *Tenebrio* tubule cells does not appear to lose its sensitivity to the bath $[K^+]$ in the presence of 6 mmol l^{-1} Ba^{2+}. Increasing the bath $[K^+]$ from 5 mmol l^{-1} to 140 mmol l^{-1} K^+ resulted in an immediate depolarization of V_{bl}, with a mean value of 75.3±2.4 mV (n=6). However, this sudden depolarization was followed by a repolarization of 30–40 mV after 3–8 min (Fig. 5A). Again, the involvement of the Na^+/K^+-ATPase and the K_{ATP} channels seems to be the explanation.

A rise in Na^+ transport and intracellular $[Na^+]$ is the primary physiological stimulus for the Na^+/K^+-ATPase in vertebrate tissue (Mauerer et al., 1998; Tsuchiya et al., 1992). In our study, a low bath $[K^+]$ (5 mmol l^{-1}), and therefore a high $[Na^+]$ (141 mmol l^{-1}), could be responsible for activating the Na^+/K^+-ATPase, thereby increasing the open probability of the K_{ATP} channels. The initial large depolarization seen when changing the bath $[K^+]$ from a low to a high value is most probably due to the following: (1) Ba^{2+}, being a competitive inhibitor of K^+ channels, is 'knocked-off' by the inward flux of K^+ ions at high bath $[K^+]$ (Eaton and Brodwick, 1980; Armstrong and Taylor, 1980) and (2) the initial intracellular [ATP] is relatively low, which means the open probability of the K_{ATP} channels is high, allowing an initial influx of K^+ ions. However, at a bath concentration containing no NaCl (140 mmol l^{-1} KCl), the Na^+/K^+ pump stops functioning, resulting in a time-dependent increase of intracellular [ATP] and therefore the closing of K_{ATP} channels. This might explain the observed repolarization of V_{bl} after a few minutes: the apical V-ATPase increases the cell negative potential, and compensation by K^+ entrance across the basolateral membrane is slowed down. Again, this response was not seen in experiments without Ba^{2+}, indicating that other highly conductive K^+ channels mask the presence of K_{ATP} channels.

To substantiate the above hypothesis, experiments were repeated in the presence of Ba^{2+} and 0.5 mmol l^{-1} glibenclamide. The hyperpolarization of V_{bl} to a mean of –66±6.2 mV, when $[K^+]$ was decreased, was far less than the –88.5±3.4 mV when only Ba^{2+} was present. Moreover, although a substantial depolarization of V_{bl} was still seen when the bath $[K^+]$ was changed from 5 mmol l^{-1} to 140 mmol l^{-1} (43 mV), this was considerably less than in experiments that involved Ba^{2+} alone. Most remarkable, however, was the sluggish response of V_{bl} in the presence of both substances. Both the hyperpolarization and depolarization of V_{bl} in response to a different bath $[K^+]$ were much slower, with the depolarization in some experiments taking more than six times longer than in earlier experiments with only Ba^{2+}. Moreover, the depolarization of V_{bl} was no longer followed by a repolarization, indicating that the putative K_{ATP} channels were blocked and therefore insensitive to the increase in intracellular [ATP] expected when the Na^+/K^+-ATPase is inhibited by a decrease in Na^+. The final depolarization (after repolarization), when only Ba^{2+} was present, was 37±4.9 mV (n=6) and is comparable with the depolarization of 43±5.7 mV (n=6) when both substances were present, possibly indicating that the K_{ATP} channels are blocked in both instances. Another marked effect of glibenclamide was that the basolateral membrane became increasingly less responsive to the surrounding $[K^+]$ with time. Reintroduction of a low bath $[K^+]$ (5 mmol l^{-1}) still elicited a hyperpolarization of V_{bl}, but to a lesser extent. In experiments where only Ba^{2+} was present, V_{bl} stayed responsive to the bath $[K^+]$ (Fig. 5A).

In summary, the effects of glibenclamide, a K_{ATP} channel blocker, on both the fluid secretion rate and basolateral membrane potentials of *Tenebrio* Malpighian tubules are strong indications of the presence of K_{ATP} channels and the involvement of these channels in ion transport.

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