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ION CHANNEL ACTIVITY FROM THE MIDGUT BRUSH-BORDER MEMBRANE OF GYPSY MOTH (*LYMANTRIA DISPAR*) LARVAE

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Summary

Ion channels from the midgut apical membrane of gypsy moth (*Lymantria dispar*) larvae were studied following mechanical fusion of brush-border membrane vesicles with planar phospholipid bilayer membranes. In symmetrical 300 mmol l^{-1} KCl (pH 9.0), nine different channels with conductances ranging from 27 to 795 pS and linear current/voltage relationships were resolved. In the presence of a KCl gradient across the bilayer (450 mmol l^{-1} *cis*/150 mmol l^{-1} *trans*), 11 different conductance levels ranging from 16 to 850 pS were detected. The channels were slightly cationic: the zero-current reversal potential was shifted by –5 mV to –21 mV compared with symmetrical KCl conditions, corresponding to p_K/p_{Cl} permeability ratios of 1.5–8.0. Most channels were neither voltage-dependent nor Ca^{2+}-sensitive and displayed complex gating kinetics. Addition of Ba^{2+} or Cs^+ to both sides of the bilayer had little effect on channel activity, but fewer distinct channels were observed when KCl was replaced by potassium gluconate, suggesting an effect of Cl^- on channel activity. A reduced number of channels was also detected when KCl was replaced by *N*-methyl-D-glucamine-HCl. Under asymmetrical *N*-methyl-D-glucamine-HCl conditions, only anionic channels were observed. They exhibited current rectification (35 pS at negative voltages and 81 pS at positive voltages) and were strongly voltage-dependent.

Key words: ion channel, Lepidoptera, larva, midgut, brush-border membrane, planar lipid bilayer, gypsy moth, *Lymantria dispar*.

Introduction

The physiology of the larval midgut epithelium of lepidopteran insects is characterized by a strong active transport of K^+ from haemolymph to lumen (Harvey et al., 1983; Dow and Harvey, 1988). This activity, which is generally thought to be mediated by a vacuolar-type H^+-ATPase coupled with an electrogenic K^+/H^+ exchanger, both located in the apical membrane of goblet cells (Wieczorek et al., 1989, 1991, 1999), maintains a large potential difference across the epithelium. The electrical component of the K^+ electrochemical gradient generated across the apical membrane serves as the main driving force for the absorption of solutes such as amino acids by columnar cells (Giordana et al., 1989).

Passage of K^+ across the basolateral membrane of the midgut epithelial cells probably occurs through K^+ channels. This membrane has a large Ba^{2+}-sensitive K^+ conductance (Moffett et al., 1982; Moffett and Koch, 1985, 1988; Schirmanns and Zeiske, 1994), and the presence of Ba^{2+}-sensitive K^+ channels has been demonstrated by noise analysis (Zeiske et al., 1986) and patch-clamp studies (Moffett and Lewis, 1990). Nevertheless, differential effects of lidocaine and Ba^{2+} on transepithelial K^+ transport suggest the existence of an additional K^+ uptake process across the basolateral membrane (Chao et al., 1990; Moffett and Koch, 1991).

There is, at present, very little evidence for ion channel activity in the apical brush-border membrane. However, in experiments in which midgut brush-border membrane vesicles were fused with artificial lipid bilayer membranes, current steps corresponding to conductances of approximately 200 pS were observed (Martin and Wolfersberger, 1995) and single-channel conductances of 31, 47 and 76 pS were resolved (Lorence et al., 1995). The channels were shown to be cation-selective, but detailed analysis of their properties was complicated by their rapid rundown (1–2 min).

In the present study, the activity of single ion channels was recorded under various conditions in brush-border membrane vesicles isolated from the midgut of gypsy moth larvae and fused to artificial planar phospholipid bilayer membranes using a mechanical procedure (Denicourt et al., 1996). Our results show that there are several types of ion channel in the apical membrane of the midgut. Most channels are slightly cation-selective, and there is at least one anionic channel exhibiting voltage-dependence.

Materials and methods

Chemicals and solutions

The phospholipids 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanolamine (POPE) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) were obtained from Avanti Polar Lipids (Birmingham, Alabama, USA). Salts, EDTA, EGTA, Hepes and *n*-decane were purchased from Sigma (St Louis, Missouri, USA).

Experiments were conducted under symmetrical conditions in 300 mmol l^{-1} KCl, 5 mmol l^{-1} $CaCl_2$, 0.5 mmol l^{-1} EDTA, 5 mmol l^{-1} Tris-HCl (pH 9.0). For ionic selectivity determinations, experiments were conducted under asymmetrical conditions with 450 mmol l^{-1}/150 mmol l^{-1} (*cis*/*trans*) KCl, potassium gluconate or *N*-methyl-D-glucamine-HCl (NMDG-Cl) instead of 300 mmol l^{-1} KCl.

Insect and brush-border membrane vesicle preparation

Second-instar larvae of the European gypsy moth *Lymantria dispar* L. (Lepidoptera: Lymantriidae) were obtained from the insect-rearing facility of the Great Lakes Forestry Center (Natural Resources Canada, Sault Ste Marie, Ontario, Canada) and reared at 18 °C on a standard synthetic diet. Whole midguts were isolated from actively feeding last-instar larvae as described elsewhere (Peyronnet et al., 1997). After removal of the Malpighian tubules and the peritrophic membrane with its food content, the midguts were pooled and stored at –80 °C until use. Brush-border membrane vesicles were prepared using a Mg^{2+} precipitation technique (Wolfersberger et al., 1987). Compared with the crude homogenate, the vesicle preparations were enriched six- to eightfold in alkaline phosphatase specific activity, an apical membrane marker, indicating that the vesicles were indeed enriched in brush-border membranes and, therefore, mostly originated from columnar cells. The final pellet containing brush-border membranes was resuspended in 10 mmol l^{-1} Hepes/KOH (pH 7.5) to a final concentration of 15–20 mg membrane protein ml^{-1} and stored at –80 °C for up to 1 month. Longer storage periods resulted in a significant reduction in channel activity. In preparation for fusion to planar lipid bilayers for electrophysiological recording, vesicles were slowly thawed on ice, briefly vortexed and sonicated for 30 s.

Planar lipid bilayers and vesicle fusion

Planar lipid bilayers were formed with a 1:1 (w/w) mixture of POPE and POPC at a final lipid concentration of 25 mg ml^{-1} in 99 % *n*-decane. The 250 µm diameter orifice drilled in a Teflon partition separating the *cis* (1 ml) and the *trans* (0.6 ml) chambers was pretreated with the same lipid mixture and dried under nitrogen. Buffer solution was added to the chambers, and the bilayer was painted over the orifice (from the *cis* side) using a 400 µm diameter stainless-steel 'finger plugger' dental probe (SDS Kerr, Orange, California, USA) previously dipped in the decane/lipid mixture. Membrane thinning was assayed by applying a triangular test pulse (40 mV peak-to-peak, 10 Hz). Typical membrane capacitance was approximately 250 pF. Under these conditions, the membrane could remain stable for several hours. Membranes were tested for the absence of channel-like activity for 15–20 min. Membrane vesicles were then fused to the lipid bilayer by gently touching the bilayer, from the *cis* side, with a 150 µm diameter dental probe previously dipped in the vesicle suspension (Denicourt et al., 1996). Vesicle fusion and channel activity were promoted by applying a holding voltage of –80 to –120 mV across the bilayer. For right-side-out vesicles, the *cis* chamber corresponded to the extracellular medium. All experiments were conducted at room temperature (22–25 °C).

Data recording and analysis

Electrical connections between the chambers and the recording instrumentation were made with Ag/AgCl electrodes and agar/salt bridges (4 % in 1 mol l^{-1} KCl). Holding voltages were corrected for liquid junction potentials, which were measured as described previously (Laprade and Cardinal, 1983). Liquid junction potentials measured under 450 mmol l^{-1}/150 mmol l^{-1} conditions (*cis*/*trans*) were 0 mV for KCl, +13.6 mV for potassium gluconate and –13.8 mV for NMDG-Cl.

Holding voltages were applied to the *cis* chamber, with the *trans* chamber connected to ground. Single-channel currents were recorded using an Axopatch-1D patch-clamp amplifier (Axon Instruments, Foster City, California, USA), displayed on an oscilloscope (Kikusui 5040, Tokyo, Japan) and stored on videotape with a CRC VR-100A digital recorder (Instrutech Corp., Great Neck, New York, USA). The currents were played back, filtered at 500 Hz by an analog eight-pole Bessel filter (Frequency Devices, Haverhill, Massachusetts, USA) and digitized at a sampling frequency of 4 kHz using a DigiData 1200 series interface and Axoscope version 1.1 software (Axon Instruments). Analysis was performed on a personal computer using pClamp version 6.02 software (Axon Instruments).

Channel conductances were estimated from the slopes of the linear regressions on the data points from the current/voltage relationships, either over the whole range of data points when the relationships were linear or in the linear regions for positive and negative voltages when the channels were rectifying. Reversal potentials V_R were obtained by interpolation of the current/voltage relationships using first- or third-order polynomial fits (depending on whether the current/voltage relationships were linear or not), and permeability ratios were calculated from V_R using the Goldman–Hodgkin–Katz equation (Hille, 1992). A quantitative estimate of channel activity was obtained either by determining the overall open state probability as 1 minus the ratio of the total time for which all channels were closed to the total recording time or, when channel open states could be reliably identified, by measuring nP_o, the ratio of the total time spent in the open state by n distinct channels to the total recording time. nP_o was used rather than the single-channel open state probability (P_o) because the total number of channels (n) in the bilayer was generally not known. Subconductance levels were identified according to the following criteria (Fox, 1987): (i) direct

transitions from subconductance levels to main conductance levels were observed; (ii) subconductance states were never observed in the absence of the main conductance; and (iii) the main conductance state did not result from the superimposition of independent channel openings. Because of the multichannel nature of the recordings, kinetic analysis of the channels was not attempted.

Results

Fusion of vesicles with planar lipid bilayers

Brush-border membrane vesicles from larval gypsy moth midguts displayed a very good propensity to fuse with lipid bilayers, as indicated by channel activity, when the vesicles were delivered directly to the *cis* side of the bilayer using a mechanical procedure (Denicourt et al., 1996). Overall, ion channels were observed in 67 % of the experiments (82 out of 123). Under symmetrical KCl conditions, the success of vesicle fusion was approximately 76 % (29 out of 38 experiments). It was not affected by making the *cis* chamber hyperosmotic relative to the *trans* chamber: under 450/150 mmol l^{-1} KCl conditions, channel activity was observed in 73 % of the experiments (27 out of 37). However, when KCl was replaced by equimolar potassium gluconate or NMDG-Cl, the yield of channel incorporation decreased to 58 % (18 out of 31 experiments) and 47 % (8 out of 17 experiments), respectively.

Following vesicle fusion to the bilayers, a variety of channel activity patterns was observed for each of the ionic conditions used in the experiments. In many cases (32 out of 82 successful fusions), a large number of channels were present which often operated in bursts: the current amplitude of the combined channels tended to saturate the recording amplifier and/or break the bilayer. In some experiments (14 out of 82), the channels ceased to operate after a period of 30 s to 5 min. In many cases where stable channel activity was achieved (36 out of 82 recordings), there were usually too many channels present in the bilayer, rendering their analysis extremely difficult. However, in 19 experiments (out of 36 displaying stable activity), the number of current levels over a 30 min period or longer was sufficiently low to allow the characterisation of the channels. All data presented below were collected from these 19 experiments conducted with vesicles obtained from eight separate batches.

Channel conductance under symmetrical KCl conditions

Representative recordings of channel activity observed after membrane fusion in 300 mmol l^{-1} KCl are illustrated in Fig. 1A. In most experiments, channel activity involved various channel types, including a large main conductance of a few hundred picosiemens, with a relatively high open state probability (Fig. 1Ai,ii). These large channels were characterised by either relatively short (Fig. 1Ai) or longer (Fig. 1Aii) open times. In other cases, the superimposition of a few small channels with a high open state probability was observed (Fig. 1Aiii). Large channels with a very short open time could also be detected (Fig. 1Ai,iii). In most recordings, a detailed examination of channel activity revealed several sublevels of conductance according to the criteria proposed by Fox (1987) (see Materials and methods).

Analysis of the data collected from seven experiments performed using two separate batches of vesicles led to the identification of at least nine main conductance levels. The conductances were linear and ranged between 27 and 795 pS (Fig. 1B).

Because of the complex nature of the recordings, voltage-dependence could only be analysed in three experiments. In one experiment, the overall open state probability was clearly voltage-dependent: it was equal to 0.7 at positive voltages and decreased to 0 at negative voltages, suggesting that at least some of the channels in the bilayer were voltage-dependent. In contrast, the channels observed in the other two experiments were not voltage-dependent, with an overall open state probability of 0.9 for voltages between −60 and +60 mV.

The role of Ca^{2+} in channel activity was investigated in two experiments. Following vesicle fusion and observation of channels, 10 mmol l^{-1} EGTA was added to both chambers to completely chelate Ca^{2+}. The channels remained active and were unaffected by the absence of Ca^{2+}, indicating that they were neither Ca^{2+}-selective nor Ca^{2+}-activated (data not shown).

Channel conductance and ion selectivity under asymmetrical KCl conditions

The ion selectivity of the channels was investigated by recording currents under asymmetrical KCl conditions (450 mmol l^{-1} *cis*/150 mmol l^{-1} *trans*). Data were collected in six different experiments using vesicles from four separate batches. At least 11 different levels of conductance were resolved (Table 1). In most experiments, they ranged between

Table 1. *Conductance and ionic selectivity of the most common current transitions observed under asymmetrical KCl conditions*

Conductance (pS)	*N*	V_R (mV)	p_K/p_{Cl}
16	1	−5	1.5
35	1	−20	5.9
56±3	3	−21±5	8.0
95±18	2	−18±1	5.2
140±25	4	−15±2	3.7
215	1	−16	4.1
275±5	2	−15±4	3.7
365±21	4	−18±5	5.2
500	1	−14	3.3
615±10	2	−13	3.0
850	1	−14	3.3

Values are means ± S.D.

The permeability ratios (p_K/p_{Cl}) were calculated from the reversal potential V_R using the Goldman–Hodgkin–Katz equation (Hille, 1992); *N* represents the number of experiments (out of a total of six) in which a given conductance was detected.

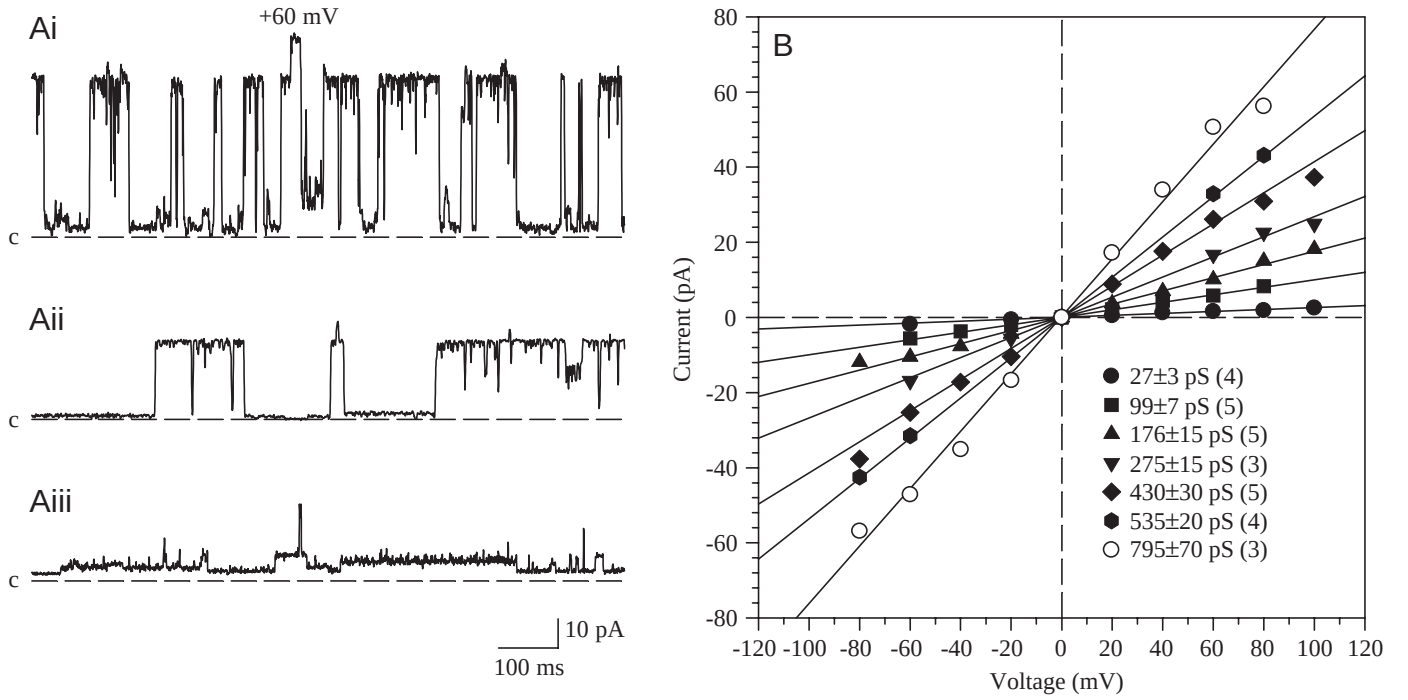

Fig. 1. (A) Representative traces of multichannel activities observed in symmetrical KCl solutions at a holding potential of +60 mV. Phospholipid bilayer membranes were formed in 300 mmol l^{-1} KCl, 5 mmol l^{-1} $CaCl_2$, 0.5 mmol l^{-1} EDTA and 5 mmol l^{-1} Tris-HCl (pH 9.0). Brush-border membrane vesicles prepared from gypsy moth midguts were fused mechanically to the bilayer membrane from the *cis* side. The three traces shown were obtained with different bilayers. The dashed line and the letter c at the left indicate the current level at which all channels were closed. Currents were filtered at 500 Hz and sampled at 4 kHz. (B) Current/voltage relationships of the most common channels observed in symmetrical KCl solutions. For each voltage, the amplitude of the resolvable current steps was measured and averaged for a number of similar current steps. Each data point represents the average of at most the number of experiments given in parentheses; error bars have been omitted for clarity. Curves were fitted to the data points by linear regression. Conductance values, corresponding to the slopes of the curves, are given as means ± S.D. Results are derived from seven experiments. For clarity, two of the smaller conductance levels, 39 pS (N=2) and 64 pS (N=5), are not shown.

16 and 365 pS, with 56, 140 and 365 pS being the most frequent channel conductances. Higher conductances of approximately 500, 615 and 850 pS were also observed in one experiment (Fig. 2A). Although it cannot be excluded that these high conductance levels could result from the simultaneous opening of several channels of smaller conductances, frequent observation of large direct transitions, as illustrated in Fig. 2A, indicated the presence of large 500 and 850 pS channels that possessed several subconducting states. The channels displayed linear current/voltage relationships. Reversal potentials V_R were shifted by 5 mV to approximately 21 mV towards negative voltages compared with symmetrical KCl conditions, demonstrating the cation selectivity of the channels (Fig. 2B; Table 1). The permeability ratio p_K/p_{Cl} was derived from V_R using the Goldman–Hodgkin–Katz equation (Hille, 1992). It ranged from 1.5 to 8.0 (Table 1), indicating low selectivity for K^+.

Addition of 10 mmol l^{-1} Ba^{2+} (N=2) or Cs^+ (N=3), two general K^+ channel inhibitors, failed to inhibit the channels (data not shown). However, because every type of channel was not necessarily present in all experiments, it cannot be excluded that some of the channels described in this study could be sensitive to these blockers.

Interestingly, in one experiment (Fig. 3), the current/voltage relationship displayed strong rectification between −100 mV and +100 mV with conductances of 270 pS at positive voltages and 63 pS at negative voltages (Fig. 3B). The reversal potential was close to −12 mV, indicating low selectivity for K^+ over Cl^-. This channel was also strongly voltage-dependent, with an nP_o close to 1 at negative voltages and falling abruptly to 0.45 at positive voltages (Fig. 3C).

Channel activity under asymmetrical potassium gluconate conditions

Replacement of KCl by equimolar potassium gluconate led to a decrease in the number of distinct conductance levels of the channels (Fig. 4). Current/voltage relationships, constructed from three experiments, remained linear under these conditions and revealed five main conductances between 26 and 280 pS with reversal potentials ranging from −10 to −16 mV (Fig. 4B). In one experiment, it was possible to estimate the nP_o of two types of channels (130 pS and 280 pS). They were strongly voltage-dependent, but in the opposite direction. While nP_o for the 280 pS channel increased sharply from zero for voltages more negative than −93 mV to almost 1 for voltages larger than −60 mV, the nP_o of the 130 pS

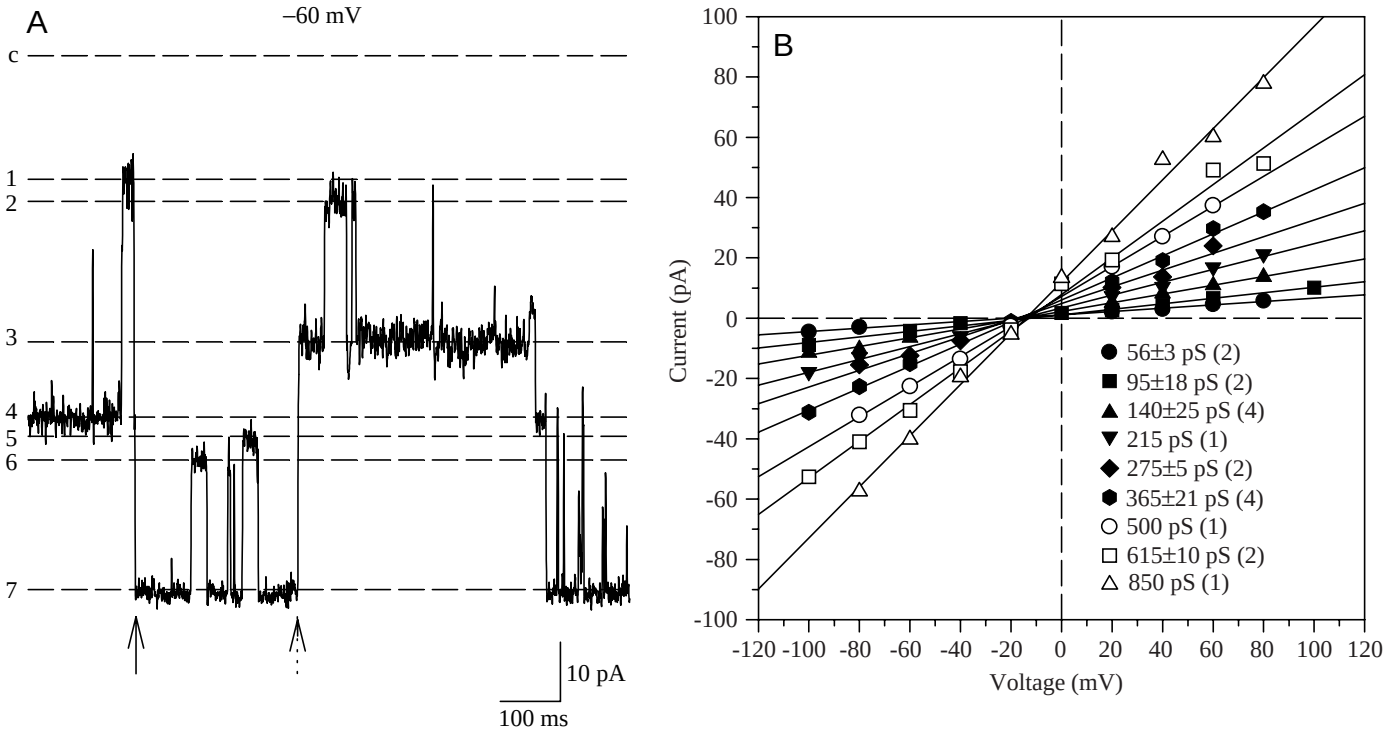

Fig. 2. (A) Current trace of large-conductance cationic channels observed under asymmetrical KCl conditions. Experimental conditions were identical to those described in the legend of Fig. 1 except that the KCl concentration was 450 mmol l^{-1} on the *cis* side of the membrane and 150 mmol l^{-1} on the *trans* side. The different levels of current transitions are indicated by dashed lines. The zero-current level is indicated by the letter c at the left. Note the presence of direct transitions of approximately 40 pA, corresponding to a channel of 850 pS, between levels 1 and 4 (solid arrow) and between levels 3 and 7 (dotted arrow). (B) Current/voltage relationships of the most frequent channels observed under asymmetrical KCl conditions. Data are derived from six experiments in the presence of a 3:1 KCl gradient across the bilayer, with the number of experiments in which a given conductance level was detected in parentheses. For clarity, data for the smaller conductance levels of 16 pS (N=1) and 35 pS (N=1) are not shown.

channels was between 0.8 and 0.95 at negative voltages, but these channels were totally inactive at positive potentials (Fig. 4C).

Channel activity under asymmetrical NMDG-Cl conditions

Replacing KCl with equimolar NMDG-Cl resulted in reduced channel activity. The channels observed, in three experiments, under these conditions were different from those described above (Fig. 5). Only one main conductance level was apparent which displayed rectification, as illustrated in Fig. 5B. The main conductance was 35 pS at negative voltages and 81 pS at positive voltages. This channel was weakly anionic, with a reversal potential of approximately +12 mV, and was strongly voltage-dependent, with nP_o decreasing sharply for voltages larger than +20 mV (Fig. 5C). A detailed analysis of the current recordings revealed the presence of smaller current steps corresponding to a rectifying conductance of 18 pS at negative voltages and 38 pS at positive voltages, with a reversal potential of approximately +8 mV (Fig. 5B).

Discussion

Incorporation of the channels

The purpose of this work was to investigate the presence and properties of ionic channels in the apical membrane of midgut cells from the larval gypsy moths. This membrane is not readily amenable to the patch-clamp technique because its brush-border structure makes it difficult to achieve a proper seal with the patch pipette. Therefore, reconstitution of the apical membrane in planar lipid bilayers by vesicle fusion was the technique of choice for investigating channel activity in this preparation. Mechanical fusion, which was successfully used to characterise the channels in the brush-border membrane of rabbit kidney distal tubules (Denicourt et al., 1996), was preferred to the standard incorporation technique in which fusion is promoted osmotically (Cohen and Niles, 1993). This mechanical method resulted in a much higher fusion rate, as demonstrated by observable channel activity under various ionic conditions in approximately 67 % of the experiments. However, ionic channel density was generally high in the brush-border membrane, and single channel transitions amenable to analysis could only be resolved satisfactorily in 23 % of the experiments in which channel activity was observed. It has been reported that vesicle fusion is promoted by the presence of channels in the open state (Woodbury and Hall, 1988). Therefore, the lower yield of channel events observed when KCl was replaced by potassium gluconate or NMDG-Cl may be due to the fact that the vesicles contained

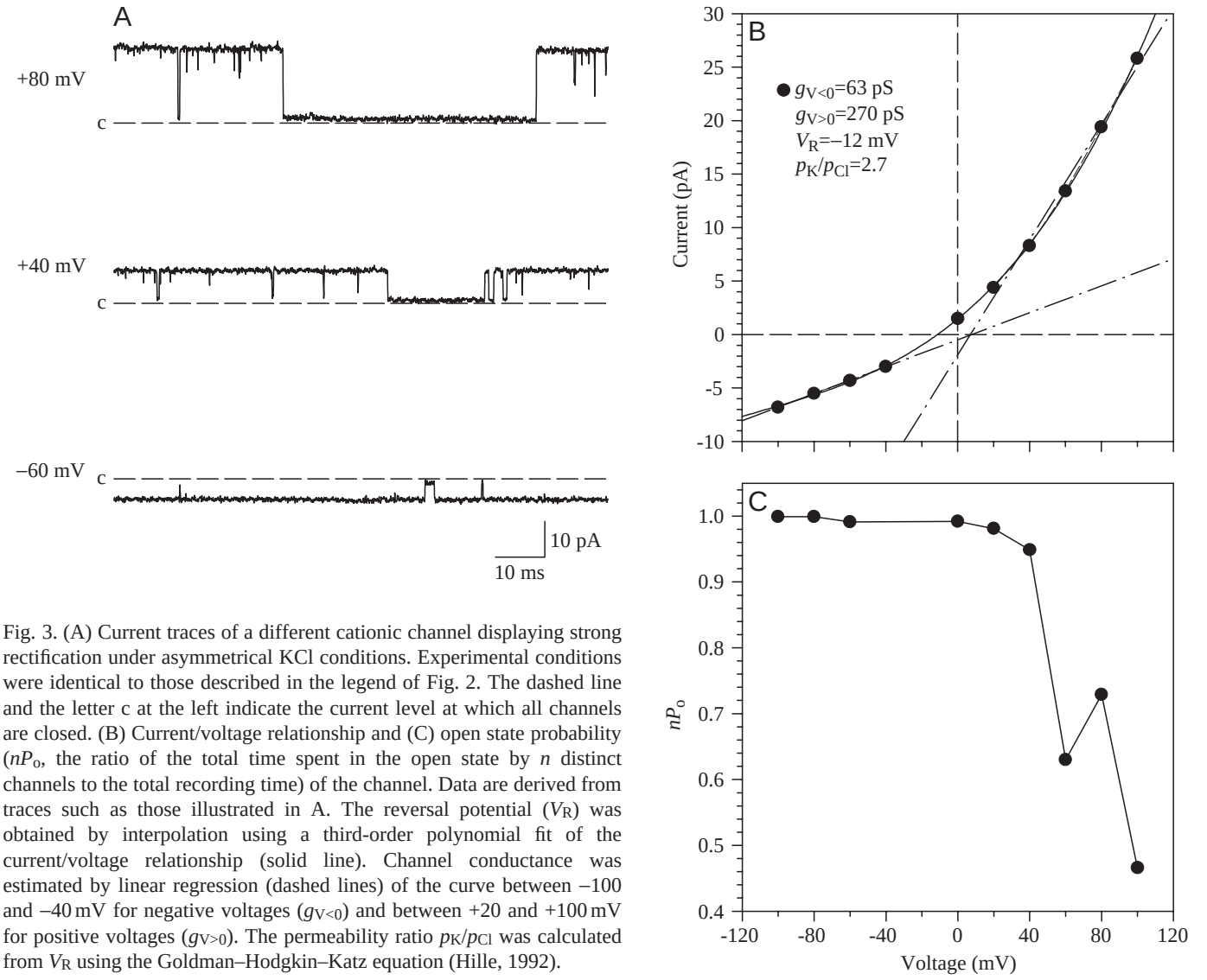

Fig. 3. (A) Current traces of a different cationic channel displaying strong rectification under asymmetrical KCl conditions. Experimental conditions were identical to those described in the legend of Fig. 2. The dashed line and the letter c at the left indicate the current level at which all channels are closed. (B) Current/voltage relationship and (C) open state probability (nP_o, the ratio of the total time spent in the open state by n distinct channels to the total recording time) of the channel. Data are derived from traces such as those illustrated in A. The reversal potential (V_R) was obtained by interpolation using a third-order polynomial fit of the current/voltage relationship (solid line). Channel conductance was estimated by linear regression (dashed lines) of the curve between −100 and −40 mV for negative voltages ($g_{V<0}$) and between +20 and +100 mV for positive voltages ($g_{V>0}$). The permeability ratio p_K/p_{Cl} was calculated from V_R using the Goldman–Hodgkin–Katz equation (Hille, 1992).

a smaller number of open channels under these conditions. In some experiments, channel activity tended to run down within a few minutes. The reason for this behaviour is unclear. A similar observation was made by Lorence et al. (1995), who reconstituted *Spodoptera frugiperda* midgut vesicles into planar lipid bilayer by osmotic fusion, and therefore it does not seem to be related to the mechanical fusion technique used in the present study, to the insect species or to the way the vesicles were prepared.

The technique used for the preparation of vesicles from insect midguts was similar to that developed for rat small intestinal brush-border membrane vesicles, which have been shown to be right-side-out (Haase et al., 1978). Therefore, the apical membrane channel proteins were probably inserted into the planar lipid bilayers with their extracellular surface facing the *cis* chamber. However, it cannot be excluded that the channels could be inserted in both orientations following fusion with planar lipid bilayers (Zweifach et al., 1991).

Origin of the channels

The channels observed in this study were probably derived from the apical membrane of *L. dispar* midguts. Several lines of evidence suggest that the vesicular material reconstituted in the bilayers was indeed made of brush-border membranes and, therefore, mostly originated from columnar cells. First, as mentioned above, compared with the crude homogenate, the vesicle preparations were enriched six- to eightfold in alkaline phosphatase specific activity, an apical membrane marker. This enrichment factor compares favourably with those obtained in several studies in which vesicles were prepared from different larval insect midguts by a variety of techniques (see Wolfersberger et al., 1987). Second, such vesicle preparations are routinely used in our laboratory to assay the pore-forming ability of *Bacillus thuringiensis* toxins (Peyronnet et al., 1996), which have been shown to act specifically on the apical membrane of gypsy moth midgut cells (Peyronnet et al., 1997). Finally, the fact that the cationic channels observed in this work were insensitive to Ba^{2+} suggests that they did not

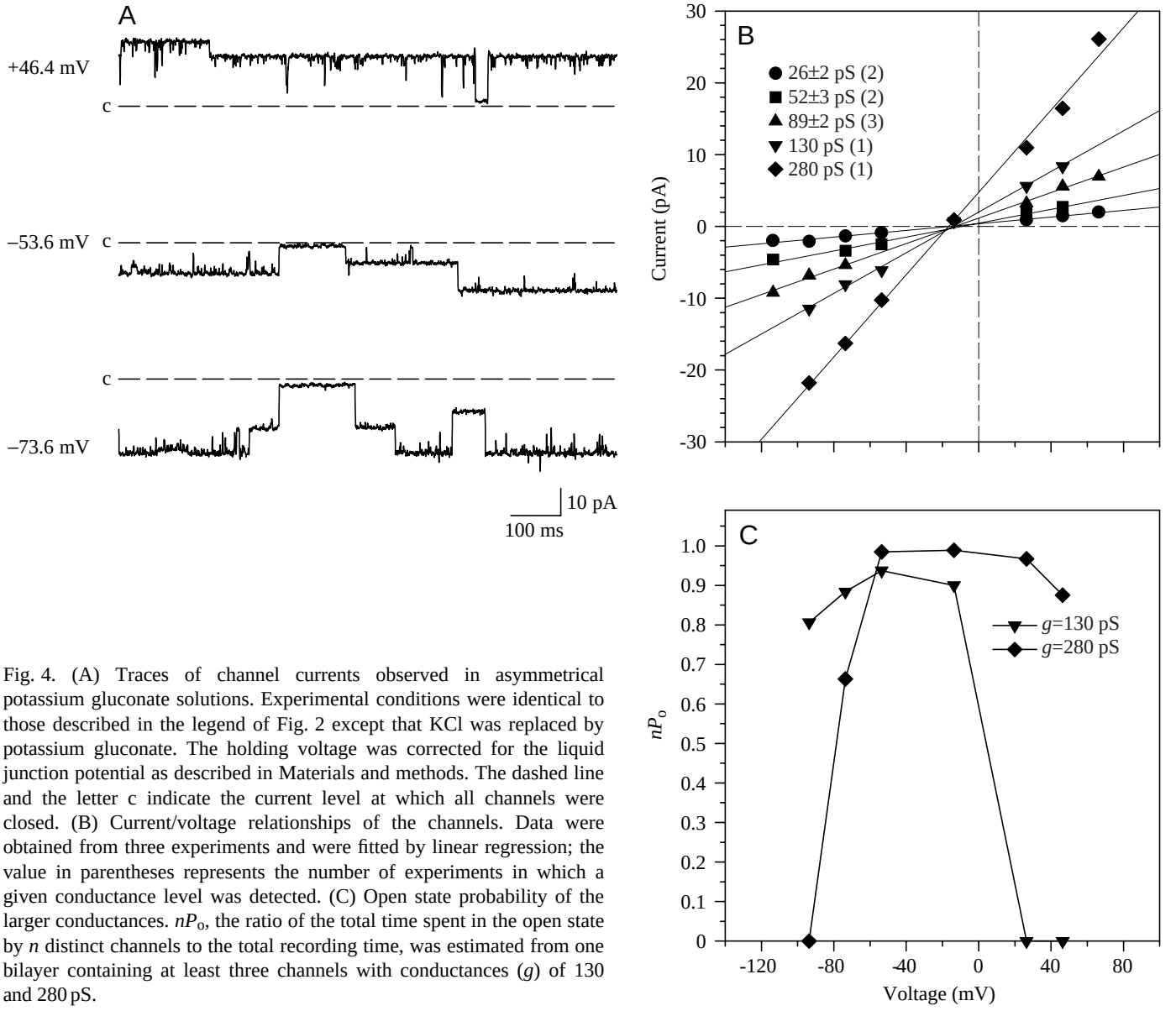

Fig. 4. (A) Traces of channel currents observed in asymmetrical potassium gluconate solutions. Experimental conditions were identical to those described in the legend of Fig. 2 except that KCl was replaced by potassium gluconate. The holding voltage was corrected for the liquid junction potential as described in Materials and methods. The dashed line and the letter c indicate the current level at which all channels were closed. (B) Current/voltage relationships of the channels. Data were obtained from three experiments and were fitted by linear regression; the value in parentheses represents the number of experiments in which a given conductance level was detected. (C) Open state probability of the larger conductances. nP_o, the ratio of the total time spent in the open state by n distinct channels to the total recording time, was estimated from one bilayer containing at least three channels with conductances (g) of 130 and 280 pS.

originate from the basolateral membrane, in contrast to the K^+ channels reported in other studies (Moffett et al., 1982; Moffett and Koch, 1985, 1988, 1991; Zeiske et al., 1986; Moffett and Lewis, 1990; Schirmanns and Zeiske, 1994). However, it cannot be totally excluded that some of the observed channels may originate from contaminating muscle layers or intracellular membranes.

Characterisation of the channels

Our results indicate that the midgut apical membrane of gypsy moth larvae possesses a large diversity of channels. In most experiments, various channels were present simultaneously, and almost all bilayers contained at least two different types of channel. This multichannel behaviour could be due to the cellular heterogeneity of the midgut. Indeed, several studies have clearly established that functional variations, such as the distribution of different enzymes (Ridgway and Moffett, 1986; Azuma and Eguchi, 1989), amino acid transport activities (Giordana et al., 1994; Wolfersberger, 1996) and transepithelial K^+ transport activity (Cioffi and Harvey, 1981), are associated with morphological differences between the anterior, central and posterior regions of the lepidopteran larval midgut. In particular, Chamberlin (1990) showed that, in *Manduca sexta*, the transepithelial flux of various ions depends on the region of the midgut. Therefore, the diversity of the channels observed in the present study may be related to their regional distribution in the midgut of *L. dispar*.

Most of the observed channels displayed low selectivity to K^+. This is consistent with selectivity data obtained for the apical membrane channels of *Spodoptera frugiperda* (Lorence et al., 1995). However, *S. frugiperda* channels were inhibited by Ba^{2+}, Ca^{2+} and Cs^+, which was not the case for the gypsy moth apical membrane channels described in the

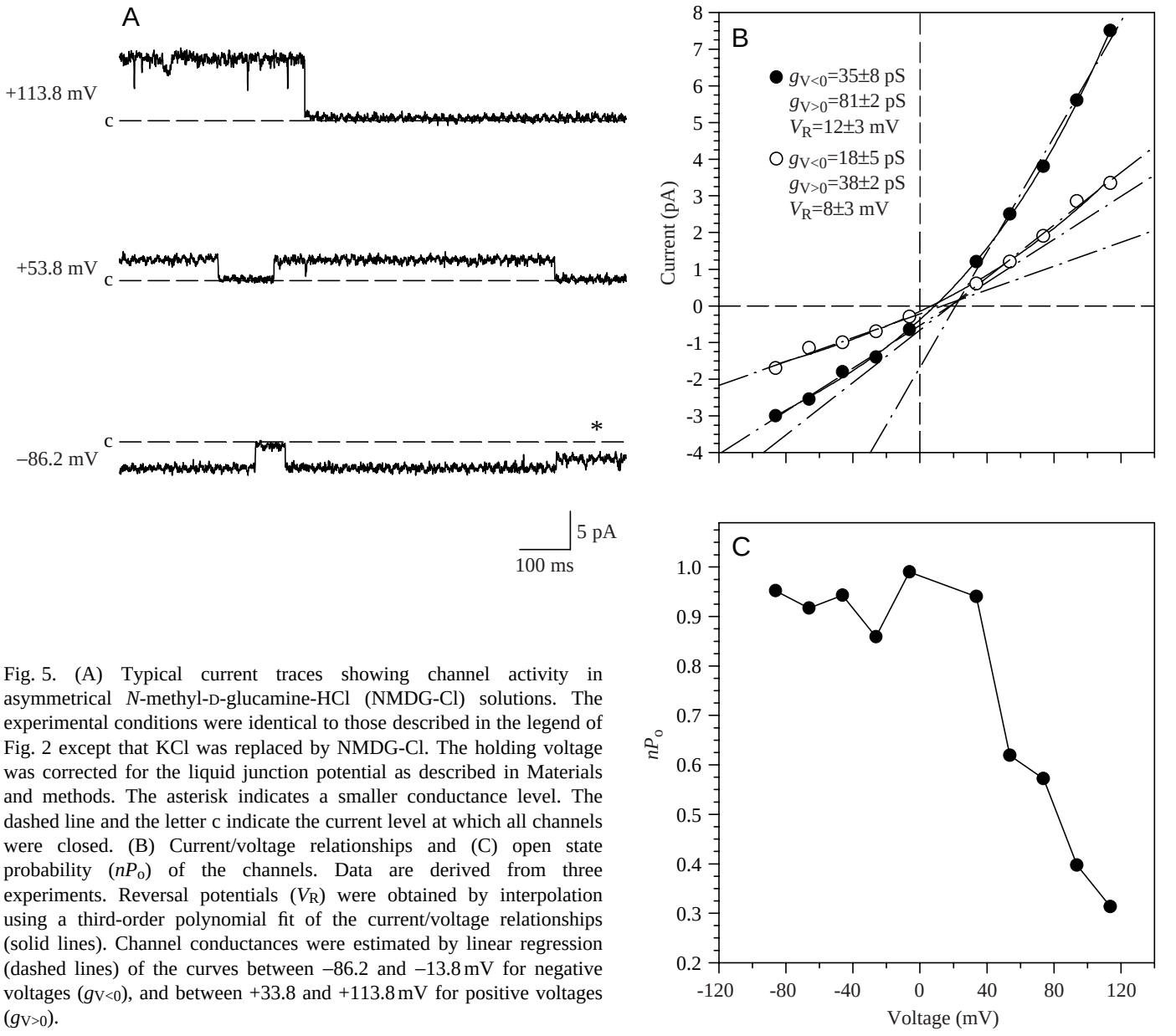

Fig. 5. (A) Typical current traces showing channel activity in asymmetrical *N*-methyl-D-glucamine-HCl (NMDG-Cl) solutions. The experimental conditions were identical to those described in the legend of Fig. 2 except that KCl was replaced by NMDG-Cl. The holding voltage was corrected for the liquid junction potential as described in Materials and methods. The asterisk indicates a smaller conductance level. The dashed line and the letter c indicate the current level at which all channels were closed. (B) Current/voltage relationships and (C) open state probability (nP_o) of the channels. Data are derived from three experiments. Reversal potentials (V_R) were obtained by interpolation using a third-order polynomial fit of the current/voltage relationships (solid lines). Channel conductances were estimated by linear regression (dashed lines) of the curves between –86.2 and –13.8 mV for negative voltages ($g_{V<0}$), and between +33.8 and +113.8 mV for positive voltages ($g_{V>0}$).

present study. This insensitivity of apical membrane transport to Ba^{2+} was also observed in the *M. sexta* midgut epithelium, in which the short-circuit current was unaffected by addition of Ba^{2+} to the luminal side (Moffett and Koch, 1985). Furthermore, the properties of *L. dispar* cationic channels described herein differ from those of midgut basolateral membrane K^+ channels, which are sensitive to Ba^{2+} but not to Cs^+ (Moffett et al., 1982; Moffett and Koch, 1985, 1988, 1991; Zeiske et al., 1986; Moffett and Lewis, 1990; Schirmanns and Zeiske, 1994). Finally, the experiments in which Ca^{2+} was excluded from both *cis* and *trans* compartments suggest that this divalent cation does not alter the activity of the channels from gypsy moth midgut apical membrane.

A smaller number of distinct channels were detected when KCl was replaced by potassium gluconate or NMDG-Cl, suggesting that the replacement of the small ions by larger ones affected the overall channel activity and possibly inhibited some of the channels. In the presence of KCl, the large majority of the channels were only slightly cation-selective and thus allowed the passage of both K^+ and Cl^-. Replacing Cl^- with gluconate did not affect the reversal potential of the channels, indicating that they remained permeable to this larger anion. However, the overall channel activity was reduced, suggesting a stimulatory effect of Cl^-, as was observed for Ba^{2+}-sensitive K^+ channels in the basolateral membrane of *M. sexta* midgut epithelium (Zeiske et al., 1992; Zeiske and Marin, 1992). Under NMDG-Cl conditions, none of the channels observed in KCl could be detected, suggesting that these slightly cation-selective channels were inhibited by $NMDG^+$. However, new channels were revealed in NMDG-Cl: they rectified the current, and

they were strongly voltage-dependent and slightly anionic. It cannot be excluded that these channels could also be permeable to larger anions such as carbonate or bicarbonate.

Most of the channels observed under a given ionic condition had similar ionic selectivities. Furthermore, the large conductances were often multiples of those derived from the measurement of smaller current steps, suggesting cooperative behaviour of clusters of small channels, as reported for various types of ion channel (e.g. Larsen et al., 1996; Kaulin et al., 1998). Moreover, the variability of observed conductances from one experiment to another may reflect the order of cooperativity in the clusters (Larsen et al., 1996).

Physiological role of the channels

The *in vivo* role of brush-border membrane vesicle channels is unclear. First, the exact origin of the proteins reconstituted in lipid bilayers is not totally certain. Second, in lipid bilayer experiments, there are none of the cellular modulatory and regulatory mechanisms found in intact cells or in the whole organ. Third, this membrane is expected to have, at most, a very low passive ion permeability so that a strong K^+ transmembrane electrochemical gradient and electrical potential can be maintained. However, a large number of active channels in the apical membrane does not necessarily imply that there would be a large activity *in vivo*, because appropriate cellular signals may be required for these channels to mediate a substantial ionic flux through the luminal wall of the midgut. Nevertheless, because of their generally poorly selective nature, these channels may be involved in regulating the membrane potential and cellular ionic gradients. For instance, they could possibly contribute to the dramatic loss of transepithelial voltage that occurs during moulting, along with a strong inactivation of the H^+-ATPase (Sumner et al., 1995). Furthermore, the slightly anionic channels observed in this study, if they were indeed permeable to carbonate or bicarbonate, may be involved in luminal alkalisation. Clearly, further studies will be required to ascertain the physiological role of these channels.

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