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LIDOCAINE AND BARIUM DISTINGUISH SEPARATE ROUTES OF TRANSBASAL K^+ UPTAKE IN THE POSTERIOR MIDGUT OF THE TOBACCO HORNWORM (*MANDUCA SEXTA*)

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Summary

The isolated posterior midgut of the tobacco hornworm maintains a vigorous transepithelial K^+ transport from the hemolymphal side to the lumen side at a rate accurately measured by its short-circuit current. Previous studies using the K^+ channel blocker Ba^{2+} suggested that partial inhibition of the short-circuit current by hemolymphal Ba^{2+} was due to blockage of one of at least two parallel transbasal entry routes for K^+ into the intracellular transport pool. The present studies show that the local anesthetic lidocaine, at a concentration of 5 mmol l^{-1} on the hemolymphal side, partly inhibits net transepithelial K^+ transport. The inhibition is accompanied by hyperpolarization of the basal membrane and an increase in transbasal resistance, suggestive of a block of transbasal K^+ conductance. The effects of lidocaine and Ba^{2+} are additive, suggesting that the inhibitors distinguish separate, parallel K^+ uptake processes.

Introduction

The posterior midgut of lepidopteran larvae consists of two major cell types, columnar and goblet, which show restricted coupling with intracellular Lucifer Yellow dye but are apparently well coupled with respect to small ions and electrical current (Moffett *et al.* 1982; Moffett and Koch, 1988*a*). The isolated, short-circuited tissue transports K^+ from hemolymph to lumen at a rate as high as 1 μmol cm^{-2} min^{-1}. Active K^+ transport involves two transmembrane steps: entry across the basal membrane followed by extrusion across the apical membrane. Studies using X-ray microanalysis (Dow *et al.* 1984) and K^+-selective microelectrodes (Moffett and Koch, 1988*b*) show that K^+ is actively transported across the goblet cell apical membrane against a large electrochemical gradient. Under typical experimental conditions the transbasal electrochemical gradient is favorable for K^+ entry (Moffett *et al.* 1982). However, the transbasal gradient becomes unfavorable when extracellular K^+ concentration is low and under hypoxia (Chao *et al.* 1990). The continuation of some net K^+ transport under these conditions is

Key words: lepidopteran insect midgut, active K^+ transport, lidocaine, barium, short-circuit current, microelectrode, *Manduca sexta*.

evidence that transbasal K^+ movement involves an active component that is masked under typical conditions *in vitro*.

Evidence for multiple parallel pathways for K^+ entry across the basal membrane was provided by the effects of Ba^{2+}. Although generally regarded as a competitive inhibitor of K^+ passage through membrane channels, Ba^{2+} partly inhibits net K^+ transport by the midgut in standard saline, but stimulates it in high-K^+ saline. This finding was interpreted as evidence that Ba^{2+} inhibits one route of transbasal K^+ entry while stimulating another; about one-third of transbasal K^+ influx appears to be Ba^{2+}-insensitive (Moffett and Koch, 1985). In standard saline, in which the inhibitory effect dominates, application of Ba^{2+} results in hyperpolarization of the basal membrane and an increase in transbasal resistance (Moffett and Koch, 1988*a*), effects that are consistent with blockage of a current-carrying process.

The present studies characterize the effect of lidocaine on net K^+ transport by the posterior midgut. Lidocaine exerts its local anesthetic effect by blocking the voltage-gated Na^+ and Ca^{2+} channels of excitable cells, but it has also been reported to block K^+ channels in the basolateral membrane of amphibian urinary bladder (Van Driessche, 1986) and turtle colon (Dawson *et al.* 1988). In the midgut, lidocaine, like Ba^{2+}, inhibits the short-circuit current (I_{sc}) and increases basal membrane resistance. However, the effect of each inhibitor is almost unaffected by the presence of the other, and the two inhibitors show different forms of dependence on extracellular K^+ concentration. These results suggest that lidocaine and barium distinguish separate routes of transbasal K^+ entry.

A preliminary account of these studies has been presented (Koch and Moffett, 1990).

Materials and methods

Insects

Larvae of *Manduca sexta* were reared on an artificial diet (Yamamoto, 1969). The morphologically distinct posterior midgut (Cioffi, 1979) was removed from cold-anesthetized fifth-instar larvae and immediately transferred to oxygenated ice-cold bathing solution. The Malpighian tubules and adhering fat body were removed and the tissue was mounted as described below.

Bathing solutions

The standard bathing solution, designated '32KS', contained, in mmol l^{-1}: 32 KCl, 5 $CaCl_2$, 5 $MgCl_2$, 5 Tris–HCl (pH 8.0), 166 sucrose. In some experiments, the concentration of KCl was varied as indicated by the solution designation; for example '70KS' contains 70 mmol l^{-1} KCl. In other experiments the solutions were also made nominally divalent-cation-free by omission of $CaCl_2$ and $MgCl_2$. Except for the experiments on side specificity, all experiments were conducted with identical solutions on both sides of the tissue. Unless indicated otherwise, bathing solutions were oxygenated with 100 % O_2.

Pure lidocaine (Sigma) and its quaternary amine congeners QX-222 and QX-314

(kindly provided by Dr Bertil Takman of Astra Pharmaceutical) were added to bathing solutions to give the concentrations indicated. At the pH of the bathing solutions used in this study (8.0) the maximum concentration of lidocaine that can be attained is 5 mmol l^{-1}. Barium chloride was added to the half-chambers as a 1 mol l^{-1} solution in distilled water to give the final concentrations indicated.

Short-circuit current studies

For studies of inhibitor effects on I_{sc}, the tissue was mounted in a modified Ussing chamber as in previous reports (Moffett and Koch, 1985). Except for the experiments on side specificity, all experiments were conducted with identical solutions on both sides of the tissue. Tissues were continuously short-circuited and I_{sc} was recorded using a strip chart recorder. Solution resistance was compensated using predetermined values.

The results of inhibitor experiments are expressed in terms of the ratio of I_{sc} after inhibition to the control value of I_{sc} measured just before addition of inhibitor (I/I_0). Recovery of I_{sc} is expressed as a percentage of the current recorded immediately before inhibitor was added. Although I_{sc} decays spontaneously with time, with our present techniques the rate of decay is less than 10 % h^{-1} after initial equilibration. Since the effects of Ba^{2+} and lidocaine are rapid and exposures to the inhibitors were brief, no corrections for time-dependent decay of I_{sc} were made.

Microelectrode studies

Microelectrodes were pulled from microcapillary glass (Fredrich Haer 1011) in a vertical puller (David Kopf) and filled with 1 mol l^{-1} KCl. Useful electrodes had tip resistances of 25–30 MΩ when measured in 32KS. The midgut was mounted in a chamber modified from the design of Thompson *et al.* (1982) and penetrated from the hemolymphal side with electrical reference to the hemolymphal solution. All experiments were conducted under short-circuit conditions with identical solutions on both sides. The microelectrode voltage was amplified by a Keithley electrometer and recorded on one channel of a Gould Brush four-channel pen recorder, with I_{sc} being simultaneously recorded on another channel.

The microelectrode was advanced at right angles to the tissue by a hydraulic drive (Friedrich Haer). Penetration of a cell was indicated by a sharp negative deflection of between −20 and −40 mV (Moffett *et al.* 1982); this is the transbasal potential (V_b). The change in transbasal resistance was measured as the change in the magnitude of deflection of V_b in response to the passage of a pulse of constant current. This is a relative measure; the absolute value of transbasal resistance cannot be measured readily by this method because of the large series resistance component presented by the bathing solution.

Statistics

All data are given ±the standard errors of their means. For each tissue in the microelectrode experiments, several impalements were made and the averages of

the values obtained were taken as best estimates for that tissue. The means presented are the averages of these best estimates.

Results

Lidocaine inhibition of I_{sc}

Bilateral addition of lidocaine partly inhibits I_{sc} (Fig. 1). In six tissues I/I_0 and the percentage reversibility of inhibition were measured as a function of the duration of exposure to lidocaine for durations of 5, 10, 20 and 40 min. In three of the tissues the order of exposure durations was ascending; in the other three the order was descending. Results from the two series were not distinguishable, and pooled results are shown in Fig. 2. The inset to Fig. 2 shows the correlation between I/I_0 and fractional recovery.

On the basis of time course and reversibility, two phases of lidocaine inhibition can be distinguished in these results. The initial phase consists of a rapid drop in I_{sc} during the first 10 min. The inhibition that occurs during this phase is almost completely reversible (Fig. 2). If exposure to lidocaine continues beyond 10 min, a slower drop in I_{sc} is seen. This phase takes up to 1 h to complete and is accompanied by a progressive decrease in reversibility (Fig. 2). To maximize reversibility, data were collected at 7.5 min after addition of lidocaine in all subsequent experiments.

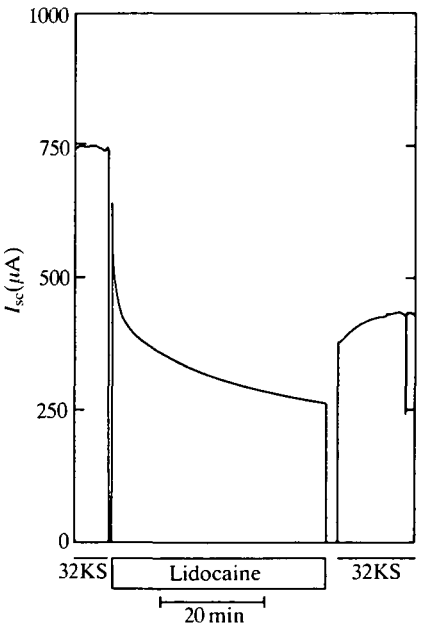

Fig. 1. Trace of the effect of 5 mmol l^{-1} lidocaine applied bilaterally. The tissue was in 32KS before and after a 40 min exposure to lidocaine. Unless otherwise noted, the short-circuit current (I_{sc}) in this and subsequent figures is the actual value for the chamber, the aperture area of which is 0.49 cm^2.

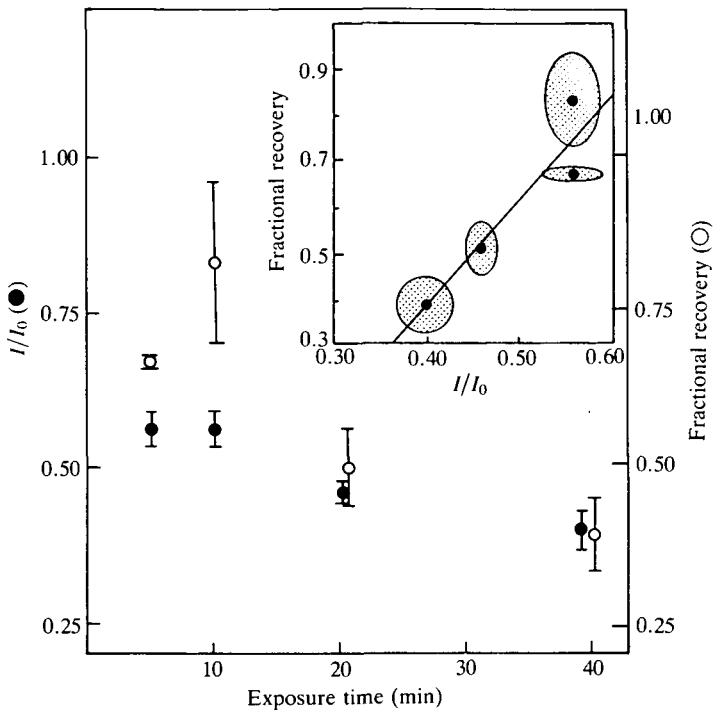

Fig. 2. Effect of exposure duration on I/I_0 (●) and on fractional recovery (○) for six midguts. Error bars are ±1 S.E. The inset shows the correlation between the two variables. Shorter exposure times are at the top right of the inset. The shaded ellipses represent the combined standard errors for the two variables represented by each point.

The limited solubility of lidocaine in bathing solutions at pH 8.0 appears to preclude the maximal effect of the inhibitor. At a concentration of 5 mmol l^{-1}, the inhibitory effect was almost exactly twice that at 2.5 mmol l^{-1} (Fig. 3).

Sidedness of lidocaine inhibition

The rapid effect of lidocaine is on the hemolymphal side (Fig. 4). In four experiments, 75±3.1% of the inhibition in effect by 7.5 min after bilateral application was attributable to the hemolymphal lidocaine, regardless of which side was exposed first.

The effects of lidocaine on transbasal voltage (V_b) and transbasal resistance (R_b) were measured in microelectrode experiments. Transbasal penetrations were made in tissues superfused with 32KS oxygenated with 100% O_2. The mean V_b was −36.5±0.84 mV in seven experiments. The superfusate was then changed to oxygenated 32KS with 5 mmol l^{-1} lidocaine and changes in V_b, R_b and I_{sc} were recorded. Fig. 5A shows typical results. The V_b hyperpolarized synchronously with the decrease in I_{sc}; the mean change in V_b was −6.35±1.63 mV. The increase in the deflection of V_b in response to the transepithelial constant-current pulse

indicates increased R_b; the mean change in basal resistance was 22±6%. The effects of lidocaine on V_b, R_b and I_{sc} are comparable to those of 6 mmol l^{-1} Ba^{2+} (Fig. 5B). As in previous studies (Moffett and Koch, 1988*a*), Ba^{2+} hyperpolarized the basal membrane with a concomitant increase in R_b and decrease in I_{sc}.

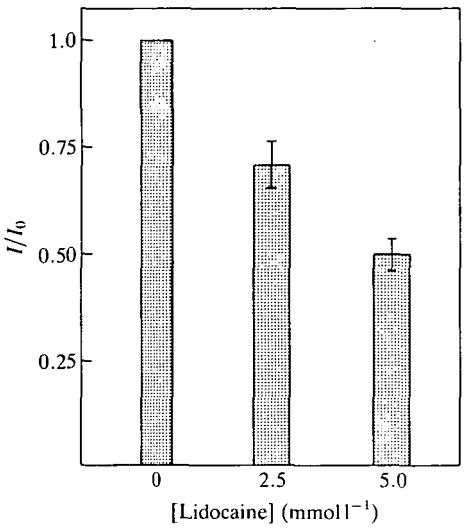

Fig. 3. Dose-response relationship of the rapid effect of lidocaine on I/I_0 (for the standard exposure duration of 7.5 min). Error bars are ±1 S.E. (N=3).

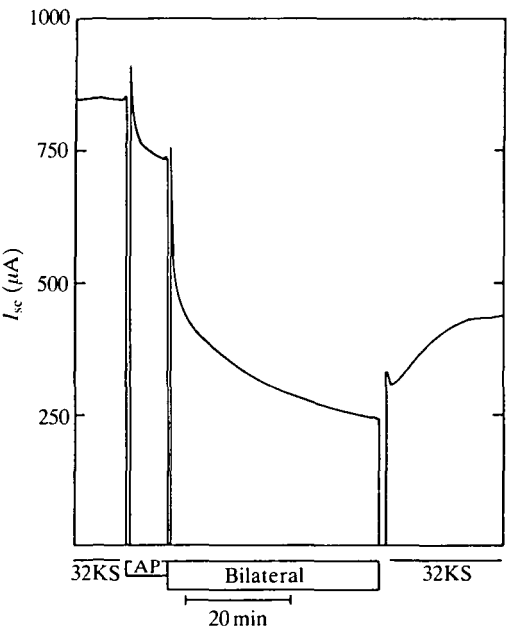

Fig. 4. Unilateral exposure to 5 mmol l^{-1} lidocaine. Apical exposure (AP) for 7.5 min followed by 40 min of bilateral exposure.

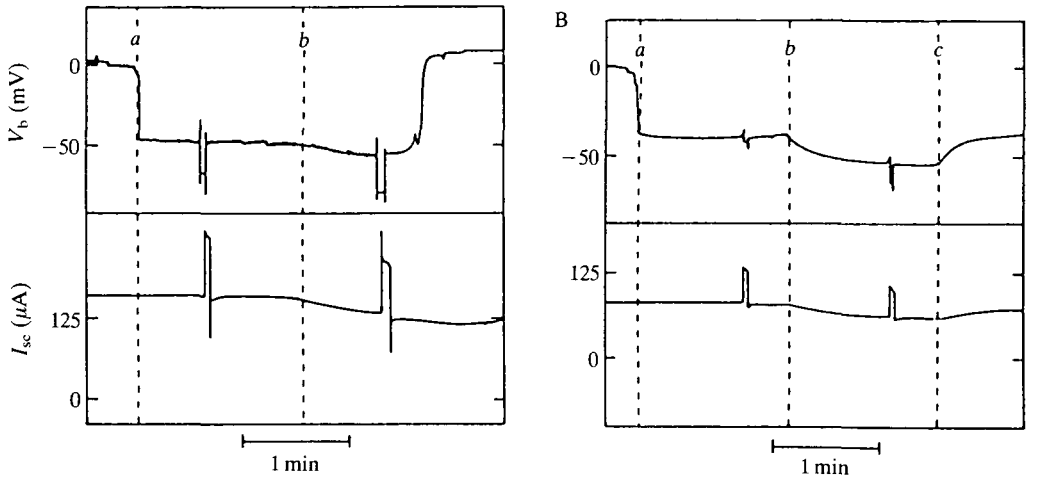

Fig. 5. Intracellular recordings of basal membrane voltage. (A) Effect of lidocaine. Upper trace, transbasal potential (V_b); lower trace, short-circuit current (I_{sc}). The tissue was incubated in 32KS and a cell was impaled at *a*. 90 μA was passed through the tissue and produced a deflection of 16.7 mV. At *b*, the superfusing solutions were changed to 32KS with 5 mmol l^{-1} lidocaine. V_b hyperpolarized and I_{sc} fell. Injection of 90 μA now gave a deflection of 22.2 mV. (B) Effect of Ba^{2+}. Upper trace, V_b; lower trace, I_{sc}. The tissue was incubated in 32KS and a cell was impaled at *a*. After 1 min, 46 μA was passed through the tissue and produced a deflection of 5.3 mV. At *b*, the superfusing solutions were changed to 32KS with 6 mmol l^{-1} Ba^{2+}. V_b hyperpolarized and I_{sc} fell. Injection of 46 μA now gave a deflection of 13 mV. The I_{sc} shown is the actual value recorded; the chamber aperture was 0.19 cm^2.

Although in previous studies we were usually able to maintain impalements through a number of solution changes, in these experiments re-introduction of lidocaine-free solution usually caused the microelectrode to be dislodged. This observation could have been due to contraction of the intestinal muscularis following washout of lidocaine.

Non-effectiveness of charged lidocaine analogues

Two quaternary amine analogues of lidocaine, QX-222 and QX-314, were used to determine whether lidocaine must permeate the cell membrane to be effective. The pKa of lidocaine is 8.1, so at pH 8.0 about half of the dissolved lidocaine is in the uncharged, lipid-soluble form. In contrast, the analogues are charged in solution and thus only weakly lipid-soluble. In two trials, neither analogue had a measurable effect on I_{sc} after 30 min of exposure to 10 mmol l^{-1}, the highest concentration tested.

Effect of bathing solution K^+ concentration

These experiments determined the effect of lidocaine on the relationship between bathing solution K^+ concentration ($[K^+]_o$) and I_{sc}. Each tissue was presented with stepwise changes in concentration over the series 10, 20, 32, 50 and

Table 1. *Effects of potassium concentrations of equilibration and test solutions on fractional inhibition of* I_{sc} (I/I_0) *by lidocaine*

	$[K^+]_o$ (mmol l^{-1})					Row average
	70	50	32	20	10	
Equilibrated in 70KS; exposed to descending $[K^+]_o$						
I_{sc} (μA cm^{-2})	1096±69	670±96	345±48	237±55	151±52	
I/I_0 (N=3)	0.43±0.03	0.41±0.02	0.45±0.03	0.44±0.01	0.46±0.04	0.44±0.01
Equilibrated in 10KS; exposed to ascending $[K^+]_o$						
I_{sc}	242±17	353±62	408±72	377±55	336±39	
I/I_0 (N=3)	0.57±0.04	0.57±0.05	0.51±0.02	0.53±0.02	0.49±0.01	0.53±0.01

Details of 70KS and 10KS are given in the text.

70 mmol l^{-1} K^+. In three of the six experiments, the tissues were equilibrated in 10KS and presented with ascending $[K^+]_o$; the other three were equilibrated in 70KS and presented with descending $[K^+]_o$. The timing of the experiments was similar to that shown in Fig. 3 of Moffett and Koch (1985), except that in the present experiments percentage recovery was also measured. Briefly, tissues were equilibrated in the initial solution until I_{sc} became stable (usually about 45 min). Then the corresponding lidocaine-containing solution was substituted. After 7.5 min of lidocaine exposure, the original solution was restored for measurement of recovery, which typically required 10–15 min. The bathing solution was then replaced with the next solution in the series, and the sequence of lidocaine exposure and recovery repeated.

The mean values of I_{sc} for each $[K^+]_o$ differed between the two protocols (Table 1), and I/I_0 was consistently lower for the tissues equilibrated at high $[K^+]_o$ than for those equilibrated at low $[K^+]_o$. Nevertheless, within each series the lidocaine-sensitive fraction of the total current was almost constant. The reversibility of the inhibition was independent of the K^+ concentration of the equilibration solution and test solutions. The mean reversibility for the series was 0.72±0.03.

Effect of hypoxia

The effect of decreased O_2 tension on lidocaine inhibition of I_{sc} was measured in 10 experiments in which the tissues were equilibrated in 32KS gassed with 100 % O_2, then switched to 32KS gassed with air (21 % O_2) and then to 32KS gassed with 5 % O_2. A 7.5 min exposure to 5 mmol l^{-1} lidocaine was interposed during each of the three O_2 tensions.

In contrast to the constancy of I/I_0 when I_{sc} was altered by changing $[K^+]_o$, reduction of O_2 tension greatly enhanced the inhibitory effect of lidocaine (Table 2). However, decreased O_2 tension reduced the reversibility of the

Table 2. *Effect of O_2 tension on I_{sc} and lidocaine inhibition*

	Oxygen tension (%)		
	100	21	5
I_{sc} ($\mu A\,cm^{-2}$)	702±49	217±30	35±10
I/I_0 ($N=10$)	0.58±0.03	0.33±0.04	0.15±0.06

inhibition. In 21 % O_2 the recovery was only 0.60±0.03 as compared to 0.73 in 100 % O_2, and currents were so low in the 5 % O_2 experiments that percentage recovery was not computed.

Non-dependence on alkaline earth ions

The experiments reported above on the dose–response relationship, the effect of varying $[K^+]_o$ and the effect of low O_2 tension were also carried out in nominally divalent-cation-free solutions. As previously reported (Moffett and Koch, 1985), initial values of I_{sc} were higher in these solutions; the mean I_{sc} in 32K was 543± $\mu A\,cm^{-2}$ for four experiments compared to the combined mean of 376 $\mu A\,cm^{-2}$ for the six experiments in the present report (Table 1). The relationship between I_{sc} and $[K^+]_o$ became quasi-saturating, as expected from previous studies (Moffett and Koch, 1985). The dose–response curve for lidocaine in divalent-cation-free solutions was similar to that in standard solution: on average, 2.5 mmol l^{-1} lidocaine inhibited 19 % of the current while 5 mmol l^{-1} lidocaine inhibited 44 %. As in standard solutions, I/I_0 for 5 mmol l^{-1} lidocaine was constant when $[K^+]_o$ was varied: the overall mean for two experiments using ascending $[K^+]_o$ was 0.68±0.02 and for two with descending $[K^+]_o$ it was 0.45±5.1. Enhancement of lidocaine inhibition by decreased O_2 tension in divalent-cation-free solution was similar to that observed in standard solutions; in two experiments, I/I_0 in 5 mmol l^{-1} lidocaine was 0.67±0.03 in 100 % O_2 but 0.06±0.02 in 5 % O_2.

Combined effects of Ba^{2+} and lidocaine

The extent of competition between Ba^{2+} and lidocaine for inhibition of I_{sc} was determined by comparing the I/I_0 of each inhibitor given singly to the I/I_0 of that inhibitor in the presence of the other. In nine of eighteen experiments 6 mmol l^{-1} Ba^{2+} was given first, followed by 5 mmol l^{-1} lidocaine; in the other nine experiments the order of inhibitors was reversed. Fig. 6 shows the average results in the form of two diagrams of changes in I/I_0, one for experiments in which Ba^{2+} was given first and one for those in which lidocaine was given first. When given first, Ba^{2+} inhibited 57 % of the current (I/I_0 was 0.43), but when given in the presence of lidocaine, Ba^{2+} inhibited 69 % of the remaining current. Similarly, when given first, lidocaine inhibited 44 % of the current, but it inhibited 68 % of the current remaining after Ba^{2+} blockade.

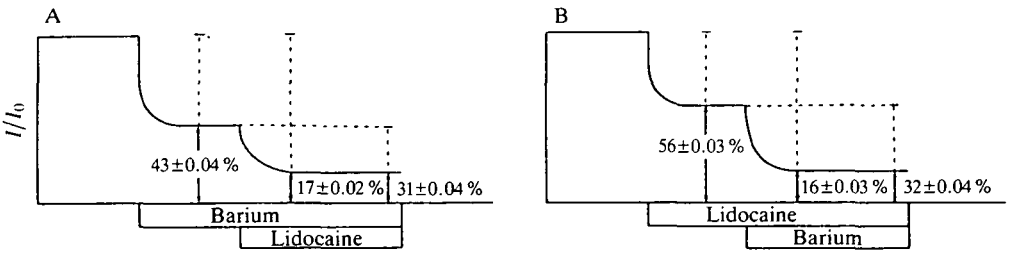

Fig. 6. Diagrams showing the interaction between Ba^{2+} and lidocaine effects on short-circuit current (I_{sc}). (A) Ba^{2+} leaves 43 % of I_{sc} when applied first. Subsequent exposure to lidocaine leaves 17 % of I_{sc}, so the lidocaine left only 31 % of the I_{sc} remaining after Ba^{2+} inhibition. (B) Lidocaine leaves 56 % of I_{sc} when applied first. Subsequent exposure to Ba^{2+} leaves 16 % of I_{sc}, so the Ba^{2+} left only 32 % of the I_{sc} remaining after lidocaine inhibition.

Discussion

Previous studies suggested that the transbasal electrical potential of midgut consists of at least two components; a Nernstian component generated mainly by the transmembrane gradient of K^+, and a contribution due to the current of net K^+ entry through conductive pathways (Chao *et al.* 1990). Cation channels in the basal membrane detected by patch-clamp (Moffett and Lewis, 1990) and noise analysis (Zeiske *et al.* 1986) are one possible route of conductive K^+ entry. These would be expected to mediate passive K^+ entry in tissues bathed in well-oxygenated solution containing normal to high K^+ concentrations, conditions under which the transbasal K^+ electrochemical gradient favors K^+ entry (Moffett and Koch, 1988*a*; Chao *et al.* 1990). An active component of K^+ uptake was inferred from observations that a short-circuit current may be maintained under low extracellular [K^+] and/or hypoxia, while under these conditions the electrochemical gradient is unfavorable for K^+ entry (Chao *et al.* 1990). If it were electrogenic, an active pathway would also contribute to the entry-related component of the transbasal potential.

As previously reported (Moffett and Koch, 1988*a*), hemolymphal Ba^{2+} hyperpolarizes the basal membrane and increases transbasal resistance. In view of the reputation of Ba^{2+} as a competitive inhibitor of K^+ channels, this effect was interpreted as the consequence of blockage of one route of K^+ entry, with diversion of some K^+ entry current to a second conductive pathway (Moffett and Koch, 1985). According to this interpretation, the increase in resistance to the K^+ entry current caused by blockage of the Ba^{2+}-sensitive pathway increases the magnitude of the entry-related component of V_b, hyperpolarizing the basal membrane. The decrease in I_{sc} that occurs at low and normal K^+ concentrations can be regarded as the result of the increased transapical potential against which the apical pump must work (Moffett and Koch, 1988*a*). In contrast, direct inhibition of the apical pump by hypoxia decreases the entry-related voltage

component, depolarizing the basal membrane (Moffett and Koch, 1988*a*; Chao *et al.* 1990).

The present studies show that the effects of lidocaine on V_b and R_b are similar to those of Ba^{2+}, arguing that both inhibitors affect the entry-related component of V_b. The fractional inhibition of either inhibitor, defined in terms of the current remaining after the first inhibitor has taken effect, is increased in the presence of the other (Fig. 6). This is evidence against competition between the two inhibitors, for such competition would have decreased the fractional inhibition of lidocaine after Ba^{2+}, rather than increasing it. However, the absolute decrease in current due to either of the inhibitors is less when it is added second than when it is added first (Fig. 6). The decrease in the amount of current abolished by either inhibitor when it is second in order of addition is consistent with an overlap between the two inhibitors of about 18 % of the total transbasal current. That is to say, if the basal membrane contained a number of channels all of equal conductance, about 18 % of the channels would be susceptible to blockage by either inhibitor. The 17 % of I_{sc} that remains in the presence of both inhibitors may be carried by an uptake pathway insensitive to either inhibitor, but could well be due to the fact that, because of its low solubility in 32KS solution, the concentration of lidocaine was suboptimal.

The concentrations of lidocaine used in these studies (2.5–5 mmol l^{-1}) are an order of magnitude higher than the range of about 200–800 μmol l^{-1} in which effects on Na^+ and K^+ channels are detectable and in which the drug is effective as a local anesthetic. The possibility that such a high dose might still be suboptimal could be explained by the likelihood that in this system, as in others, the binding of lidocaine to its active site is reversible. To achieve maximal inhibition of I_{sc}, the inhibitor must be concentrated enough to block the susceptible channels essentially all the time. In contrast, blockage of neuronal conduction requires only that some critical fraction of the voltage-gated channels be blocked at any instant.

The relationship between I_{sc} and $[K^+]_o$ has been analyzed as the sum of a saturating and a linear component (Moffett and Koch, 1983, 1985). These components are believed to reflect different concentration dependences of parallel transbasal uptake processes. According to this analysis, under standard conditions about two-thirds of the transported K^+ enters the cells through the saturating component. The relative contribution of the saturating component to total K^+ uptake is greatest at low $[K^+]_o$ and decreases as $[K^+]_o$ increases.

The control values of I_{sc} measured in the experiments in which a series of $[K^+]_o$ was presented (Table 1) are not comparable to results obtained in our previous studies of the K^+ concentration dependence of I_{sc} (Moffett and Koch, 1985), which used a similar protocol. The difference is assumed to reflect the accumulation of irreversible lidocaine block over the course of the repeated exposures to lidocaine in the present experiments, making control values of I_{sc} measured later in each experiment progressively lower than would be expected from the previous studies. Nevertheless, the fractional inhibition of the remaining I_{sc} by lidocaine was remarkably constant over the range of extracellular K^+ concentrations,

regardless of whether the concentrations were presented in ascending or descending order (Table 1). This result indicates that, unlike the situation for the divalent-cation-sensitive uptake process, the relative contribution of the lidocaine-sensitive uptake process to total uptake is independent of $[K^+]_o$.

The values of I/I_0 for the ascending series of $[K^+]_o$ are significantly greater than those of the descending series (Table 1). Although no definite conclusion can be drawn, the difference could reflect an effect of the equilibration $[K^+]_o$ on intracellular $[K^+]$. Previous studies gave values of about 70 mmol l^{-1} for tissues superfused in 10KS, but greater than 100 mmol l^{-1} for tissues superfused in 70KS (Moffett *et al.* 1982). Although any difference in the cytoplasmic K^+ concentration ($[K^+]_i$) of the tissues at the start of the two experiments undoubtedly diminished as the experiments progressed, the greater degree of inhibition occurred in the descending concentration series in which tissues could be expected to have higher $[K^+]_i$ values. The result implies that the fraction of total uptake that is lidocaine-inhibitable is more sensitive to $[K^+]_i$ than to $[K^+]_o$. Although higher $[K^+]_i$ is associated with more negative values of V_b, voltage-sensitivity of lidocaine block can be ruled out because I/I_0 is constant over a range of $[K^+]_o$ previously shown to result in large changes in V_b (Moffett and Koch, 1988*a*).

Deletion of Ca^{2+} and Mg^{2+} from bathing solution favors the saturating component of K^+ uptake, while addition of Ba^{2+} favors the linear component at the expense of the saturating component, particularly if the bathing solutions are also Ca^{2+}- and Mg^{2+}-free (Moffett and Koch, 1983, 1985). In Ca^{2+},Mg^{2+}-free solution, Ba^{2+} stimulates I_{sc} when $[K^+]_o$ is 50 mmol l^{-1} or higher. Lidocaine inhibition, unlike that of Ba^{2+}, was not affected by removal of Ca^{2+} and Mg^{2+}, and lidocaine inhibited I_{sc} at high as well as normal and low $[K^+]_o$. The insensitivity of the lidocaine effect to $[K^+]_o$ and to deletion of alkaline earth ions is additional evidence that lidocaine affects a component of transbasal K^+ uptake insensitive both to Ba^{2+} and to the presence or absence of Ca^{2+} and Mg^{2+}, unlike the pathway identified by Moffett and Koch (1985).

Both Ba^{2+} and lidocaine are regarded as blockers of membrane channels. Such blockers of passive movement would be expected to inhibit I_{sc} only under conditions in which the transbasal K^+ electrochemical gradient favors K^+ entry, and to have no (or perhaps a stimulatory) effect when the transbasal gradient is unfavorable for K^+ entry. In 32KS gassed with 5% O_2, the transbasal K^+ electrochemical gradient rapidly becomes unfavorable for K^+ entry. Under these conditions the inhibitory effect of Ba^{2+} actually increased. This result was taken to suggest that the entry process inhibited by Ba^{2+} is thermodynamically active (Chao *et al.* 1990). The similar increase in effectiveness of lidocaine in hypoxia found in the present studies suggests that lidocaine, like Ba^{2+}, inhibits a thermodynamically active K^+ uptake process.

Quaternary analogues of lidocaine are effective when applied to the cytoplasmic side of axons but not when applied to the outside (Frazier *et al.* 1970). The failure of extracellular quaternary analogues to inhibit the I_{sc} of midgut suggests that lidocaine must dissolve in the membrane lipid to reach its site of action. Lidocaine

competes favorably with K^+ for detection by resin-based K^+-selective microelectrodes, and was detected in the cytoplasm with such microelectrodes after application to the hemolymphal side (K. F. Moffett and A. Koch, unpublished observations). However, the block of I_{sc} probably does not come primarily from free intracellular lidocaine, since the drug is markedly more effective when applied to the hemolymphal side of the tissue, although lidocaine would be expected to enter the cytoplasm with equal ease from either side of the tissue. Thus, lidocaine may reach its blocking site from within the lipid component of the basal cell membrane, as has been suggested for lidocaine block of Na^+ channels in frog muscle (Schwarz *et al.* 1977).

The effects of lidocaine on transepithelial K^+ transport described here add to the evidence that K^+ enters the intracellular transport pool of posterior midgut by multiple pathways, rather than by a single readily characterizable mechanism. The substantial effect on I_{sc} of partial inhibition of K^+ uptake by Ba^{2+} and lidocaine suggests that regulation of the uptake pathways could be a potent mechanism for physiological control of transport of K^+.

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