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Effects of lidocaine on transport properties of renal brush-border membranes

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The effect of lidocaine was examined in membrane vesicles from rabbit renal brush borders. Changes in the ionic permeability and the kinetics of Na^+-dependent metabolite transport were observed at different concentrations of anesthetic. Lidocaine was found to alter the membrane permeability of all inorganic cations examined (Li^+, Rb^+, K^+, and Na^+). At low lidocaine concentrations, there was a saturable decrease in permeability, whereas at higher concentrations (> 0.2 mM) there was a non-saturable general increase in cation permeability. Lidocaine (1.0 mM) inhibited Na^+-coupled transport of all ten substrates examined (sugars, amino acids and Krebs cycle intermediates). The affinity for the substrate decreased in the presence of the anesthetic.

Introduction

Despite their widespread use, anesthetics act on membranes by mechanisms which are poorly understood. Local anesthetics are characterized by their ability to block the conduction of electrical impulses in excitable membranes, presumably by affecting Na^+ channels. The one striking difference between these anesthetics and other drugs which act at cell membranes is the relatively high concentration required for anesthetic activity [1,2]. Furthermore, unlike many site-specific drugs where particular chemical structures are required, anesthetic activity depends largely on lipid solubility [3]. While lipid solubility is known to be a major factor, the role of specific protein interactions is not clear. The non-specificity of anesthetic action is supported by the observation that pressure has an antagonistic effect on anesthesia [4,5], and that most anesthetics are active at a pH which maximizes the concentration of lipid-soluble species [6,7]. However, other studies suggest that the effects of pH may reflect the drug's ease of insertion into the lipid environment rather than its blocking activity [8].

This study examines the effects of local anesthetic on a non-excitable biological membrane, renal brush-border membrane vesicles using the potential sensitive dye 3,3′-dipropylthiadicarbocyanine iodide (diS-C_3-(5)) [9]. This method offers the ability to observe changes in ionic permeability and Na^+-dependent cotransport systems [10,11]. Through observing the kinetics of these systems in the presence of anesthetic, information can be obtained concerning the specificity of drug–membrane protein interaction.

Abbreviations: diS-C_3-(5), 3,3-dipropylthiadicarbocyanine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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Materials and Methods

Preparation of purified plasma membrane vesicles. Purified rabbit kidney brush-border membrane vesicles were prepared using the Ca^{2+} precipitation and centrifugation method [12]. Vesicles were suspended in one of three buffers containing 10 mM potassium gluconate, 10 mM sodium gluconate or 10 mM choline chloride with 90 mM choline gluconate, 100 mM mannitol and 10 mM Tris-Hepes (pH 7.5). Membranes were stored at a protein concentration of 10–15 mg/ml under liquid nitrogen until used [13]. These membranes demonstrate multiple electrogenic Na^+-coupled transport systems as well as membrane permeabilities that are inhibited by channel blocking agents [14]. External solutions were made 100 mM in the ionic salt of interest and buffered to pH 7.5 with 10 mM Tris-Hepes. All solutions including the purified membrane solution were made iso-osmotic at 310 mosmol $\cdot l^{-1}$ using the appropriate concentration of mannitol. The pH and osmolarity of solutions were monitored using an Orion Model 801 pH meter and a Westcor 5100 C vapor pressure osmometer respectively.

Measurement of membrane potential using the voltage sensitive dye diS-C_3-(5). The electrical potential across the plasma membrane was determined using the fluorescent dye diS-C_3-(5) as previously described [10,11,15]. For each potential measurement, vesicles (0.3–0.8 mg protein) were added to 2.5 ml of external buffer solution containing 1.5 μM diS-C_3-(5). All experiments were carried out at 23°C in polystyrene cuvettes. The voltage response of the dye was calibrated by measuring the K^+ diffusion potentials in the presence of valinomycin, and bi-ionic diffusion potentials were obtained as previously described [10]. All potential measurements were derived from fluorescence measurements made by exciting at 620 nm and recording the emission at 669 nm.

Ionic permeability measurements in purified membrane vesicles. Ionic permeability measurements were made by placing vesicles in various external buffered solutions with the ion of interest substituted in place of choline. Membrane potentials were calculated from the observed fluorescence. Ionic permeabilities were calculated from the membrane potential and the concentration of both internal and external ions using the constant field equation [10]. The effects of local anesthetics on ion permeability were then calculated from the resulting change in fluorescence. The ionic permeabilities of the four cations (Li^+, Na^+, K^+, Rb^+) were examined in the presence of ten different concentrations of lidocaine (0.1 mM–1.0 mM). Each permeability measurement was determined as an average of eight independent observations. The means of the eight observations were found to have a standard error of $\pm 3\%$ of the mean at lidocaine concentrations 0.1 mM–1.0 mM (Fig. 1). A multivariable non-linear regression analysis was performed using a program run on a NorthStar computer (NorthStar, Inc., Berkeley, CA). This program applies a modification of the Gauss-Newton method of non-linear regression used by Duggleby [16].

Measurement of Na^+-coupled transport systems. The transport of amino acids, glucose and metabolic intermediates were measured as a change in potential generated by the electrogenic cotransport of Na^+. The effects of anesthetic on these cotransport systems were observed through changes in membrane potential. The kinetic parameters ΔF_{max} and K_f, i.e., the maximum increase in fluorescence and the concentration of substrate producing $0.5\Delta F_{max}$, were calculated at various concentrations of lidocaine.

The effects of anesthetic on the Na^+-coupled cotransport systems were examined by analysing the apparent Michaelis-Menten kinetic parameters ΔF_{max} and K_f. These values were calculated by using eight different substrate concentrations and observing the resulting change in fluorescence [11]. For each substrate concentration three triplicate observations were made. Substrate concentrations were chosen such that they were well distributed both above and below the K_f. The parameters ΔF_{max} and K_f were calculated using an iterative non-linear regression program similar to that used for ionic permeabilities. Kinetic parameters and estimates of standard errors were calculated using this program directly from fluorescent measurements as a function of substrate concentration.

Results

Effect of lidocaine on ionic permeability rabbit renal brush border membrane vesicles

Ionic permeabilities were measured in the presence of lidocaine at concentrations ranging from 0.1 mM to 3.0 mM. The effect of the local anesthetic on the permeabilities of Li^+, Rb^+, K^+, and Na^+ is shown in Fig. 1A. In the absence of lidocaine, the ionic permeabilities relative to choline (Cho^+) were Li^+ $(35 \pm 1) > Rb^+$ (20 ± 1) $= K^+$ $(20 \pm 1) > Na^+$ $(6.6 \pm 0.4) > Cho^+$ (1.0 ± 0.3). These relative values are similar to those reported elsewhere for renal brush borders using voltage-sensitive dyes [14,17,18]. Furthermore, estimates of absolute ion permeabilities are comparable with those obtained by radioactive tracer measurements, e.g., P_{Br}, which is about 5-times more permeable than P_K, is estimated to be approx. $1 \cdot 10^{-8}$ cm $\cdot s^{-1}$ [18]. Preliminary experiments revealed that 1–3 mM lidocaine had no effect on the permeability of the brush-border membrane to choline or gluconate. The experimental approach was to measure the time course of the dissipation of potassium gradients in the presence of valinomycin (Ref. 10, Fig. 1; Ref. 17, Fig. 2; Ref. 18, Fig. 7). Under these conditions, the dissipation of the K^+ gradient is a function of the gluconate and choline permeabilities. For example, with 100 mM potassium gluconate outside and 10 mM potassium gluconate + 90 mM choline gluconate, the potential declined by 2.5 mV in 60 s; whereas with external KBr, the p.d. declined by 25 mV in 60 s. Lidocaine (3 mM) had no effect on the dissipation of K^+ gradients in choline gluconate solutions (-2.0 ± 0.5 vs. 2.4 ± 0.4 mV in 60 s in four experiments. All subsequent experiments to test the effect of lidocaine on cation permeability were carried out in choline gluconate solutions.

Fig. 1A shows that for the four cations examined (Li^+, Rb^+, K^+, Na^+) there was a significant change in ion permeability which was dependent on the concentration of lidocaine present. At low concentrations of lidocaine (0.2 mM), the relative ionic permeabilities were found to be Li^+ $(34 \pm 1) > Rb^+$ $(18 \pm 0.5) = K^+$ $(17 \pm 0.5) > Na^+$ $(4.4 \pm 0.3) > Cho^+$ (1.0 ± 0.4). For Rb^+, K^+, and Na^+, there was a significant decrease in ionic permeability at low (0.2 mM) concentrations of

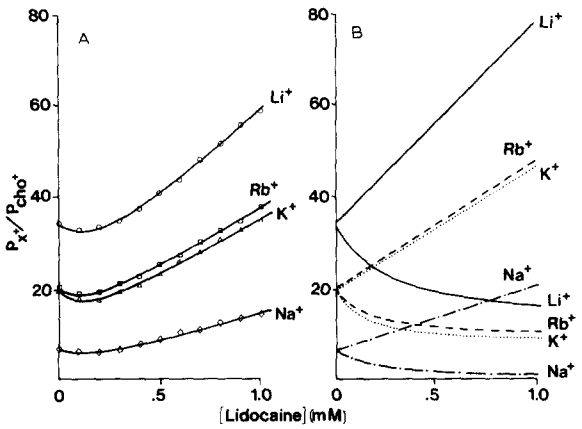

Fig. 1. (A) The effect of lidocaine on the ionic permeability of rabbit renal brush-border membrane vesicles. Membranes were loaded with 10 mM sodium gluconate, 90 mM choline gluconate, 100 mM mannitol and 10 mM Tris-Hepes (pH 7.5). The external buffers contained 100 mM sodium gluconate (Na^+), potassium gluconate (K^+), rubidium gluconate (Rb^+) or lithium gluconate (Li^+), 100 mM mannitol and 10 mM Tris-Hepes (pH 7.5). The fluorescence signal was obtained for each ion at 11 different lidocaine concentrations at 100 μM intervals from 0 to 1 mM. Each reported ionic permeability represents the average of eight separate observations. The curve represents a three-variable least squares non-linear regression analysis to the expression:

$$P_L = P - [(P_{max} \cdot L)/(K_i + L)] + [C \cdot L]$$

where P is the ionic permeability relative to Cho^+ in the absence of lidocaine.

(B) Regression analysis of lidocaine effect on ionic permeability. Using the data of part (A), the two effects of lidocaine are isolated mathematically for each ion from the above expression as given in Eqns. 2 and 3. The regression analysis of ion permeability vs. L gave the following:

Ion	P	C	P_{max}	K_i (mM)
Na^+	6.6 ± 0.4	1.4 ± 0.2	7.4 ± 0.4	0.23 ± 0.03 mM
K^+	20.0 ± 1	2.6 ± 0.4	12.4 ± 0.3	0.15 ± 0.02 mM
Rb^+	20.0 ± 1	2.8 ± 0.4	13.2 ± 0.2	0.17 ± 0.01 mM
Li^+	35.0 ± 1	4.4 ± 0.6	24.2 ± 0.6	0.29 ± 0.02 mM

lidocaine, compared to the observed ionic permeabilities in the absence of anesthetic as demonstrated using a t-test ($P < 0.05$) with sample sizes $n = 8$ (Fig. 1). At higher concentrations of lidocaine (> 0.2 mM), the permeabilities of all inorganic cations were found to increase linearly. At 3.0 mM lidocaine, the relative permeability of these cations increased 8-fold over what they were in the ab-

sence of anesthetic. Eleven concentrations of lidocaine were examined at 100 μM intervals from 0 to 1.0 mM. From this data, it was observed that the resultant changes in the relative ionic permeabilities was the sum of two independent responses: a saturable high-affinity, low-capacity inhibition, and a non-saturable low-affinity activation. This response could be modeled using a three variable least squares non-linear regression analysis using the expression:

$$P_L = P - [(P_{max} \cdot L)/(K_i + L)] + [C \cdot L] \quad (1)$$

where P_L and P are the ionic permeabilities of X^+ relative to choline in the presence and absence of lidocaine, respectively. P_{max} is the maximal inhibition of relative permeability, K_i is the concentration of lidocaine required to inhibit ion permeability 50%, L is the concentration of lidocaine and C is the coefficient of the linear increase in membrane permeability. The resultant kinetic constants P_{max} and K_i from this analysis are given in the legend for Fig. 1.

Fig. 1B shows the plots of the ionic permeabilities that are derived from Eqn. 1 when the two independent portions of the equation are plotted separately. The results are the expressions 2 and 3.

$$P_L = P - [(P_{max} \cdot L)/(K_i + L)] \quad (2)$$

$$P_L = P + [C \cdot L] \quad (3)$$

where Eqn. 2 is the saturable inhibition of ionic permeability and Eqn. 3 represents a non-specific linear increase in ion permeability which is proportional to the concentration of anesthetic.

Effect of lidocaine on Na^+-dependent co-transport systems

Purified renal brush-border membrane vesicles demonstrate Na^+-dependent electrogenic transport systems for the uptake of sugars, amino acids, and metabolic intermediates. In the presence of 100 mM Na^+, all ten substrates significantly increased (depolarized) the membrane potential; while in the absence of Na^+, no detectable change could be observed. While the ten transported substrates caused a depolarization of the membrane, the non-transported substrates L-glucose and D-phenylalanine showed no effect on membrane

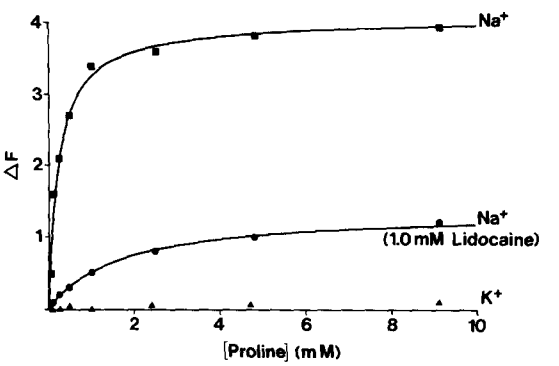

Fig. 2. The effect of lidocaine on the kinetics of Na^+-coupled proline transport in renal brush-border membrane vesicles. In each experiment 0.2 mg of protein was added to 2.5 ml of buffer containing 10 mM sodium gluconate (or 100 mM potassium gluconate), 0.0 or 1.0 mM lidocaine, 10 mM Tris-Hepes (pH 7.5), 1.5 μM diS-C_3-(5) and sufficient mannitol to make the buffer iso-osmotic with the internal loading buffer. Vesicles were loaded with 10 mM sodium gluconate, 90 mM choline gluconate, 100 mM mannitol and 10 mM Tris-Hepes (pH 7.5). The fluorescence signal was obtained by activating at 620 nm and recording the emission at 669 nm. 1.0 mM lidocaine inhibited the proline-induced change in fluorescence by significantly increasing the K_m and decreasing the maximal signal ΔF_{max}.

potential in the presence of Na^+ or K^+. As an example, Fig. 2 illustrates that in the presence of Na^+, proline causes a concentration-dependent change in the optical response of the voltage-sensitive dye, while in the absence of Na^+ (100 mM K^+), no detectable change was observed. The results of similar experiments for all ten substrates are summarized in Table I.

Five concentrations of lidocaine were used to examine the effect of local anesthetic on the Na^+-dependent glucose cotransport. The results of these experiments are illustrated in Figs. 2 and 3. At each lidocaine concentration there was a linear relationship between ΔF and $\Delta F/$[glucose]. Lidocaine at all concentrations examined (0.25–2.0 mM) changed both the K_f and ΔF_{max} of Na^+/glucose cotransport. In Fig. 4A, the K_f for glucose rises exponentially with increasing lidocaine concentration up to 2.0 mM. Over this range the K_f rose from 0.26 ± 0.03 in the absence of drug to 40.0 ± 11.0 in the presence of 2.0 mM lidocaine. The ΔF_{max} decreased from 6.2 ± 0.2 in the absence of lidocaine to 0.15 ± 0.2 at 2.0 mM lidocaine. Fig. 4B shows the relationship of both ΔF_{max} and

TABLE I

THE EFFECT OF LIDOCAINE ON Na^+-DEPENDENT COTRANSPORT SYSTEMS

The kinetic parameters K_f and ΔF_{max} were determined from the observed change in fluorescence upon the addition of substrate in the presence and absence of 1.0 mM lidocaine. Vesicles were loaded with 10 mM sodium gluconate, 90 mM choline gluconate, 100 mM mannitol and 10 mM Tris-Hepes (pH 7.5). The external buffer contained 100 mM sodium gluconate, 0.0 or 1.0 mM lidocaine, 10 mM Tris-Hepes (pH 7.5), 1.5 mM diS-C_3-(5), and sufficient mannitol to make the buffer iso-osmotic with the internal loading buffer. Six to eight substrate concentrations were chosen from 50 μM to 9.1 mM. The observed change in fluorescence at each substrate concentration was measured in triplicate. A least squares non-linear computer analysis demonstrates that lidocaine alters both the K_f and ΔF_{max}. Values in parentheses are the ratio of the kinetic parameters K_f and ΔF_{max} in the presence and absence of 1.0 mM lidocaine.

	K_f		ΔF_{max}	
	control	1.0 mM lidocaine	control	1.0 mM lidocaine
D-Glucose	0.26 ± 0.03	1.4 ± 0.2 (5.4)	6.3 ± 0.2	2.3 ± 0.1 (0.37)
L-Proline	0.24 ± 0.04	1.7 ± 0.2 (7.1)	4.0 ± 0.2	1.4 ± 0.1 (0.35)
L-Serine	0.23 ± 0.1	6.0 ± 0.5 (2.6)	14.6 ± 0.3	5.0 ± 0.3 (0.34)
L-Threonine	3.2 ± 0.6	4.6 ± 0.3 (1.4)	13.2 ± 1.3	5.0 ± 0.2 (0.38)
L-Phenyl-alanine	0.76 ± 0.09	2.5 ± 0.3 (3.3)	10.1 ± 0.5	3.9 ± 0.2 (0.39)
L-Alanine	3.0 ± 0.2	8.6 ± 3.0 (2.9)	7.0 ± 0.2	2.7 ± 0.6 (0.39)
L-Valine	0.67 ± 0.03	4.4 ± 0.8 (6.6)	7.0 ± 0.1	2.2 ± 0.2 (0.31)
L-Isoleucine	0.60 ± 0.03	3.9 ± 0.4 (6.5)	5.7 ± 0.1	1.6 ± 0.8 (0.28)
Citrate	0.21 ± 0.01	0.9 ± 0.1 (4.3)	6.8 ± 0.2	1.5 ± 0.2 (0.22)
Succinate	0.25 ± 0.01	1.2 ± 0.1 (4.8)	5.1 ± 0.1	1.8 ± 0.1 (0.35)

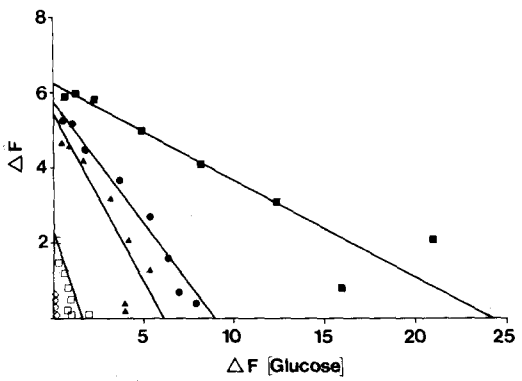

Fig. 3. The effect of lidocaine on the kinetics of Na^+-coupled glucose transport in renal brush-border membrane vesicles. In each experiment, 0.40 mg of protein was added to 2.5 ml of buffer containing 100 mM sodium gluconate, 10 mM Tris-Hepes (pH 7.5), 1.5 μM DiS-C_3-(5) and sufficient mannitol to make the buffer iso-osmotic with the internal loading buffer. Vesicles were loaded with 10 mM sodium gluconate, 90 mM choline gluconate, 100 mM mannitol and 10 mM Tris-Hepes (pH 7.5). The fluorescence signal was obtained by exciting at 620 nm and recording the emission at 669 nm. Eight glucose concentrations varied from 50 μM to 9.1 mM. Lidocaine concentrations were 0.0 mM (■), 0.25 mM (●), 0.50 mM (▲), 1.0 mM (□), 1.5 mM (◇). Each point represents the average of four observations. At each glucose concentration, the standard error was less than 10% of the mean. Non-linear regression analysis of data was fit to the expression: $\Delta F = (\Delta F_{max} \cdot (S))/(K_f + (S))$.

Na^+ permeability with increased lidocaine concentration. The ΔF_{max} is inversely proportional to the relative Na^+-permeability over the range of lidocaine concentrations examined (0–2.0 mM).

The kinetics of Na^+-coupled transport was examined for ten different transported substrates in the presence and absence of 1.0 mM lidocaine. In every case, lidocaine decreased both the ΔF_{max} and the affinity for substrate (Table I). The average ΔF_{max} values decreased uniformly to 33% of control values, while the K_f increased 1.4 to 7 times that found in the absence of substrate (average 4.5 ± 1 fold).

Discussion

Renal brush-border membrane vesicles demonstrate an ion selectivity sequence similar to those previously found in intestinal brush-border membranes [10] and choroid plexus [17]. The permeability sequence $Li^+ > Rb^+ = K^+ > Na^+ > Cho^+$ is similar to the Eisenman sequence I with the exception of Li^+ which is more permeable than predicted based on a selective filter with low field strength [19]. Lidocaine alters the permeability of the brush-border membranes in a complex fash-

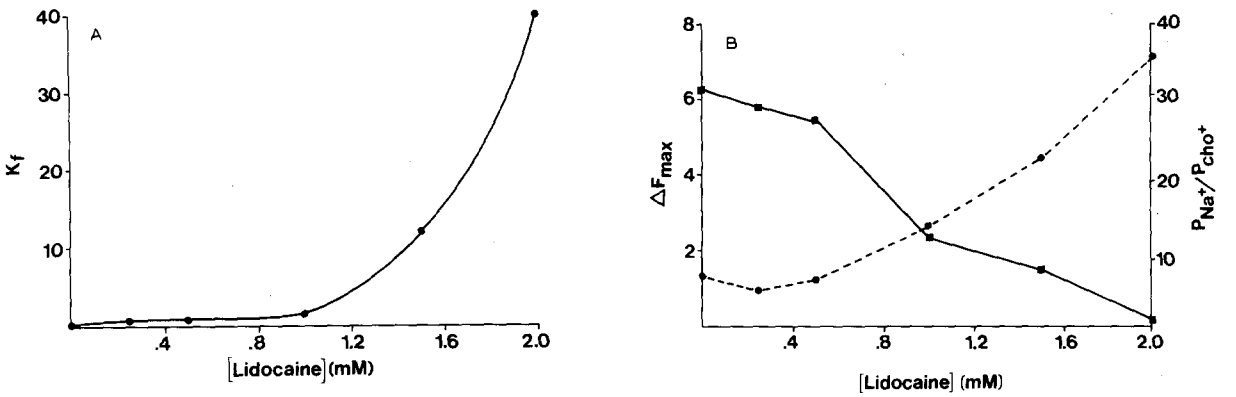

Fig. 4. The effect of lidocaine concentration on the kinetic parameters K_f and ΔF_{max} of Na^+-coupled glucose transport in renal brush-border membranes. Kinetic values were derived using the conditions and observations of Fig. 2. (A) The effect of lidocaine concentration on the observed K_f of glucose transport. The K_f was observed to increase exponentially with increasing concentrations of lidocaine. (B) Effect of lidocaine concentration on the ΔF_{max} of Na^+-dependent glucose transport. Superimposed is the Na^+ permeability in the absence of glucose. The decrease in ΔF_{max} is inversely proportional to the increase in Na^+ permeability as lidocaine concentration is increased. The relationship between lidocaine concentration, Na^+ permeability, the K_f and ΔF_{max} of Na^+-dependent glucose transport was determined.

Lidocaine (mM)	0	0.25	0.5	1.0	1.5	2.0
P_{Na^+}/P_{Cho^+}	6.6 ±0.4	4.8 ±0.6	6.2±0.6	13.6±1	22 ±2	36 ± 4
K_f	0.25±0.03	0.64±0.08	0.9±0.1	1.4±0.2	12.1±3	40 ±11
ΔF_{max}	6.2 ±0.2	5.8 ±0.2	5.5±0.1	2.4±0.1	1.5±0.2	0.15± 0.2

ion. The simplest model is that of the two independent effects contained in Eqn. 1 and demonstrated separately in Eqns. 2 and 3 as graphed in Fig. 1B. At low anesthetic concentrations (< 300 μM), there was a saturable decrease in permeability, presumably through its action on specific membrane proteins such as ion channels. This is supported by the observation that anesthetics block electrical conduction in excitable membranes. In addition, K^+-channel blockers have been shown to inhibit K^+ permeability in renal brush-border membranes at 37°C [14].

Rb^+ and K^+ demonstrate similar ionic permeabilities throughout the range of lidocaine concentrations (0–3 mM), while the Li^+ and Na^+ response is quite different, suggesting that Rb^+ and K^+ cross the membrane through a pathway distinct from that utilized by Li^+ and Na^+. Consistent with this model is the resultant inhibition constants K_i which were found to be 0.17 ± 0.01 mM for Rb^+ and 0.15 ± 0.02 mM for K^+ while those for Li^+ and Na^+ were significantly different, being 0.29 ± 0.02 mM and 0.23 ± 0.03 mM, respectively.

The second effect (Eqn. 3) plays a major role in determining the ion permeability at higher concentrations (> 300 μM) since it increases ion permeability proportionately to the lidocaine concentration. No evidence of saturation was observed for the increase in membrane permeability at high lidocaine concentrations up to 3 mM, and this increase did not appear to be a specific interaction since the membrane permeability of all four inorganic cations changed equally resulting in an 8-fold increase at 3 mM over what they were in the absence of lidocaine. Singer and Jain [20] have shown that while local anesthetics inhibit ion conduction through membrane proteins, their effect on pure lipid membranes is that of increasing ionic permeability non-specifically.

Lidocaine profoundly alters the kinetics of Na^+-dependent glucose transport. Both the affinity for substrate and the apparent maximal rate of transport were decreased in the presence of anesthetic (Fig. 4). The K_f for glucose transport increased, and the ΔF_{max} decreased in the presence of 1 mM lidocaine. Because lidocaine increases the membrane permeability to cations, Na^+ can act to

short circuit the transport induced change in membrane potential resulting in an apparent decrease in the ΔF_{max}. Fig. 4B demonstrates that the ΔF_{max} changes inversely with the change in Na^+ permeability at varying lidocaine concentrations. Therefore, we conclude that the local anesthetic decreases the affinity for substrate in Na^+ glucose transport directly as measured by K_f, while increased Na^+ permeability is, at least in part, responsible for the apparent decrease in ΔF_{max}.

The kinetic parameters K_f and ΔF_{max} for 10 different substrates found in the absence of anesthetic were consistent with those found using both potential sensitive dyes and radioactively labeled substrates [11]. Table I shows that lidocaine significantly altered both the K_f and F_{max} for all ten substrates. 1.0 mM lidocaine increased the K_f an average of 4.5-fold while decreasing the apparent ΔF_{max} 60%. Interestingly, there was a great deal of variation (42%) in the lidocaine induced change in K_f from substrate to substrate. In contrast, the change in ΔF_{max} due to 1.0 mM lidocaine varied little between substrates (14%). This again supports the model that lidocaine acts directly on Na^+-dependent systems to increase the K_f of transport while the decrease in ΔF_{max} is due in part to an increase in membrane permeability to Na^+.

In conclusion, lidocaine altered the ionic permeability of renal brush borders in a dose-dependent manner. One explanation is that lidocaine has the effect of both inhibiting ion permeability through membrane proteins directly and by increasing ionic permeability through the lipid portion of the membrane non-specifically. Na^+-dependent transport of organic substrates was also inhibited by the local anesthetic. While the increase in K_f is believed to be a direct protein interaction, the decrease in the observed ΔF_{max} is partially due to the increased permeability of the membrane to Na^+ resulting in a short circuiting of the transport induced depolarization of the membrane.

Acknowledgments

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