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Effect of Local Anesthetics on Chloride Transport in Erythrocytes

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Summary. The self-exchange of chloride isotopes across the human erythrocyte membrane was inhibited by tetracaine, benzocaine, and lidocaine. The inhibition by tetracaine was increased at higher pH values, but was not exclusively due to the uncharged form of tetracaine. The inhibition was effective within 5 seconds, was reversible, was noncompetitive with chloride ions, and was not reversed by calcium ions. These findings indicate that local anesthetics react with the erythrocyte chloride carrier at a site separate from the chloride site and cause inhibition of anion transport by a specific mechanism not involving changes in the surface charge density on the erythrocyte membrane.

An analysis of the effect of local anesthetics on the transport of chloride across human erythrocytes was carried out to obtain information concerning the role of surface charges in anion transport and the interaction between local anesthetics, and the specific chloride carrier mechanism. Previous investigations have shown that local anesthetics expand the erythrocyte membrane (Seeman, 1966) and reduce both active and passive cation fluxes (Andersen, 1968). They, furthermore, reduce the enhancement of passive sodium fluxes caused by butanol (Burt & Green, 1971) or caused by Ca^{++} in both erythrocytes (Seeman, Kwant, Goldberg & Chau-Wong, 1971) and phosphatidylserine vesicles (Papahadjopoulos, 1970). These findings appear to be in accord with the evidence that the hydrophobic tail of these molecules can become incorporated into cell membranes (Cerbón, 1972) thereby causing expansion, and the polar end of these molecules when positively charged can contribute to the net surface charge. This mechanism can quantitatively explain the effects

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of local anesthetics on the rates of ionophore-mediated cation transport across planar phospholipid bilayers (McLaughlin & Harary, 1975), and can qualitatively explain the reduction of the net negative zeta potential of erythrocytes (Hardy & Shah, 1972).

If these effects of local anesthetics are mediated solely by altering net membrane surface charge density, they would be expected to result in opposite changes in anion and cation transport. The work described in this paper was undertaken to evaluate this hypothesis by measuring the effect of local anesthetics on chloride transport in human red cells (Gunn, Dalmark, Tosteson & Wieth, 1973). The effects of three local anesthetics, tetracaine, lidocaine, and benzocaine, upon the self-exchange flux of chloride across erythrocytes were studied. The influences of pH and Ca^{++} concentration on the action of the anesthetics were also investigated. Deuticke and Gruber (1970) have previously reported that phosphate transport in erythrocytes is inhibited by tetracaine (10^{-3} M).

Materials and Methods

Fresh human red blood cells, drawn by venipuncture into a heparin-coated flask, were washed in 170 mM NaCl, titrated with CO_2 or $NaHCO_3$ to the desired pH at 0 °C, then transferred to the desired medium by repeated resuspension and centrifugation with fresh medium in the cold. The cells were loaded with radioactive chloride-36 at 50% hematocrit by equilibration for more than six half-times of the subsequent efflux, then packed in small nylon tubes as previously described (Gunn *et al.*, 1973). The efflux was initiated by injected packed cells (~200 mg wet cells) into a well-stirred thermostated chamber containing medium (20 ml) identical with that in which the cells had been packed except free from isotope. The efflux was followed by the rapid filtration technique of Dalmark and Wieth (1972). The results are expressed as the unidirectional flux of chloride, M_{Cl} in mEq/(kg cell solids × min). No net chloride flux occurred; therefore, the self-exchange of chloride isotopes was measured.

Solutions and Chemicals

Media. The inhibition by local anesthetics was evaluated in a medium containing (mM): NaCl 145, $MgCl_2$ 1.0, $CaCl_2$ 1.5, glycylglycine 27, D-glucose 5 and titrated with NaOH to the desired pH value. When the concentration of Ca^{++} was varied, both $MgCl_2$ and $CaCl_2$ were deleted from the control medium, and 10^{-3}M ethylenediamine-tetraacetate (EDTA) was added. Additions of $CaCl_2$ were made to this control medium. When the chloride concentration dependence of M_{Cl} in 10^{-3}M tetracaine was studied, the media contained (mM): Na-acetate 141, $Mg(acetate)_2$ 1.0, $Ca(acetate)_2$ 1.5, glycylglycycine 27, D-glucose 5, and different concentrations of NH_4Cl to vary both intra- and extracellular chloride concentration together.

Chemicals. The salts used were reagent grade. Tetracaine-HCl (Pontocaine® hydrochloride, Wintrop Laboratories, New York, N.Y.), tetracaine (Sigma Laboratories, St. Louis,

Mo.), benzocaine (Sigma Laboratories), or lidocaine (courtesy of Dr. G. Rosen, Duke University) were prepared as concentrated stock solutions in water and used in the preparation of the media.

Radioactivity was counted in a liquid scintillation counter (Beckman, LS-30 or Intertechnique, CG30) after pipetting equal aliquots of serial samples of cell-free supernatant into a toluene-ethanol (7:3 v/v) mixture with 3.5 g of PPO (Kodak, Rochester, N.Y.) per liter. The efflux rate coefficient was evaluated from the slope of the semilogarithmic plot of $(1\text{-}a(t)/a(\infty))$ verses time where a (t) was the counts per minute outside at time t, and $a(\infty)$ at equilibrium of the tracer. No correction of this slope was made since the extracellular chloride compartment was always at least 100-fold greater than the intracellular compartment during the efflux (Funder & Wieth, 1967). Cell water and chloride per kg dry cell solids were measured and the flux calculated as previously reported (Gunn *et al.*, 1973).

Results

In all the experiments presented, the outflux of chloride-36 was measured at 0 °C (±0.1 °C) from fresh human red blood cells without the net flux of chloride, hydrogen or hydroxyl ions, or water.

A. *Inhibition of Chloride Self-Exchange Flux By Local Anesthetics*

Two local anesthetics, tetracaine and benzocaine, were shown to be inhibitors of the chloride efflux as shown in Figs. 1 and 2. Fig. 1 shows inhibition by tetracaine with increasing concentrations. With no tetracaine present, the control efflux was about 880 mEq Cl^-/(kg cell solids $\times$ min). In a medium with 10^{-4}M tetracaine, but otherwise the same, the flux was reduced about 8%. With concentrations of 4×10^{-4}, 10^{-3} and 5×10^{-3}M tetracaine, the flux decreased approximately 35, 55 and 85%, respectively. This inhibition was reversible by repeatedly washing the cells in tetracaine-free medium before loading them with tracer and measuring the chloride efflux.

In Fig. 2 the inhibition by benzocaine is shown. Here the control flux was about 860 mEq Cl^-/(kg cell solid $\times$ min). With a concentration of 10^{-4}M benzocaine, there was a noticeable reduction in the flux (about 26%); and this was further reduced in 10^{-3}M benzocaine (66%).

Fig. 3 shows the effects of lidocaine on the chloride self-exchange flux. The control flux was 870 mEq Cl^-/(kg cell solid $\times$ min), and only in the presence of 5×10^{-3}M lidocaine was there significant inhibition (about 14%).

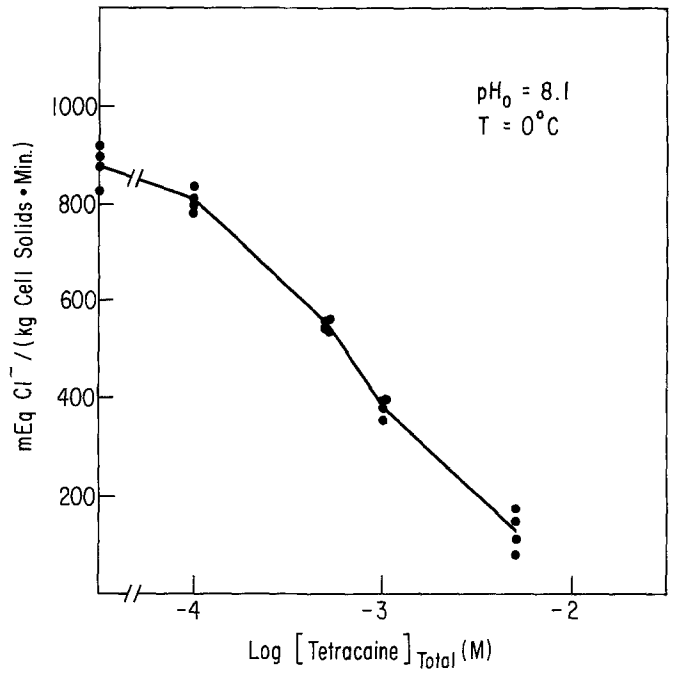

Fig. 1. Inhibition of chloride self-exchange flux by tetracaine. The external chloride was 150 mM, the ratio of internal to external chloride concentration r_{Cl}, was 0.73 in one series and 0.67 to 0.70 in the other series of experimental over the concentration range

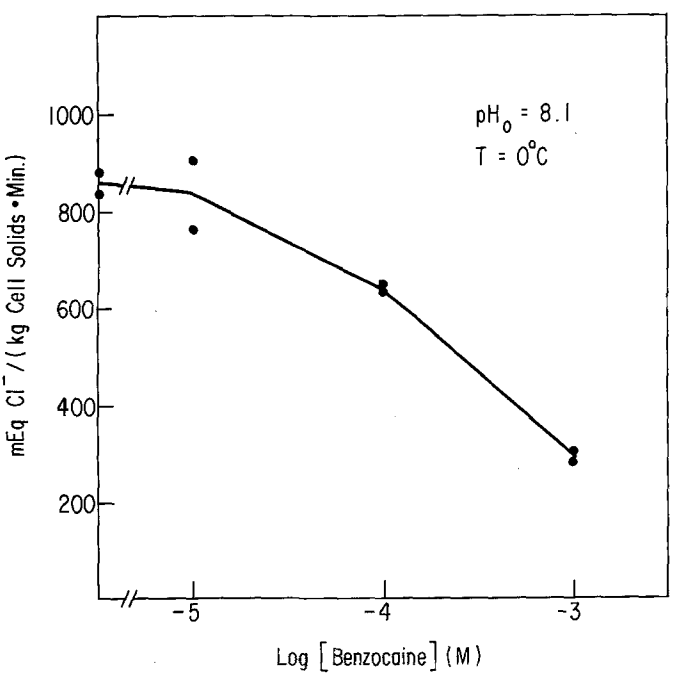

Fig. 2. Inhibition of chloride self-exchange flux by benzocaine. The external chloride concentration was 149 mM; the chloride ratio, r_{Cl}, was 0.74 in these experiments

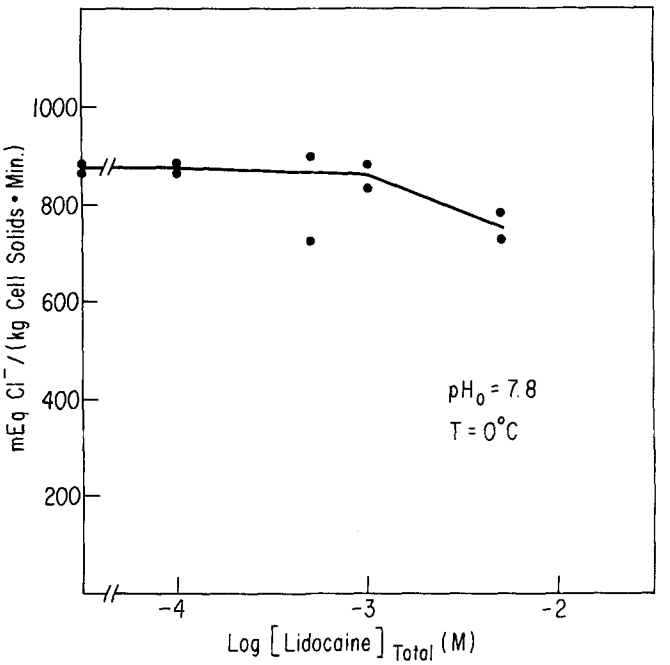

Fig. 3. Inhibition of chloride self-exchange flux by lidocaine. The external chloride concentration was 146 to 153 mM; the chloride ratio, r_{Cl}, was 0.82 to 0.89

B. *Effects of Time of Exposure to Local Anesthetics on Inhibition*

The length of time of exposure to the drug did not seem to affect the inhibition of chloride efflux by tetracaine (10^{-3}M) as can be seen in Fig. 4. In three experiments the cells were not exposed to the local anesthetic until they were injected into the tracer-free medium, and the efflux was initiated with tetracaine present. The fluxes here were shown to be about 590 mEq Cl^-/kg cell solid × min. Erythrocytes prewashed and loaded with isotope in 10^{-3}M tetracaine for between a half-hour and four hours prior to the flux measurement at the same tetracaine concentration showed no further inhibition. Since, in the first instance, the first efflux sample was taken within 5 sec, the full effect of tetracaine on chloride transport was complete within this time under these conditions.

C. *Effects of External Calcium Concentration on Chloride Self-Exchange Flux*

Differing amounts of calcium chloride were used in otherwise similar media to study the effects of external Ca^{2+} on the efflux. The control

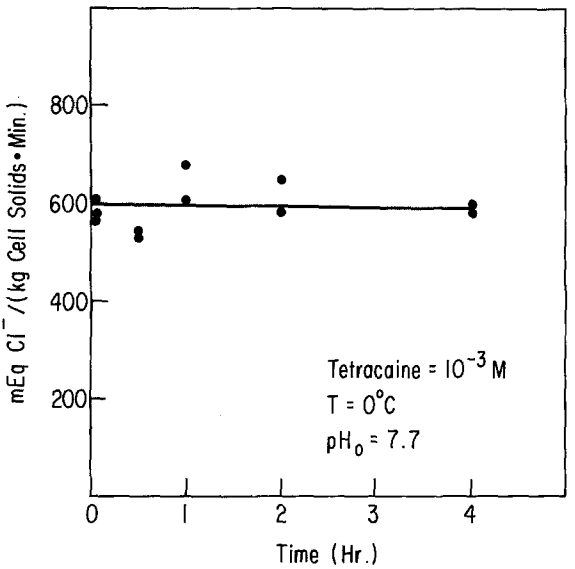

Fig. 4. The self-exchange flux of chloride in erythrocytes after different periods of exposure to tetracaine. Cells exposed to tetracaine for the first time during initiation of the tracer efflux are graphed nearest the ordinate line

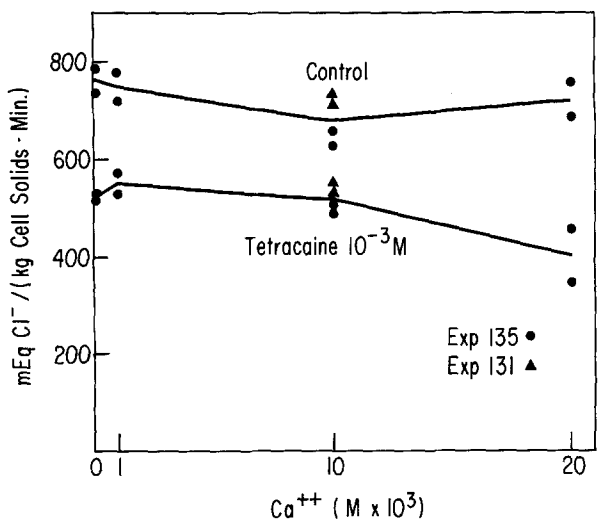

Fig. 5. The chloride self-exchange flux in the presence and absence (control) of tetracaine as a function of total calcium concentration

efflux rate in 10^{-3} M EDTA was approximately 760 mEq Cl^-/kg cell solid × min (Fig. 5). Concentrations of 10^{-3}, and 10^{-2} and 2×10^{-2} M total external Ca^{2+} did not seem to affect the efflux significantly either

in the absence or presence of tetracaine. The slight inhibition observed in 20mM $CaCl_2$ (total chloride: 188 mEq/liter) and no tetracaine may be due to the elevated chloride concentration or ionic strength. Comparable inhibition without local anesthetics has been seen when NH_4Cl was added to the medium (Gunn *et al.*, 1973). Thus, a specific calcium inhibition was probably not observed here in the control cells and no calcium reversal of the tetracaine inhibition was observed.

D. *pH Dependence of Chloride Self-Exchange Flux in the Presence of Local Anesthetics*

The chloride self-exchange flux in the presence of either tetracaine (10^{-3}M) or benzocaine (10^{-3}M) showed a maximum as a function of pH around pH 7.5 (Fig. 6) and the values at pH 8.1 agree with those in Figs. 1 and 2. The shape of the curves relating flux to pH were similar in the presence of either local anesthetic, but the maximum flux was lower (about 390 mEq Cl^-/kg cell solids × min) in benzocaine than in

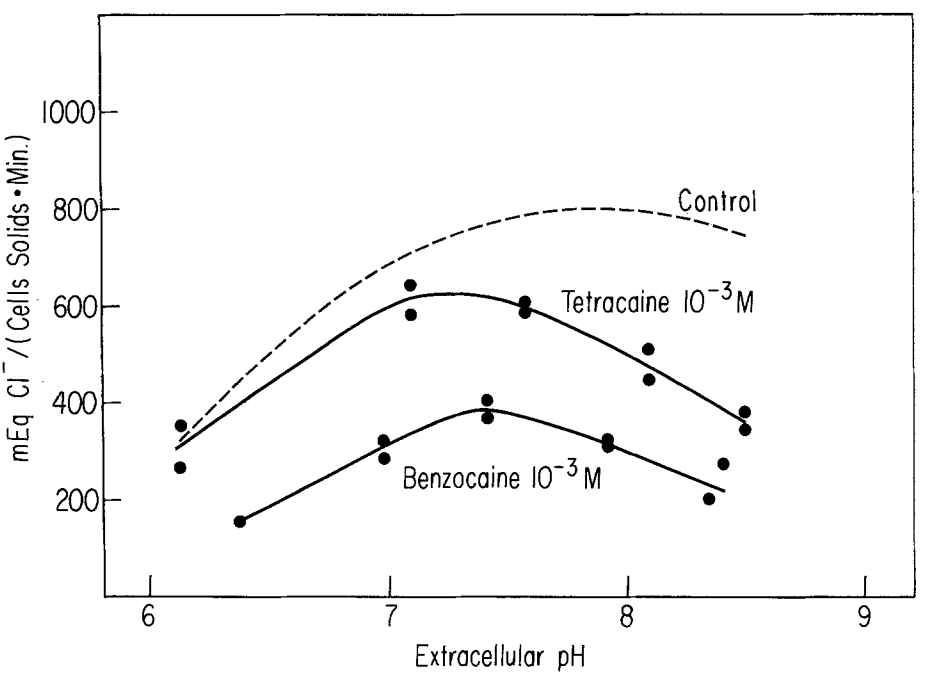

Fig. 6. pH dependence of chloride self-exchange flux in the presence of 10^{-3}M tetracaine or benzocaine

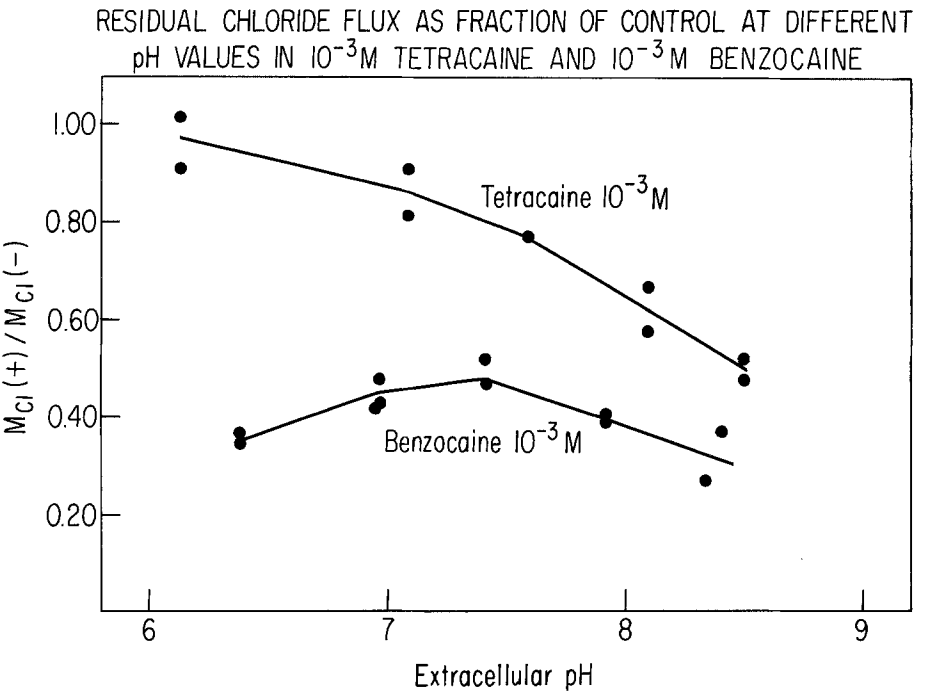

Fig. 7. The ratio of the chloride self-exchange flux in the presence ($M_{Cl}(+)$) of local anesthetic to that in the absence ($M_{Cl}(-)$) of local anesthetic as a function of extracellular pH

tetracaine (about 620 mEq Cl^-/kg cell solids × min) and both were lower than previously observed in the absence of local anesthetics (*see* Gunn *et al.*, 1973).

To demonstrate more clearly the effects of the local anesthetics over this pH range, this data is regraphed in Fig. 7. It shows the fractional residual flux expressed as the ratio of the flux in the presence to that in the absence of local anesthetic plotted as a function of extracellular pH. Between pH 8.6 and 6.1 the extent of tetracaine inhibition decreases with pH while the inhibition by benzocaine remained between 30 and 50%. Benzocaine is uncharged over this pH range while the fraction of ionized tetracaine increases at lower pH values. This pH dependency is compatible with the idea that the uncharged form of the local anesthetic is the most potent inhibitor of chloride self-exchange.

The dependence of chloride self-exchange on the total tetracaine concentration was then measured at two pH values. The control flux at pH 8.1 was 745 with internal chloride concentration/external chloride concentration, $r_{Cl}=0.661$ and at pH 7.2 was 682 with $r_{Cl}=0.944$. The fraction of these control fluxes remaining at different tetracaine con-

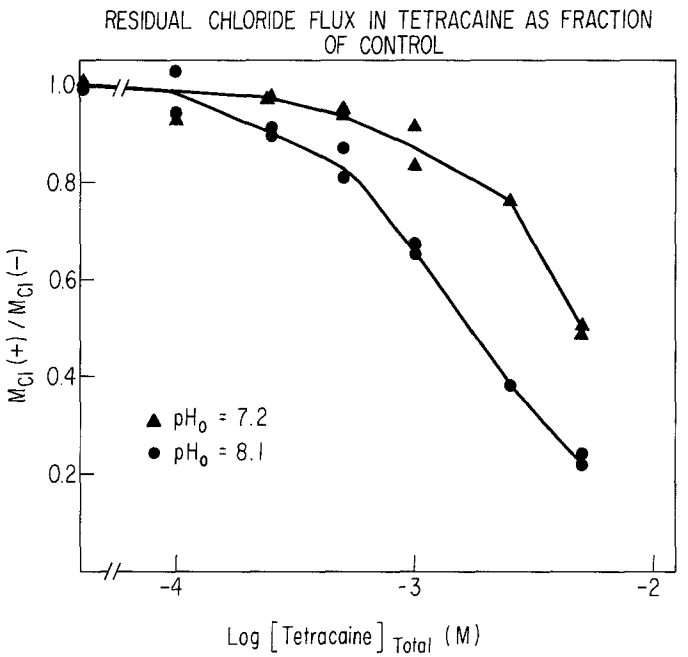

Fig. 8. The residual chloride flux in tetracaine as the fraction of the flux in the absence of tetracaine at two pH values. At pH 7.2 the control flux was 682 mEq Cl^-/(kg cell solid × min) with $r_{Cl}=0.944$; at pH 8.1 the control flux was 745 with $r_{Cl}=0.661$

centrations is shown in Fig. 8. The reduction of the external pH (and internal pH) shifted the dose-response curve toward higher concentrations. The average shift of the dose-response curve, calculated from the average area between the curves and ordinate values of 0.95 and 0.50, was 0.48 log units of total tetracaine concentration. The shapes of the curves in Fig. 8 like that given in Fig. 1 closely follow the theoretical dose-response curve for a one to one reaction between tetracaine and a receptor site; for example, when the concentration was one log unit above and below the concentration causing 50% inhibition, the flux was 91 and 9% inhibited, as expected.

E. *Chloride Concentration Dependence of Chloride Flux in the Presence of Tetracaine*

The chloride self-exchange was measured at different chloride concentrations (Fig. 9) but always with chloride at steady-state distribution and nearly equal internal and external chloride concentration (internal

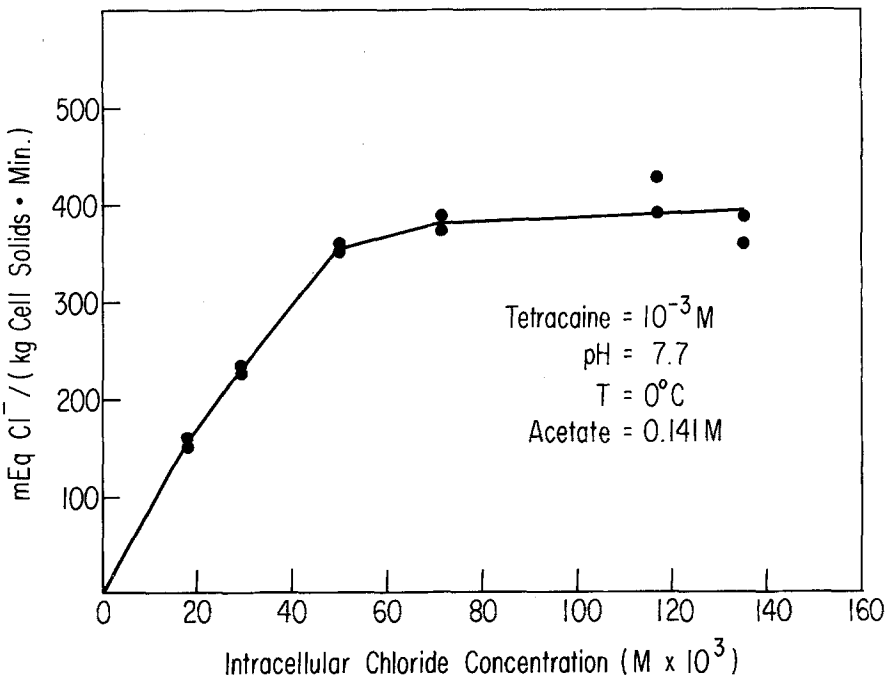

Fig. 9. Residual chloride self-exchange in the presence of 10^{-3} M tetracaine as a function of intracellular chloride concentration. Maximum flux was about 400 mEq Cl^-/(kg cell solid × min) and half-maximal flux was achieved when the intracellular chloride concentration was about 25 mM

chloride concentration/external chloride concentration, r_{Cl}, equalled 1.1 at most concentrations but was 0.91 at the highest concentration) in the presence of tetracaine (10^{-3} M). This data showed the saturation characteristics observed previously without tetracaine (Gunn *et al.*, 1973) but with the maximum flux reduced from 460 to 400 mEq Cl^-/kg cell solid × min) with tetracaine. This value is also reduced compared with the values shown in Figs. 4 and 6 at the same pH = 7.7 and the same tetracaine concentration. This additional reduction is believed to be due to the additional noncompetitive inhibition produced by the presence of 141 mM Na-acetate in all the solutions used in Fig. 9 experiments. The concentration of chloride at which flux was half-maximal in Fig. 9 was 25 mM, the same as in the absence of tetracaine. The reduction in the chloride fluxes caused by the tetracaine is not due to desaturation of the chloride transport system in 150 mM chloride, but due rather to a reduction in the maximum fluxes. Thus, the interaction between chloride and the residual functioning transport system in the presence of tetracaine was unaltered and this is called noncompetitive inhibition

in analogy with enzyme inhibitor kinetics. The fraction of the functioning transport system blocked by tetracaine is, however, reduced in Na-acetate solutions. Tetracaine alone at pH 7.7 gave a 25% inhibition of chloride self-exchange (Fig. 7); but in the presence of acetate, this concentration of tetracaine appeared to reduce the maximum flux by a lesser extent (13%) [M_{Cl} = 460 mEq Cl^-/(kg cell solid × min) in the presence of acetate without tetracaine (Gunn *et al.*, 1973)].

Discussion

Chloride transport in human erythrocytes contains two operationally, but not necessarily mechanistically, distinct fluxes: the forced self-exchange or hetero-exchange of anion equivalents without the possibility of net charge transfer (which has been called the exchange pathway) and the net flux of anions which may accompany net cation charge movement throught the membrane (which has been called the conductance pathway). The former pathway dominates the self-exchange measurements reported here by a factor greater than 10^4 at 0 °C (Lassen, Pape & Vestergaard-Bogind, 1973). This forced self-exchange pathway presumably involves a limited number of membrane (protein) molecules since the fluxes show saturation, competition with HCO_3^- and noncompetitive inhibition by phloretin (Gunn, *unpublished observation*), and since an irreversible anion transport inhibitor, DIDS (4,4′-diisothiocyano-2,2′-stilbene disulfonic acid), binds to a specific membrane protein (Cabantchik & Rothstein, 1974).

The reduction by tetracaine of chloride self-exchange at different chloride concentrations phenomenologically appears to be noncompetitive inhibition (Fig. 9). The maximum chloride flux was reduced, but the concentration of chloride at half-maximum flux was little altered. In molecular terms this may mean that tetracaine reacts in a reversible manner with a site distant from the chloride transport site yet prevents transport. The remaining transport molecules without tetracaine attached to these sites appear to function normally. In analogy with the inhibition of enzymes, the site of action of tetracaine would be distant from the chloride site since both tetracaine and chloride, inhibitor and substrate, should be simultaneously complexed with the transporting mechanism to cause noncompetitive kinetics by a reversible inhibitor. If either tetracaine or chloride prevented the complexation of the other by proximity of their respective binding sites, competitive inhibition would have been

observed. In this sense the "distance" between the sites is functional rather than a precise spatial measurement.

The effect of tetracaine is not mediated through a reduction of local chloride concentration at the chloride loading site because an increase in chloride concentration did not overcome the tetracaine inhibition. Furthermore, any positive charged tetracaine molecules would enhance the local counter-anion concentration. In further support of the noncompetitive mechanism for tetracaine inhibition, the inhibition of chloride flux by tetracaine and acetate together is predicted by a simple model of two independent noncompetitive inhibitors in which the chloride flux is proportional to the membrane concentration of chloride-carrier complexes, C-Cl.[1] In this case the flux in acetate, M_A, in tetracaine, M_T, or in both M_{AT} can be related to the control flux in saturating concentrations of chloride, M_0. $M_A/M_0 = 460/870$ (Gunn *et al.*, 1973) $= 0.53 = (1+K_1A)^{-1}$; $M_T/M_0 = 0.75$. (Fig. 7) $= (1+K_3T)^{-1}$, and therefore $M_{AT}/M_A = (1+K_1A)\cdot(1+K_3T+K_1A)^{-1} = 0.85$. This value is the same as observed experimentally: 400 (Fig. 9)/460 = 0.87.

From the observation that increasing Ca^{++} concentrations have no significant effect on the inhibition of chloride self-exchange by tetracaine, we concluded that either the inhibition is caused by the neutral form of the molecule or that Ca^{++} does not compete with the charged form of tetracaine for binding in this system. The previous explanation offered for the antagonism between Ca^{++} and nerve blockade by local anesthetics (Blaustein & Goldman, 1966) involves competition with, and displacement of, the cationic form of the local anesthetics. If the nonionized form of tetracaine is the anion transport inhibitor, no competition with Ca^{++} would be expected. Therefore, since Ca^{++} does not reduce the tetracaine inhibition of chloride fluxes it seems unlikely that the mechanism of this effect involves ionic interactions with head groups of membrane phospholipids since the elegant studies of Ohnishi and Ito (1974) show the condensation of phosphotidyl serine in a mixed phospholipid bilayer by calcium and its reversal by local anesthetics.

It should be pointed out that many of our results on chloride self-

[1] Acetate (141 mM) reduced the chloride flux to 53% of control and has previously been shown to be a noncompetitive inhibitor (Gunn *et al.*, 1973). Tetracaine reduced the chloride flux to 75% of control while reducing the flux in acetate to 87% of that in acetate alone. The latter value can be calculated from the former values by assuming that the chloride-loaded, C-Cl, and chloride-unloaded carrier molecule, C, react with acetate, Ac, or tetracaine, T, independently but not simultaneously by equilibrium reactions $C + A \overset{K_1}{\rightleftharpoons} C-A$; $C + T \overset{K_3}{\rightleftharpoons} C-T$; $C-\mathrm{Cl} + A \overset{K_1}{\rightleftharpoons} C-A-\mathrm{Cl}$; $C-\mathrm{Cl} + T \overset{K_3}{\rightleftharpoons} C-T-\mathrm{Cl}$. *See* Gunn (1972) for outline of titratable carrier model of anion transport.

exchange flux on red blood cells bear similarities to those found by Århem and Frankenhaeuser (1974) on the cation conductances in crab nerves. As others have found on the squid giant axon (Shanes, Freygang, Grundfest & Ametniek, 1959; Narahashi, Frazier & Moore, 1972) and frog nerve (Hille, 1966) the tertiary amine local anesthetics had a major effect on reducing specific cation conductances, g_{Na} and g_K. In addition, Århem and Frankenhaeuser (1974) found Ca^{++} had little effect on the inhibition and low pH reduced the inhibition. They also found that the reduction of g_{Na} and g_K by benzocaine was unaffected by pH. These three points are also true of chloride fluxes in erythrocytes. 5×10^{-4}M benzocaine half-inhibited both g_{Na} in crab nerves at 25 °C and chloride self-exchange in erythrocytes at 0 °C (Fig. 2).

Skou (1961) showed that there was a strong correlation between anesthetic potencies and the ability to alter several surface properties of monolayer films but was careful to disclaim that correlation proved that those properties of the local anesthetics were causally related to the anesthetic effects. In a like manner, the small expansion of erythrocyte membrane area at which osmotic lysis occurs in the presence of local anesthetics (Seeman, Kwant, Sauks & Argent, 1969) may reflect the insertion of the agents into the membrane but does not establish that this is the basis of their anesthetic potency. Nuclear magnetic resonance studies suggest that both electrostatic (Hauser, Penkett & Chapman, 1969) and hydrophobic (Cerbón, 1972) interactions play a role in the attachment of local anesthetics to the membranes of phospholipid vesicles. In both studies there seemed to occur a "burying" of the hydrophobic tail of the local anesthetic in the phospholipid layer. The higher potency of tetracaine as compared with analogues has been postulated to be due to its longer hydrophobic tail which causes greater penetration into the membrane. Our studies show that local anesthetics can produce inhibition of anion exchange which is a quite different effect from those reported thus far on nerves and phospholipid vesicles.

From the analysis of the data in Fig. 8 we can conclude that both charged and uncharged tetracaine are effective in reducing the chloride self-exchange flux. The argument assumes that only the unionized form is active and shows that this leads to an incorrect pK_a for tetracaine. The concentration of an unionized form at a site is independent of the surface potential or transmembrane potential and only differs from that in either the bulk medium or cell water by a partition factor independent of potentials. Since tetracaine acts rapidly and the cells were prewashed with tetracaine solutions we may assume that the medium

concentration of unionized tetracaine (BH_m) equals that at the reactive site (BH_S) (or $BH_S = k\ (BH_m)$ where k is the partition coefficient if the site is within the membrane). The data curves in Fig. 8 show that the ratio of total tetracaine concentration (at pH 7.2/ at pH 8.1) equals 3.0 (=log 0.48) when the concentrations of the active form at the reactive site are equal. If the unionized form is the only active form at the reactive site, its concentration at the site and its concentration in the bulk solution are equal when the ratio of total tetracaine concentrations is 3.0 in the medium. This implies that the pK_a of tetracaine in the medium must be 7.7 as calculated by the Henderson equation ($B = B_{total}$ $(1 + 10^{pK_a - pH})$). This does not agree with the published $pK_a = 8.47$ (Eisenbrand & Picher, 1938) nor with our calculation that the pK_a is greater than 9.0 at 0 °C in 150 mM KCl (Fig. 10). Because of the discrepancy

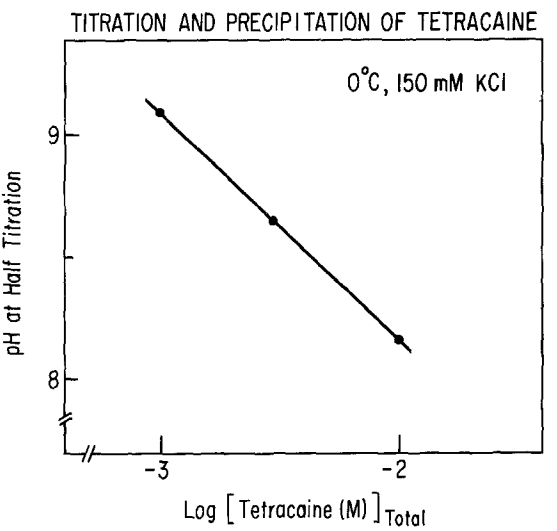

Fig. 10. The $pH_{1/2}$ at which half of the tetracaine is titrated from the protonated to the uncharged form as a function of total tetracaine concentration. The low solubility of the unionized tetracaine prevented a direct determination of the pK_a, but a lower bound could be calculated. The $pH_{1/2}$ can be measured even in the presence of a precipitate of the unionized drug. If the solubility limit of the unionized tetracaine (B_{SL}) is constant and is less than half of the total concentration, the graph of $pH_{1/2}$ versus log $(\text{tetracaine})_{total}$ should have a slope of -1.0, since by Henderson's equation $pH_{1/2} = pK_a + \log B_{SL} - \log (\text{tetracaine})_{total} + \log 2$. The graph has a slope of -0.94, therefore the assumption that B_{SL}/K_a is nearly constant can be made and the value calculated from the data. B_{SL}/K_a is approximately 5.6×10^5. With this value and the observation that tetracaine solubility is less in the cold and Eisenbrand and Picher's (1938) observation that $B_{SL} = 5.9 \times 10^{-4}$ M at 25 °C, we may place an upper limit on K_a of 10^{-9} and a lower limit on the pK_a for tetracaine of 9.0

between this measured pK_a and the calculated value of 7.7, we deduce that the assumption that only the unionized form is active must be false. Since the inhibition of chloride flux increased but the concentration of ionized tetracaine decreased with pH, the ionized form cannot be the only active form. We therefore can conclude that both forms of tetracaine were inhibitory in our system. Furthermore, using the observations that H_3O^+ (Gunn, Wieth & Tosteson, 1975) and tetracaine (Fig. 9) behave as noncompetitive inhibitors of chloride flux, the decreased potenty of tetracaine at lower pH values cannot be quantitatively explained without further assuming that the uncharged tetracaine is more potent than the charged form[2].

In conclusion, tetracaine inhibits chloride self-exchange in both charged and uncharged forms but with different potencies and in a reversible, noncompetitive fashion. This inhibition is not calcium sensitive and is probably not due to changes in surface charge density.

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[2] Following the same line of argument given in footnote 1, the fractional residual flux at pH 7.2 as a function of tetracaine may be calculated from the fractional residual flux at pH 8.1 in tetracaine and the ratio of the two fluxes in the absence of tetracaine. The calculated fractional residual flux at 5×10^{-3}M tetracaine, for example, would be 0.25 at pH 7.2 if both charged and uncharged tetracaine were equally potent inhibitors of chloride flux, whereas the residual flux is 0.50 (Fig. 8).

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