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Amphipathic amines affect membrane excitability in *Paramecium*: Role for bilayer couple

(excitable membrane/amphipathic drugs/anesthetics/Ca^{++} permeability)

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ABSTRACT **Amphipathic amines and local anesthetics stimulated reversal of the ciliary beating direction in wild-type *Paramecium*. Ca^{++} influx across the surface membrane and the consequent increase in internal Ca^{++} causes ciliary reversal and backward swimming. Mutant cells of the "Pawn" class, which lack a "gating" mechanism for regulating Ca^{++} influx, did not swim backwards in the presence of local anesthetics. Local anesthetics stimulated the *passive* efflux of K^+, but had no effect on the *active* transport of K^+ or Ca^{++}. Apparently *passive* influx of Ca^{++} also was stimulated by local anesthetics as evidenced by their effects on swimming direction. These data can be interpreted in terms of the "bilayer couple" hypothesis of Sheetz and Singer [(1974) *Proc. Nat. Acad. Sci. USA* 71, 4457–4461]: amphipathic drugs affect cells by asymmetric insertion into one face of the lipid bilayer.**

As predicted by this hypothesis, the drugs' effects were seen only after a short time lag, and quaternary amines were less effective than tertiary amines. The effect on behavior was caused by any of several amphipathic cations, and the relative potency was a function of their hydrophobicity. Amphipathic anions, which according to the hypothesis would insert into the opposite face of the lipid bilayer, had little effect on ciliary reversal. Asymmetric perturbation of the lipid bilayer with amphipathic cations may trigger the opening of the Ca^{++} gate.

The ciliated protozoan *Paramecium aurelia* has an excitable membrane which mediates the transfer of information from receptors for various stimuli to the numerous cilia located over the cell surface. The cell membrane produces a graded, Ca^{++}-mediated, regenerative depolarization in response to several stimuli (1). Ca^{++} serves both as a current-carrying ion and as an intracellular messenger conveying information from the surface to the cilia. Increasing the intracellular Ca^{++} concentration causes the cilia to reverse their beating direction and as a result the cell swims backward (1). Hence, the swimming behavior of the cell serves as a visual correlate of the electrical state of the cell surface membrane. Genetic analysis is relatively simple in *Paramecium* and behavioral mutants have been selected and studied which are defective in components of this system (2). For these reasons, *Paramecium* is an excellent organism for the study of phenomena of excitation.

Ca^{++} and K^+ movements in excitability have been studied by electrophysiological methods and by measurement of isotope fluxes (3, *, †). The excitable membrane of *Paramecium* resembles that of neurons, except that in *Paramecium* Ca^{++}, not Na^+, carries the initial current. Ca^{++} influx is the basis for the membrane depolarization induced by any of several stimuli, while a concurrent K^+ efflux short-circuits the response.

Dryl studied the effects of several detergents and drugs on *Paramecium* behavior. He found that chloropromazine (a hydrophobic tertiary amine) would trigger ciliary reversal. Cetyltrimethylammonium bromide weakly stimulated ciliary reversal, and was effective in blocking ciliary reversal in response to several negative chemotactic stimuli (4). He reported that an anionic detergent, sodium dodecyl sulfate, had an effect opposite to that of the cationic detergent cetyltrimethylammonium bromide (5). A brief report by Ozeki and Grundfest stated that procaine caused membrane depolarization in crustacean muscle fiber, which most likely was Ca^{++} mediated (6). Friedman and Eckert (7) investigated the response to electrical stimulation in cells which had been exposed to the local anesthetic tetracaine; it increased membrane resistance and the amplitude of the Ca^{++}-mediated response.

We have found that local anesthetics and amphipathic amines are effective in stimulating ciliary reversal in *Paramecium*. We report the relative effectiveness of several drugs in promoting ciliary reversal and in stimulating ion fluxes in wild-type and mutant strains of *Paramecium*. The effects of these drugs on *P. aurelia* have a striking similarity to the interaction of these agents with erythrocytes. Due to the asymmetric arrangement of various phospholipids across the erythrocyte membrane, the inner layer bears a net negative charge (8). Sheetz and Singer have proposed that, because of this charge asymmetry, amphipathic molecules partition preferentially into one side of the bilayer, depending on their charge (9). They showed that in erythrocytes hydrophobic cations partition primarily into the inner lipid layer, and induce specific cell shape changes, and they suggested that some cellular functions may be sensitive to changes in one half of the bilayer. The term "bilayer couple" was used to describe this phenomenon. We have interpreted our results with *Paramecium* in terms of the bilayer couple hypothesis.

MATERIALS AND METHODS

Firefly lanterns, tetracaine, procaine, benzocaine, diphenhydramine·HCl, papaverine, nicotine, propranolol, hexamethonium bromide, benzoyl choline, tetraethyl ammonium chloride, acetylcholine, and choline were obtained from Sigma Chemical Co. Phenoxybenzamine was a gift of Smith, Kline and French. *n*-Ethylamine, *n*-octylamine, *n*-dodecylamine, *n*-hexadecylamine, *n*-decanol, *n*-decanoic acid, and diphenylamine (all reagent grade) were obtained from Eastman Organic Chemicals. Other chemicals used were sodium

Abbreviations: ED, effective dose; NMR, nuclear magnetic resonance; s, t, q, m, singlet, triplet, quadruplet, multiplet in NMR; DMAB, *N,N*-dimethylaminoethyl benzoate.

* J. Browning and D. Nelson (1975) "Biochemical studies on the excitable membrane of *Paramecium aurelia* I. $^{45}Ca^{++}$ fluxes across the excited and resting membrane," submitted for publication.

† J. Browning and D. Nelson (1975) "Biochemical studies on the excitable membrane of *Paramecium aurelia* II. Direct measurement of monovalent cation fluxes," submitted for publication.

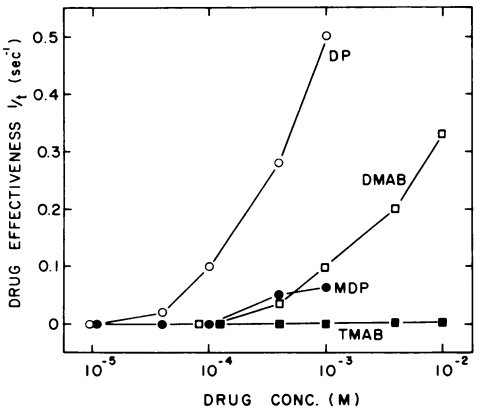

FIG. 1. Comparison of the ability of two quaternary:tertiary amine pairs to stimulate ciliary reversal in *P. aurelia*. The swimming behavior of the cells was observed as described in *Materials and Methods*. Drug effectiveness is represented as the inverse of the time elapsed between exposure to the drug and the initiation of backward swimming. DP and MDP were diphenhydramine and *N*-methyldiphenhydramine, respectively; DMAB and TMAB are *N,N*-dimethylaminoethyl benzoate and *N,N,N*-trimethylaminoethyl benzoate (benzoylcholine), respectively.

dodecyl sulfate (Pierce Chemicals, sequanal grade); methyl iodide, *N,N*-dimethylaminoethanol, *N,N*-diethylaminoethanol (Aldrich); benzoyl chloride (Mallinckrodt); and lidocaine and dibucaine (K & K Fine Chemicals). All cations were used as the chloride salt where not mentioned. The wild-type cell line of *Paramecium aurelia* was syngen 4, stock 51, non-kappa bearing. All cell lines were the gift of Dr. C. Kung. The mutant lines used were: Pawn A (genotype *PwA PwA*, stock d4-94), Pawn B (genotype *PwB PwB*, stock d4-95), Fast-1 (genotype *fA fA*, stock d4-98), Fast-2 (genotype *fna fna*, stock d4-91), and Paranoiac (genotype *PaA PaA*, stock d4-90). These mutations are described by Kung (10).

Drug Effects on *Paramecium*. Cells were grown in cerophyl medium* with 1 μg/ml of sitosterol at room temperature, washed, and transferred into 1 mM Ca^{++}, 1 mM K^+, 1 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) buffer, 0.1 mM Na_2EDTA, or 0.5 mM citric acid and Tris to pH 7.0. Cells were equilibrated in this solution for at least 1 hr prior to observation, then approximately 20 μl of cells (50–100 cells) were transferred into 0.5 ml of the same solution plus the agent to be tested. The whole solution was placed on an obliquely illuminated glass slide and viewed with a low power (×15) dissecting microscope. Cells were photographed on an obliquely illuminated glass plate with a Polaroid MP-4 Land Camera in a manner similar to that described by Chang and Kung (11). There was a time lag between exposure to the drug and the first behavioral responses, and the length of this lag depended on the concentration of the drug. The time when the first cells began to respond to the drug and the time by which the entire population had responded were noted, and the average of these two values is the response time used in Fig. 1. The drug concentration to which the cells responded within 3–5 sec was defined as the effective dose (ED). To avoid artifacts due to O_2 exhaustion in cell suspensions held in a pipette, a fresh aliquot of cells was withdrawn immediately prior to each observation.

ATP Assays. ATP levels were determined by the firefly luciferase assay (12). Cells (20,000/ml) were exposed to tetracaine in a solution of the same composition used in behavioral observations. Oxygen was bubbled through the flask during the experiment. To 2.0 ml of cells was added 0.1 ml of 1 M KCl, and the suspension was centrifuged at 1500 × *g* for 20 sec (the KCl prevented the cells from swimming out of the pellet). The supernatant was removed by aspiration, and the pellet was suspended in 0.3 ml of ice-cold 50% trichloroacetic acid. The acid was extracted twice with 3 volumes of ether, and the excess ether was removed by passing air over the suspension. To determine released ATP, the pellet was removed with a pasteur pipette and ice-cold trichloroacetic acid was added to the supernatant and treated as described above. ATP determinations on the supernatant were sensitive enough to detect leakage of 1% of the total intracellular ATP.

Triton-Extracted Models of *Paramecium*. Triton-extracted *Paramecium* were prepared by the method of Naitoh and Kaneko (13). They were reactivated in the presence and absence of 200 μM tetracaine and observed in the light microscope.

Ion Flux Assays. K^+ and Ca^{++} influx and efflux were measured as previously described*†.

Drug synthesis

***N*-Methyl-diphenhydramine Perchlorate (2-Diphenylmethoxy-*N,N,N*-trimethyl Ethylamine Perchlorate).** Six grams of diphenhydramine·HCl was dissolved in 50 ml of acetone and refluxed for 5 hr; 4 ml of methyl iodide was added slowly. The solution was poured into 2 M sodium perchlorate at 0°, and the precipitate was collected. The perchlorate salt was recrystallized from ethanol–water, mp (uncorrected) 173–175°; nuclear magnetic resonance (NMR): δ 3.4 (9 H, s), 3.9 (4 H, m), 5.7 (1 H, s), 7.4 (10 H, m). (δ in ppm relative to tetramethylsilane.)

***N,N*-Dimethylaminoethyl Benzoate·HCl (DMAB).** Six milliliters of benzoyl chloride were added slowly to 5.4 ml of *N,N*-dimethylaminoethanol in 50 ml of anhydrous pyridine at 22°. The mixture sat for 1 hr and the precipitated hydrochloride salt was collected and washed with diethyl ether. Recrystallization from acetone yielded 10 g of white crystals, mp (uncorrected) 149–150°, (literature, 148°; ref. 14); NMR: δ 3.0 (6 H, s), 3.5 (2 H, t; J = 5 Hz), 4.5 (2 H, t; J = 5 Hz), 7.5 (5 H, m).

***N,N*-Diethylaminoethyl Benzoate·HCl.** Synthesis was identical to the preparation of DMAB except that 7.0 ml of *N,N*-diethylaminoethanol was reacted with 6.0 ml of benzoyl chloride at 0°. After 1 hr, the mixture was transferred to −20° and allowed to sit for another hour. The precipitated HCl salt was collected and recrystallized three times from acetone (yield 9.5 g) mp (uncorrected) 124–125° (literature 124°; ref. 14); NMR: δ 1.2 (6 H, t; J = 7 Hz), 3.2 (4 H, q, J = 7 Hz), 3.4 (2 H, t, J = 5 Hz), 4.5 (2 H, t, J = 5), 7.5 (5 H, m).

RESULTS

Effects of Hydrophobic Amines on *Paramecium aurelia*. When paramecia were mixed with relatively low concentrations of tetracaine, we usually observed no change in behavior in 10–20 sec. Then the cells began swimming backward in a manner similar to the response to monovalent cations. The backward swimming lasted about 20–40 sec, after which forward swimming resumed. After 30–60 sec of exposure to the drug, the cells became pear-shaped, with the posterior end being contracted. In higher drug concentrations (above 100 μM) cells eventually stopped swimming. The effect of the drug was reversible; cells exposed to 100 μM tetracaine for 30 min were motile after the drug was washed away.

Varying the level of external Ca^{++} from 1 to 20 mM or

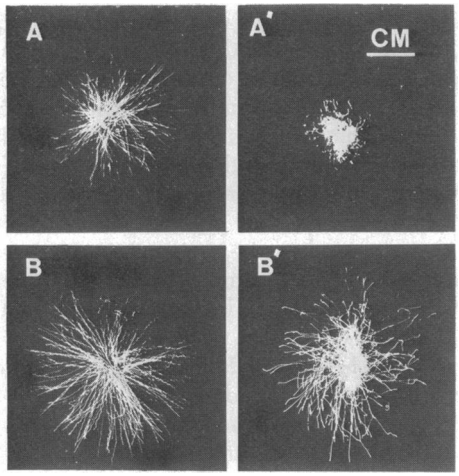

FIG. 2. Behavioral responses to tetracaine by wild-type and Pawn (d4-95) mutant *P. aurelia*. A thin film of the cell equilibration solution (described in *Materials and Methods*) at 22–23° was placed on a glass plate and obliquely illuminated. Approximately 50 cells were placed in a drop onto the center of the glass plate and the camera shutter was opened. The film was exposed for the 10 ± 1 sec immediately after the cells were dropped onto the plate. (A) Wild-type and (B) Pawn cells in the absence (A, B) and presence (A′, B′) of 100 μM tetracaine. "Star" patterns (A, B, B′) result from the forward swimming of the cells from the original drop. Wild-type cells, when placed into 100 μM tetracaine, undergo ciliary reversal on contact with the drug and make no forward progress (A′) and remain confined to the original drop. When a small drop of a cell suspension was placed in a solution of tetracaine, wild-type cells underwent ciliary reversal upon approaching the boundary between the original drop and the surrounding solution of tetracaine. As a consequence of this avoiding response, cells seldom swam into the region of high tetracaine concentration, but instead remained in the original drop. This experiment, therefore, differs from that described above, in which cells and tetracaine were thoroughly mixed, causing continuous backward swimming. A high concentration of tetracaine was used to minimize the time lag between exposure to the drug and the behavioral response (100 μM tetracaine was a high concentration for these particular cell preparations). Bar = 1 cm.

external K^+ from 0 to 4 mM had no effect on either the time lag for initiation of ciliary reversal or the duration of the response.

The concentration of tetracaine required to elicit a given response differed somewhat with different preparations of *Paramecium;* it appears that the sensitivity to such drugs differs with growth stage. With some preparations of *Paramecium* (those shown in Fig. 2, for example) 100 μM tetracaine was sufficient to cause changes in swimming behavior almost immediately.

Table 1. Comparison between the ability of various local anesthetics to stimulate ciliary reversal in *Paramecium aurelia* and their local anesthetic potency

Drug	Relative potency* for ciliary reversal	Relative potency† for local anesthesia
Procaine	1 (3)	1
Benzocaine‡	1 (3)	1
Lidocaine	6 (0.5)	4
Tetracaine	40 (0.075)	36
Dibucaine	46 (0.065)	53

* Values in parentheses are the ED (mM) for stimulation of ciliary reversal by the various anesthetics.
† Compiled from Blaustein and Goldman (18) and Adriani *et al.* (19).
‡ Cells responded very weakly to benzocaine.

Structure–Activity Relationships. The ability of various local anesthetics to stimulate ciliary reversal paralleled their potency as local anesthetics (Table 1). A variety of drugs were found to cause ciliary reversal; all were amphipathic amines. Among the highly effective agents were phenoxybenzamine·HCl, papaverine, nicotine, diphenhydramine·HCl, propranolol, diphenylamine, and several *n*-alkyl amines. Somewhat similar compounds which were relatively ineffective were hexamethonium bromide, acetylcholine, choline, and tetraethylammonium chloride.

The requirement for an anion or a cation was tested by comparing the following related compounds: *n*-dodecylamine, *n*-dodecanoic acid, *n*-decyl alcohol, and sodium *n*-dodecyl sulfate (Table 2). The neutral and positively charged (alcohol and amine) molecules were effective; the carboxylic acid affected behavior only weakly; and the sulfate was completely ineffective. The role of the hydrophobic portion of the compound was examined by varying the chain length of the *n*-alkyl amine series (Table 2); the effectiveness of a compound increased with increasing chain length. Comparison of the methyl-substituted amine (DMAB) and the ethyl-substituted amine (diethylaminoethyl benzoate) indicated that the more hydrophobic the amine, the more potent the compound (Table 2). To determine whether tertiary or quaternary amines were more effective in stimulating ciliary reversal, the tertiary-quaternary amine pairs diphenhydramine (tertiary): methyldiphenhydramine (quaternary) and DMAB (tertiary): benzoylcholine (quaternary) were tested. In both cases the tertiary amines were more effective in stimulating ciliary reversal (Fig. 1).

Effects of Hydrophobic Amines on Behavioral Mutants of *Paramecium aurelia*. Tetracaine stimulated ciliary reversal with all of the behavioral mutants (Fast-1, Fast-2, and Paranoiac) except those designated Pawn (Fig. 2). Pawn mutants at both loci (*PwA* and *PwB*) lacked a ciliary reversal response to tetracaine, although in these strains the same drug concentrations affected cell shape and eventually caused immobilization.

Effects of Hydrophobic Amines on Membrane Permeability. Tetracaine (100 μM) had no effect on either K^+ influx or Ca^{++} efflux, while K^+ efflux was stimulated by tetracaine (100 μM) (Fig. 3). The data for Ca^{++} influx are not shown, because Ca^{++} influx had to be measured at 0° (to inhibit a Ca^{++} efflux pump) and the effects of tetracaine at this temperature were anomalous. At 0° and at low concentrations (10–50 μM) tetracaine caused cell lysis in 15–30 min; however, the concentration dependence for stimulation

Table 2. Ability of various compounds to stimulate ciliary reversal in *Paramecium aurelia*

Compound	ED (mM)
n-Ethylamine	Not effective up to 0.3
n-Octylamine	0.2
n-Dodecylamine	0.05
n-Hexadecylamine	0.01
n-Dodecanoic acid	>0.50
n-Decyl alcohol	0.10
n-Dodecyl sulfate (sodium)	Not effective up to 0.2
N,N-Dimethylaminoethyl benzoate	5.5
N,N-Diethylaminoethyl benzoate	0.8

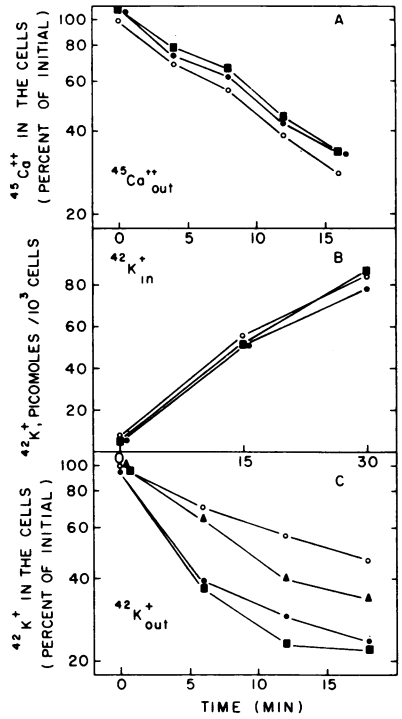

FIG. 3. Effects of tetracaine on the influx of $^{42}K^{+}$ and efflux of $^{45}Ca^{++}$ and $^{42}K^{+}$. Assays were done as described elsewhere*† $^{45}Ca^{++}$ efflux was measured at 25 μM Ca^{++}; $^{42}K^{+}$ influx, at 100 μM K^{+}; and $^{42}K^{+}$ efflux, at 1 mM K. Tetracaine was added immediately before the initial point. Control O, tetracaine 10 μM ▲, 50 μM ●, and 100 μM ■.

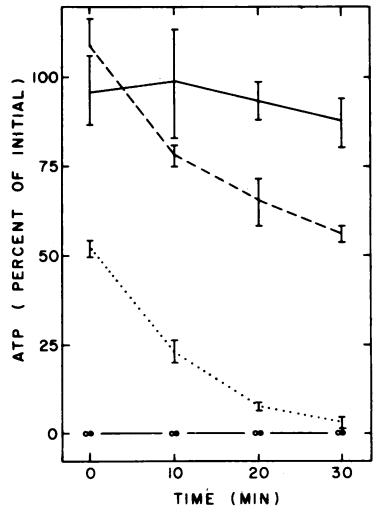

FIG. 4. Effects of tetracaine on ATP levels and ATP permeability. ATP in the cells: control (—), 100 μM tetracaine (- - -), and 1 mM NaN_3 (• • •). Both tetracaine and NaN_3 were added immediately prior to the initial point. (NaN_3 was added to show that this ATP pool was sensitive to an energy poison.) ATP in the medium outside of the cells is shown in the absence of tetracaine (O) and in the presence of 100 μM tetracaine (●). Error bars indicate the range of duplicate determinations.

of ciliary reversal was unchanged. At 0°, tetracaine caused changes in cell shape which were different from those observed at room temperature. To test whether the increased membrane permeability was due to destruction of the membrane by hydrophobic amines and the consequent nonspecific leakage of various compounds down their concentration gradients, we examined the levels of intracellular ATP and the presence of ATP leaked into the medium (Fig. 4). Intracellular ATP levels decreased following exposure to tetracaine; however, no ATP was detected in the medium.

Effects of Hydrophobic Amines on Triton-Extracted Models of *Paramecium*. Cells extracted with Triton X-100 lose their membrane permeability barriers, but they regain motility upon the addition of ATP and Mg^{++}. The cilia of these freely permeable "models" retain their ability to respond to increased Ca^{++} levels by reversing their beating direction (13). In the presence of 200 μM tetracaine models swam forward, just as in the absence of tetracaine (data not shown). Control preparations in the presence of 0.1 mM Ca^{++} swam backwards as previously described by Naitoh and Kaneko (13).

DISCUSSION

Friedman and Eckert (7) studied the effect of tetracaine upon the *Paramecium* membrane's response to electrical stimulation, and concluded from electrophysiological data that tetracaine probably decreased K^{+} conductance.

We have presented several types of evidence which indicate that stimulation of ciliary reversal in *Paramecium* by local anesthetics results from the opening of "gates" controlling passive ion movements across the membrane.

(*1*) Gentle treatment with Triton X-100 destroys the permeability barrier of the cytoplasmic membrane, but leaves cilia intact and able to beat when provided with MgATP. With such Triton-extracted models, tetracaine does not cause ciliary reversal, suggesting that the drug acts on the surface membrane, not directly on the cilia.

(*2*) Local anesthetics appear to affect passive ion fluxes but not active ion pumping. Neither Ca^{++} efflux nor K^{+} influx, both active processes*†, was affected by tetracaine. K^{+} efflux, which probably occurs through a gated pore†, was stimulated by tetracaine (Fig. 3C). Unfortunately, we were unable to measure directly the effects of local anesthetics upon passive Ca^{++} influx; our assay for $^{45}Ca^{++}$ influx requires incubation at 0° to inactivate the Ca^{++} efflux pump, and at this low temperature tetracaine has anomalous effects on *P. aurelia*. Relatively low anesthetic concentrations bring about morphological distortions and lysis at 0°. However, from the observation that tetracaine induces ciliary reversal in wild-type cells but not in Pawn mutants at room temperature (no lysis occurs at room temperature), we infer that the passive influx of Ca^{++} through the gated pore is stimulated by the anesthetic. Although local anesthetics only affected those ion movements which occurred passively down electrochemical gradients, these drugs did not appear to alter gross membrane leakiness, as judged by unaltered ATP permeability in the presence of tetracaine.

(*3*) All of the mutant cell lines with defective behavior, except those cells bearing the Pawn mutation, showed ciliary reversal induced by local anesthetics. Pawn mutants lack a functional Ca^{++} "gate" (15, 16), but all other cell lines which were tested (Fast-1, Fast-2, and Paranoiac) have a normal Ca^{++} "gate" (10). The local anesthetics bind to Pawn-type cells, since Pawn cells exhibit effects of the drugs (eventual immobilization and cell shape changes).

The drugs could bind to a protein "receptor", indirectly affecting the Ca "gate", with consequent ciliary reversal. However, we consider it more likely that the drugs interact primarily with the membrane lipids. Papahadjopoulos (17) has summarized much evidence that the potency for local anesthesia by various local anesthetics exactly parallels their effects on model phospholipid membranes (phospholipid

Ca^{++} binding, Ca^{++} permeability in phospholipid vesicles, monolayer surface area, and the reduction of zeta potentials). Thus, local anesthetic action in nerves probably occurs at the lipid level, and does not involve specific drug–receptor interactions. We have shown that local anesthetics stimulate ciliary reversal and block excitability in nerves with the same relative potency. We consider it very probable that the drugs also act at the lipid level in *Paramecium*.

Sheetz and Singer's bilayer couple hypothesis (9) suggests a drug–lipid interaction which explains many previous observations on local anesthetics. According to this hypothesis, the two faces of a closed membrane bilayer differ in composition, and may respond differently to various perturbations while still remaining coupled as a bilayer. Presumably, protein-mediated membrane functions could be controlled by the bilayer couple. In erythrocyte membranes the asymmetric distribution of membrane phospholipids (8) is thought to produce an asymmetry in the head group regions of the two faces of the bilayer; the negative inner face is more likely to attract and hold a lipophilic cation such as tetracaine. One consequence of the asymmetric distribution of tetracaine in the two faces of the bilayer is a characteristic alteration of erythrocyte shape (9). Our observation that the effects of a series of hydrophobic amines on ciliary reversal in *Paramecium* parallel the effects of the same drugs on erythrocyte shape suggests to us that the "bilayer couple" may play a role in the excitable membrane of *Paramecium*.

Although an asymmetric transmembrane phospholipid distribution is an essential component of the hypothesis and has not been shown in any biological membrane except erythrocytes, the bilayer couple hypothesis allows several testable predictions.

(*1*) Because the amphipathic drug must cross the bilayer to be effective, there should be a time lag between exposure to the drug and manifestation of its effects.

(2) Cationic molecules should be most effective, whereas anionic molecules should be inactive. Sheetz and Singer showed that the quaternary amines were less effective in the erythrocyte system than the corresponding tertiary amines, presumably because quarternary amines were unable to deprotonate to a neutral form which could diffuse to the inner face of the bilayer.

(3) Increasing the lipid solubility of the drug should increase its effectiveness.

All of these predictions were confirmed in our studies of the effects of local anesthetics on ciliary reversal. We observed a delay of several seconds before the drug effect was evident. Cationic molecules were effective, whereas the anionic compounds (carboxylic acid and sulfate forms) were inactive (Table 2). Tertiary amines were more effective than the corresponding quaternary forms (Fig. 1), and increasing the hydrophobicity of both the non-ionic part of the drug (increasing alkyl chain length) and the amine (dimethyl versus the diethyl substituted amine) increased a drug's effectiveness.

The induced changes in erythrocyte morphology persist for as long as the local anesthetic is present (9), and one might expect that ciliary reversal induced by tetracaine also would persist until the removal of the drug. It does not; some adaptation mechanism, perhaps related to the mechanism responsible for adaptation to K^+ stimulation, must be operative.

We conclude that these drugs cause ciliary reversal in *Paramecium* in a manner consistent with the hypothesis that the effect of hydrophobic amines on swimming behavior results from their insertion into the inner face of the membrane's lipid bilayer. Because these agents appear to affect the Ca^{++} "gate" while interacting with the lipid bilayer, the functional properties of the Ca^{++} "gate" must be affected by membrane lipids. We suggest that the Ca^{++} "gate" responds differentially to the two halves of the lipid bilayer. Local anesthetics affect *Paramecium* through perturbation of one half of the lipid bilayer and hence opening the Ca^{++} "gate". The drugs may affect Ca^{++} binding to the phospholipids in some asymmetric manner leading to membrane depolarization. Alternatively, the extra bulk of the asymmetrically distributed drug might affect the ion "gates". If these drugs artificially induce a normal physiological process, then a plausible mechanism for membrane excitability is suggested. The lipid bilayer could "sense" the potential difference across the membrane and communicate this information via the bilayer couple to a protein ion "gate".

Although the bilayer couple hypothesis explains our observations, it is not the only possible explanation for these results. For example, tetracaine may directly affect some membrane protein or alter some ion permeability to cause membrane depolarization; the depolarization *per se* may trigger the opening of the voltage-sensitive Ca^{++} gate.

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