INFLUENCE OF LOCAL ANESTHETICS UPON HUMAN POLYMORPHONUCLEAR LEUKOCYTE FUNCTION IN VITRO

Reduction of Lysosomal Enzyme Release and Superoxide Anion Production*

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Cationic local anesthetics (tertiary amines) recently have been demonstrated to influence membrane-dependent responses to surface stimuli in a variety of cultured cells (1–4). In murine BALB/3T3 cells, for example, dibucaine, procaine, and tetracaine caused enhanced susceptibility to agglutination by concanavalin A (1, 2) as well as disorganization of membrane-associated cytoskeletal elements (2, 3). These, and other effects of local anesthetics, such as inhibition of ligand-induced capping (2, 4), could be mimicked by treatment of cells simultaneously with colchicine and cytochalasin B, agents which putatively interfere with the function of microtubules and microfilaments, respectively (1–3, 5–7). Consequently, it has been suggested that local anesthetics interact with these structural and contractile elements of cells so as to interfere with normal transmembrane control of surface receptor mobility (1–3).

Microtubules and microfilaments are now known to be essential for several normal responses to surface stimulation of human polymorphonuclear leukocytes (PMN).[1] Treatment of these cells with cytochalasin B and/or colchicine alters their morphology (8, 9) and interferes with directed motility (10, 11) and particle ingestion (10, 12, 13). Colchicine, in micromolar concentrations, inhibits microtubule assembly in both normal and cytochalasin B-treated PMN (14). Furthermore, colchicine partially inhibits release into the surrounding medium (exocytosis) of lysosomal enzymes from PMN exposed to ingestible particles and nonphagocytosable stimuli (9, 14–18). Since these effects of colchicine and cytochalasin B have been documented and quantitated, it was of interest to compare

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; EACA-ZTS, zymosan-treated serum containing epsilon-aminocaproic acid; LDH, lactate dehydrogenase; PMN, polymorphonuclear leukocyte(s); STZ, serum-treated zymosan.

the effects of local anesthetics with those of colchicine and cytochalasin B with respect to their capacity to influence human PMN function and morphology in vitro.

Materials and Methods

Leukocyte Suspensions. Leukocytes (approximately 85% PMN) were prepared from venous blood as previously described (19) and suspended in phosphate (10 mM)-buffered 140 mM NaCl, pH 7.4, supplemented with 0.6 mM $CaCl_2$ and 1.0 mM $MgCl_2$. This buffer was used throughout. Aliquots of cell suspensions containing 2–4 × 10^6 PMN were transferred to 10 × 75 mm polypropylene tubes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). Some cells were preincubated with cytochalasin B (5.0 μg/ml) (ICI Research Laboratories, Alderly Park, Cheshire, England) in 0.1% dimethyl sulfoxide (Matheson, Coleman, and Bell, East Rutherford, N. J.) at 37°C for 10 min before addition of appropriate compounds and stimuli. This concentration of dimethyl sulfoxide did not influence cytochrome *c* reduction, enzyme release, enzyme asays, or morphology of PMN (see below).

Stimuli. Zymosan (ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio) was boiled and washed with 140 mM NaCl and then incubated with fresh human serum at a concentration of 10 mg/ml for 30 min at 37°C. After centrifugation and washing twice, this preparation of serum-treated zymosan (STZ) was suspended in buffer at a concentration of 10.0 mg/ml.

Fresh autologous serum containing 250 mM epsilon-aminocaproic acid (EACA) (Sigma Chemical Co., St. Louis, Mo.) was incubated with zymosan (1.0 mg/ml) at 37°C for 15 min and then rendered free of particles by centrifugation (3,000 *g*). Previous studies have established that the bulk of lysosomal enzyme releasing activity in zymosan-treated serum containing EACA (EACA-ZTS) can be attributed to its content of the low molecular weight complement component, C5a (18). EACA-ZTS was employed at a final concentration of 10% (vol/vol). Concanavalin A (Con A) was obtained from Sigma Chemical Co.

Superoxide Anion Production. The generation of superoxide anion by PMN was measured as previously described (20). Briefly, duplicate reaction mixtures containing 0.075 mM horse heart ferricytochrome *c*, type III (Sigma Chemical Co.) were incubated with compounds and stimuli for 15 min at 37°C. Incubations were terminated by sedimenting the cells at 755 *g* for 10 min at 4°C. Supernates were diluted with buffer and the absorbance spectrum measured from 540 to 560 nm. With the aid of ferricyanide and dithionite, the amount of cytochrome *c* that was reduced and the total amount of cytochrome *c* present were calculated, using an absorbance coefficient of 21.1 mM^{-1} cm^{-1} at 550 nm (reduced − oxidized) (20, 21). The specificity of cytochrome *c* reduction was checked by assaying supernates from reaction mixtures to which had been added 0.01 mg/ml superoxide dismutase (Truett Laboratories, Dallas, Texas) in addition to appropriate compounds and stimuli. Superoxide generation is expressed as nanomoles cytochrome *c* reduced per 10^6 PMN.

Lysosomal Enzyme Release. The extracellular release of PMN granule-associated enzymes was measured as previously described (16–18) in duplicate reaction mixtures identical to those employed for the determination of superoxide generation, but in the absence of cytochrome *c*. After incubation, the reaction mixtures were centrifuged in the cold and cell-free supernates removed for enzyme assays. Beta-glucuronidase was determined after 18 h of incubation with phenolphthalein glucuronidate (Sigma Chemical Co.) as substrate (22). Lysozyme was determined by the rate of lysis of *Micrococcus lysodeikticus* (Worthington Biochemical Corp., Freehold, N. J.) measured by decrease in absorbancy at 450 nm (23). Crystalline hen egg-white lysozyme (Worthington Biochemical Corp.) was used as a standard. The cytoplasmic enzyme, lactate dehydrogenase (LDH) was measured as described previously (24) and used as an indicator of cell viability (16–18, 25). Total enzyme activities were determined in simultaneously run duplicate reaction mixtures to which had been added the detergent Triton X-100 (0.2%, vol/vol) (Rohm and Haas Co., Philadelphia, Pa.).

Binding of Con A to PMN. For these experiments, purified preparations (98 ± 1%) of PMN were obtained by means of Hypaque/Ficoll gradients (26). To these (5 × 10^6 PMN) were added 0.16 μCi (0.26 μg) ^{3}H-Con A (New England Nuclear, Boston, Mass.) plus 30 μg of unlabeled lectin. After 15 min of incubation, the PMN were sedimented (155 *g* for 10 min), washed three times with buffer, and allowed to dry at room temperature. The dried pellets were digested with 0.5 ml of 0.2

TABLE I

Enzyme Release from Human PMN Exposed to STZ: Effects of Lidocaine and Tetracaine

Additions to PMN*	Enzyme released‡			
	(n)	β-Glucuronidase	Lysozyme	LDH
None	(11)	2.6 ± 0.3	1.6 ± 0.2	8.8 ± 1.8
STZ (control)	(11)	15.4 ± 0.6	8.1 ± 0.4	9.6 ± 2.2
STZ + lidocaine (14 mM)	(5)	8.9 ± 1.1 <0.001§	4.9 ± 0.3 <0.001§	23.4 ± 4.4 <0.005§
STZ + lidocaine (7 mM)	(5)	12.3 ± 1.9 <0.05	6.7 ± 0.3 <0.025	18.1 ± 2.3 <0.025
STZ + tetracaine (1.0 mM)	(6)	4.5 ± 0.4 <0.001	2.1 ± 0.2 <0.001	15.2 ± 2.4 >0.05
STZ + tetracaine (0.5 mM)	(6)	8.2 ± 0.5 <0.001	5.0 ± 0.3 <0.001	12.8 ± 2.2 >0.1
STZ + tetracaine (0.25 mM)	(6)	11.4 ± 0.7 <0.001	6.4 ± 0.3 <0.01	10.3 ± 2.1 >0.5

* PMN were incubated with STZ (1.0 mg/ml) ± local anesthetic for 15 min at 37°C.

‡ β-glucuronidase, micrograms phenolphthalein/18 h/2 × 10^6 PMN; lysozyme, micrograms crystalline hen egg-white lysozyme equivalents/2 × 10^7 PMN; LDH, absorbance units/2 × 10^6 PMN. Total activity released by 0.2% Triton X-100: β-glucuronidase = 280 ± 24; lysozyme = 79.5 ± 8.9; LDH = 895 ± 26. Mean ± SEM. (n) = number of experiments.

§ *P* vs. control (Student's *t* test).

N NaOH for 2 h at 56°C, followed by the addition of 0.2 ml of 3.0% acetic acid and 0.5 ml of distilled water. Aliquots (1.0 ml) were added to 20 ml Bray's solution (New England Nuclear) and counted in a Beckman LS-100 liquid scintillation counter (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Results have been expressed as counts per minute per 5 × 10^6 PMN.

Other Compounds. Lidocaine HCl (Astra Pharmaceutical Products, Inc., Worcester, Mass.) and tetracaine HCl (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) were prepared daily in buffer. Xanthine and xanthine oxidase were from Sigma Chemical Co.

Electron Microscopy and Morphology. Replicate cell suspensions were fixed at room temperature as described previously (8), and embedded in Spurr's low viscosity epoxy. Lead citrate-stained sections were viewed in a Zeiss EM 9S Microscope (Carl Zeiss, Inc., New York). All centrioles visible on six sections cut from each of three experiments were photographed at × 17,000 and printed at × 50,000 on high contrast paper. Pericentriolar microtubules were counted from all of the centrioles photographed as described (18). Profiles were considered to be microtubules if they had straight, parallel sides, 24–28 nm apart, were at least 55 nm long, and were more electron dense than the ground cytoplasm. Microtubules were counted in a 2 × 2 μm square centered upon a centriole.

Results

Lysosomal Enzyme Release. As has been reported previously from this laboratory (16, 17, 20, 25, 27), normal human PMN selectively release, or secrete, lysosomal but not cytoplasmic enzymes upon exposure to phagocytosable particles such as STZ. Such enzyme release very likely occurs by a mechanism which has been termed "regurgitation during feeding" (25, 27), whereby fusion of lysosomal granules with incompletely closed, newly forming, phagosomes results in the leakage of granule constituents into the extracellular environment. Enzyme release from cells exposed to STZ in the presence or absence of local anesthetics is shown in Table I. Depending upon the anesthetic agent and the concentration at which it was employed, enzyme release in this experimental system could be significantly reduced. Appropriate control experiments excluded the possibilities that particles or other test reagents interfered with the enzyme assays or that there was selective adsorption of enzymes to cells or particles after their release into the suspending buffer. In several experiments, reduction of lysosomal enzyme release by the local anesthetics was associated with increased leakage of the cytoplasmic enzyme, LDH into the surrounding medium. This indication of cytotoxicity was most noticeable with

TABLE II

Enzyme Release from Cytochalasin B-Treated Human PMN: Effect of Tetracaine

Additions to PMN*	Enzyme released‡			
	(n)	β-Glucuronidase	Lysozyme	LDH
None	(14)	2.9 ± 0.3	1.8 ± 0.2	10.4 ± 1.9
STZ (Control)		24.6 ± 1.7	15.5 ± 0.6	13.8 ± 2.2
STZ + tetracaine (1.0 mM)	(7)	10.3 ± 1.3 <0.001§	6.6 ± 0.4 <0.001§	16.9 ± 2.5 >0.1§
STZ + tetracaine (0.5 mM)	(7)	17.2 ± 1.5 <0.01	10.5 ± 0.4 <0.01	15.4 ± 2.1 >0.5
STZ + tetracaine (0.25 mM)	(7)	19.8 ± 1.7 <0.05	12.3 ± 0.5 <0.05	14.5 ± 2.2 >0.5
EACA-ZTS (Control)	(7)	63.7 ± 2.4	24.8 ± 0.7	14.4 ± 2.1
EACA-ZTS + tetracaine (1.0 mM)	(7)	8.6 ± 0.7 <0.001	2.4 ± 0.3 <0.001	16.1 ± 1.9 >0.1
EACA-ZTS + tetracaine (0.5 mM)	(7)	33.7 ± 1.9 <0.001	12.3 ± 0.6 <0.001	15.0 ± 2.1 >0.5
EACA-ZTS + tetracaine (0.25 mM)	(7)	48.9 ± 2.0 <0.01	19.7 ± 0.7 <0.05	14.0 ± 2.3 >0.5

* PMN were incubated at 37°C for 10 min with cytochalasin B (5.0 μg/ml) before the addition of STZ (1.0 mg/ml) or EACA-ZTS (10%, vol/vol). Cells were incubated with stimuli ± tetracaine for 15 min at 37°C.

‡ As described in Table I. Mean ± SEM. (n) = number of experiments.

§ *P* vs. control (Student's *t* test).

high concentrations of lidocaine; consequently, only tetracaine was employed in the experiments described below.[2]

In preliminary experiments, we observed that lidocaine and tetracaine significantly reduced the initial rate of ingestion by PMN of opsonized paraffin oil/oil red O droplets (13) (36% with 14 mM lidocaine, 37% with 1.0 mM tetracaine). Consequently, the local anesthetics could have reduced lysosomal enzyme release from PMN exposed to STZ simply by diminishing phagocytosis; particle ingestion is probably a prerequisite for maximal enzyme release in this experimental system. In order to examine this possibility, we measured the effects of tetracaine upon selective lysosomal enzyme release in experimental systems in which phagocytosis could be excluded as a variable.

Cytochalasin B-treated PMN respond to the particulate stimulus, STZ, and to the soluble stimulus, EACA-ZTS by selectively releasing lysosomal enzymes (8, 14, 16–18, 20, 25). Surface stimulation of these cells, in the absence of phagocytosis, facilitates membrane fusion between lysosomal membranes and the plasma membrane leading to the discharge of granule contents into the external environment as if into a phagocytic vacuole (exocytosis). As was the case with normal cells exposed to STZ, tetracaine significantly reduced enzyme release from cytochalasin B-treated PMN exposed either to STZ or to EACA-ZTS (Table II).

Another stimulus for enzyme secretion by PMN which does not require phagocytosis (or cytochalasin B) is Con A (19, 28). In contrast to other stimuli, however, Con A provokes the discharge from these cells only of lysozyme; azurophil granule constituents (e.g., beta-glucuronidase) are not released. Con A-mediated lysozyme release from normal human PMN was also reduced significantly by tetracaine (data not shown).

Thus, it appeared that tetracaine, at concentrations which were not cytotoxic, was capable of interfering with the complex process of exocytosis in PMN regardless of the manner in which the cells were stimulated. Inhibition of particle ingestion could be excluded as accounting for this effect. Whereas these

[2] Dibucaine, procaine, and butacaine similarly caused leakage of LDH from PMN at concentrations which reduced lysosomal enzyme release.

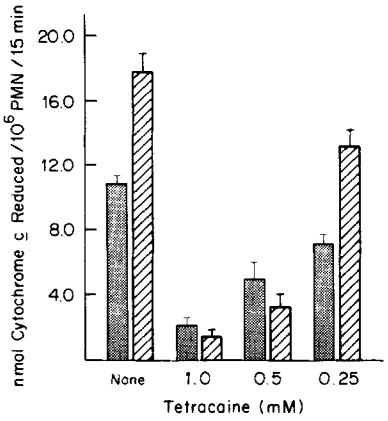


FIG. 1. Influence of tetracaine upon the generation of superoxide anion by normal (stippled bars) and by cytochalasin B-treated (hatched bars) PMN exposed to STZ. PMN were preincubated with cytochalasin B (5.0 μg/ml) for 10 min before exposure to STZ (1.0 mg/ml) for 15 min at 37°C. Superoxide anion generation was measured as superoxide dismutase-inhibitable cytochrome *c* reduction. Mean ± SEM, number of experiments (n), 7. Superoxide anion generation was reduced significantly by tetracaine ($P < 0.001$ with 1.0 mM and 0.5 mM, $P < 0.025$ with 0.25 mM).

findings were quite consistent with either direct or indirect effects of this anesthetic agent upon cytoskeletal elements and/or membrane fusion as the mechanism whereby it inhibited enzyme release, they did not rule out the possiblity that this agent acted by influencing the manner in which the various stimuli interacted with the cell surface to generate the "signals" that provoke exocytosis. Therefore, we examined the effect of tetracaine upon another consequence of PMN cell surface stimulation.

Superoxide Anion Generation. Human PMN respond to appropriate particulate and soluble stimuli with a marked increase in oxidative metabolism (29). PMN generate superoxide anion (measured as superoxide dismutase-inhibitable cytochrome *c* reduction) in a time- and concentration-related fashion independently of phagocytosis (19, 20). The responses of normal and of cytochalasin B-treated PMN to STZ are shown in Fig. 1. As was the case with lysosomal enzyme release, superoxide generation was reduced significantly by the local anesthetic, tetracaine. This effect of tetracaine was not due simply to its ability to interfere with the assay system since it was found not to influence the measurement of superoxide generated in an experimental system employing xanthine and xanthine oxidase (30). Tetracaine also significantly reduced superoxide generation by normal PMN exposed to Con A (data not shown). The effect of tetracaine upon the generation of superoxide by PMN, as well as its effect upon release of lysosomal enzymes, was completely reversible. Cells exposed to tetracaine for 15 min at 37°C and then washed only once with buffer, responded comparably to normal cells when subsequently challenged with either STZ or Con A.

Binding of Con A to PMN. The possibility that tetracaine may have influenced contact between the various stimuli and the PMN surface membrane was examined by measuring the effect of tetracaine upon binding of ^{3}H-Con A to these cells. Tetracaine (1.0 mM) had virtually no effect upon lectin-binding at 4°C. At 37°C, however, the uptake of ^{3}H-Con A by PMN was reduced (4,160 ± 340

cpm vs. 5,530 ± 480 cpm, $P < 0.05$) in the presence of the anesthetic. This reduction, which may have reflected either diminished endocytosis of labeled lectin or fewer binding sites as a consequence of diminished exocytosis, was very likely insufficient by itself to account for the markedly diminished response to Con A by tetracaine-treated cells as judged from previously reported dose-response and binding studies (19, 28).

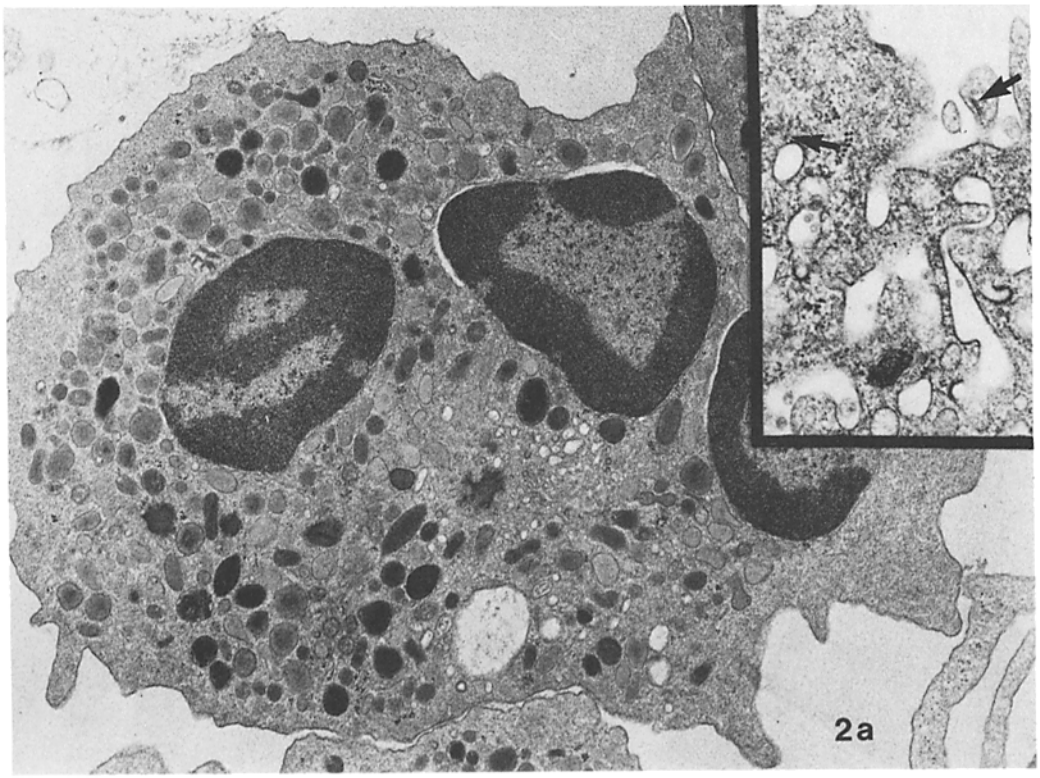
Electron Microscopy. PMN exposed to tetracaine were conspicuously different from their untreated counterparts when examined with the electron microscope. Compared to normal cells which have membrane ruffles and pseudopodia (Fig. 2 *a*), tetracaine-treated PMN were smooth and rounded with centrioles located in the geometric center of the spherical cell (Fig. 2 *b*). This change was similar to that reported in anesthetic-treated mouse macrophages (31), but was quite different from the changes in shape seen in PMN treated with cytochalasin B and/or colchicine. Cytochalasin B-treated PMN are rounded but not smooth surfaced, and in thin sections the retracted plasma membrane appears as invaginations toward centrally located centrioles (8, 9, 14). Colchicine-treated cells are irregular in shape with centrioles and Golgi apparatus displaced from their usual position and have a markedly reduced number of microtubules (9, 14, 16). Microtubules in tetracaine-treated PMN were not distinguishable in appearance, location, or number from those in corresponding control, unstimulated cells. Microtubule assembly in response to stimulation, however, was affected by pretreatment with tetracaine. Although the number of microtubules visible within a 2 × 2 μm square centered over the centriolar regions of treated and untreated cells was similar in the absence of stimulation (25.6 ± 2.5 vs. 28.2 ± 1.6, $P > 0.5$), the number visible after stimulation with Con A, for example, was increased only in untreated cells (39.9 ± 2.3). Tetracaine (1.0 mM) prevented this increase (25.6 ± 2.6, $P < 0.001$). A single wash with buffer was sufficient to restore the ability of tetracaine-treated PMN to respond normally to Con A.

Other morphologic responses to stimulation with Con A were very different in tetracaine-treated as compared to untreated PMN. In untreated cells, areas of the plasma membrane were invaginated to form narrow channels which in cross-section appeared as vacuoles (insert Fig. 2 *a*). In contrast, the plasma membrane of tetracaine-treated PMN exposed to Con A remained smooth and unruffled. Numerous microfilaments were visible in the subplasmalemmal cytoplasm (insert Fig. 2 *b*).

Discussion

The cationic local anesthetic agent, tetracaine reversibly inhibited two mem-

FIG. 2. (*a*) An untreated, normal human PMN sectioned through a centriole. The cell is irregular in profile with agranular pseudopodic extensions. × 11,000. Insert: A portion of the peripheral cytoplasm of a Con A-stimulated, untreated PMN. The membrane is thrown up into folds and regions bearing Con A-binding sites are being internalized. Microfilaments (arrows) are visible in the cytoplasm. × 24,000. (*b*) A similar section of a tetracaine-treated PMN. The cell profile is smooth and the granules appear larger and more densely stained than controls. × 11,000. Insert: The peripheral cytoplasm of a Con A-stimulated, tetracaine-treated PMN showing a circumferential arrangement of microfilaments but no change in the shape of the plasma membrane. × 24,000.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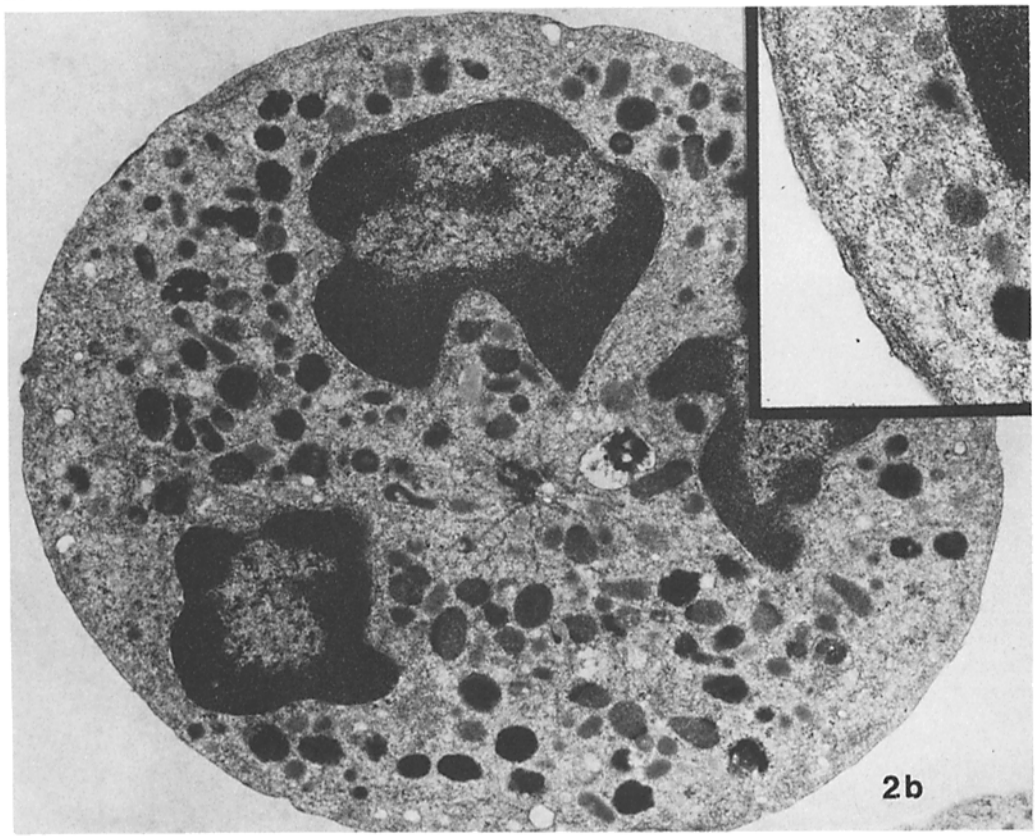
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brane-dependent responses of human PMN to surface stimulation; exocytosis of granule-associated enzymes and generation of superoxide anion. These two responses occur independently in stimulated cells (19, 20) and can also be influenced by pharmacologic agents which directly or indirectly affect microtubules, microfilaments, and cell membranes. For example, inhibition of microtubule assembly by colchicine modestly reduces release of lysosomal enzymes but has little, if any, influence upon the generation by stimulated PMN of superoxide anion (14, 32). Cytochalasin B, by inhibiting interaction of actin-binding protein and actin, interferes with subplasmalemmal microfilaments (7). Consequently, it enhances exocytosis and has variable effects upon PMN metabolism (depending upon the stimulus) (20, 32). Membrane-stabilizing agents, such as certain adrenal corticosteroids, reduce both enzyme release as well as superoxide generation (33). Thus, the effects of tetracaine upon PMN exposed to surface stimuli resemble most the effects of corticosteroids.

Whereas tetracaine may have influenced the function of cytoskeletal and/or contractile elements of PMN to produce the altered responses to surface stimuli which we observed, our results are also consistent with an effect of this compound upon the membranes of the cell. Indeed, ultrastructural alterations were observed primarily in the surface membranes of treated PMN. Gross changes in the morphology of microtubules and microfilaments, as observed in 3T3 cells (3), were not seen in tetracaine-treated human PMN, although microtubule assembly in response to stimulation was inhibited.[3] This supports the possibility that the function of these structures was affected so as to alter normal transmembrane control of surface receptor mobility. In fact, such an effect is consistent with the alterations of cell shape observed after tetracaine treatment as well as with some of the results of our biochemical studies demonstrating diminished exocytosis. Nevertheless, some of the most dramatic changes attributable to tetracaine treatment were metabolic and were unlikely to be due to effects of this compound upon either microtubules or microfilaments. Our data suggest, therefore, that tetracaine may act upon the membranes of PMN so as to modify stimulus-membrane interactions and retard membrane fusion much in the same fashion as corticosteroids (33).

Cationic local anesthetics affect biological membranes in a variety of ways (reviewed in reference 35). In excitable tissues, for example, they block sodium channels and, consequently, inhibit membrane depolarization by reducing sodium and potassium conductances (36). More general effects of these agents upon membranes have been attributed to both hydrophobic and electrostatic interactions with acidic phospholipids (37, 38) as well as to their ability to displace calcium ions from membrane sites (35, 39, 40). These effects produce expansion of membranes and promote membrane stability (35). It should be noted, however, that at high concentrations, cationic anesthetics enhance the fluidity of membrane phospholipds and may even cause lysis of perturbed bilayers (35, 38). Local anesthetics also have been reported to prevent mobilization of internal stores of calcium in various types of cells (41). These effects of local anesthetics can be manifested by a variety of altered cellular functions. They reduce osmotic fragility of erythrocytes (35, 42), inhibit cell fusion (43, 44),

[3] Lidocaine has been reported to inhibit rabbit brain microtubule assembly in vitro (34).

decrease cell-to-cell as well as cell-to-substrate adhesion (45, 46), and inhibit exocytosis in such secretory cells as platelets (47), mast cells (48), chromaffin cells of the adrenal medulla (49), and beta-cells of the pancreas (50). In phagocytic cells, such as macrophages and PMN, they alter cell shape (31) and diminish spreading (31, 45), locomotion (51), and particle ingestion (with associated metabolic changes) (52).

The effects of tetracaine upon PMN responses to surface stimulation which are described in this report can be explained by several of the aforementioned effects of this compound upon biomembranes, particularly those that interfere with stimulus-response coupling and membrane fusion. Whereas it is also possible that tetracaine acted upon microtubules and microfilaments, or their membrane attachments in human PMN, the data suggest that the actions of tetracaine in this cell type are not mimicked, as they appear to be in others (1–3), by the combined actions of cytochalasin B and colchicine. The reasons for this are unclear. Whatever the explanation may be, our results provide an exception to a recently hypothesized "general" mechanism of action of local anesthetics.

Summary

Cationic local anesthetics have been reported to influence cellular responses to surface stimuli by interfering with the function of microtubules and microfilaments. Since unimpaired microtubule and microfilament functions are required by human polymorphonuclear leukocytes in order to respond normally to surface stimulation, we have studied effects of the local anesthetic, tetracaine on the function and morphology of these cells in vitro. Tetracaine (0.25–1.0 mM) significantly reduced extracellular release of the lysosomal enzymes, beta-glucuronidase and lysozyme from polymorphonuclear leukocytes exposed to serum-treated zymosan (a particulate stimulus), zymosan-treated serum (a soluble stimulus), and to the surface-active lectin, concanavalin A. Tetracaine also significantly reduced superoxide anion production (superoxide dismutase-inhibitable cytochrome *c* reduction) by these cells. Tetracaine was not cytotoxic and its effects could be reversed completely by washing cells once with buffer. Electron microscope examination of tetracaine-treated cells revealed marked alterations of surface membranes. Microtubules and microfilaments appeared normal in "resting" polymorphonuclear leukocytes, but the increase in microtubules normally observed in stimulated cells was not seen after tetracaine treatment. These results suggest that tetracaine interferes with those interactions between immune reactants and the polymorphonuclear leukocyte cell surface which provoke exocytosis and increased oxidative metabolism.

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