Biochemical Pharmacology, Vol. 27, pp. 2447–2453.

0006-2952/78/1015-2447$02.00/0

INHIBITION BY TERTIARY AMINE LOCAL ANESTHETICS OF PHAGOCYTOSIS IN CULTURED MOUSE PERITONEAL MACROPHAGES

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(*Received* 21 *November* 1977; *accepted* 26 *January* 1978)

Abstract—The tertiary amine local anesthetics, dibucaine, tetracaine and procaine, have been shown to exert a reversible inhibition of phagocytosis of opsonized sheep red blood cells by cultured mouse peritoneal macrophages. The observed inhibition was due primarily to a functional alteration of the macrophage. The potencies of the three local anesthetics for inhibition of phagocytic uptake were proportional to their lipophilic nature as measured by octanol–water partition. Inhibition of phagocytosis by tetracaine was antagonized by Ca^{2+}. The above observations suggest that the general properties which characterize the action of tertiary amines as anesthetics, namely lipophilic nature and antagonism by Ca^{2+}, are also those attributable to the inhibition of macrophage phagocytosis *in vitro*.

The pharmacologic effects of tertiary amine local anesthetics have generally been considered to result from their interaction with membrane lipids[1–5], particularly the acidic phospholipid head group[2–5]. Recent observations indicate that local anesthetics can also affect cellular cytoskeletal organization[6–8]. Hence, it has been suggested[6] that the previously reported inhibitory effects of local anesthetics on cell spreading and motility[9–11], cell aggregation[12] and exocytosis[13] might be reasonably reevaluated in terms of the action of these drugs on cellular cytoskeletal organization in addition to membrane lipids, since similar inhibition of these processes can be produced by treatment with drugs acting on microtubules and/or microfilaments[13–16].

Examination of phagocytizing macrophages has revealed a system of microtubules and microfilaments surrounding the particle being ingested[17]. The active participation of microtubules and microfilaments in phagocytosis has been suggested through the inhibition of this process by disruption of either microtubule or microfilament organization. Alkaloid drugs such as colchicine and vinblastine, which cause a depolymerization of microtubule protein, do not affect phagocytosis *per se*, but modify the internalization of membrane receptors[18, 19]. Cytochalasin B, which disrupts microfilament organization, has been shown to reversibly inhibit phagocytosis[20, 21].

The inhibition of phagocytosis by tertiary amines has been mentioned briefly in connection with other studies[22, 23]; however, detailed information on their action is lacking. The present study was designed to evaluate the effects of the tertiary amine local anesthetics, tetracaine, dibucaine and procaine, on macrophage phagocytic uptake of opsonized sheep red blood cells (SRBC), and to gain some understanding of the action of tertiary amine local anesthetics on biological membranes.

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MATERIALS AND METHODS

Reagents. Dibucaine HCl and procaine HCl were a generous gift of Dr. George Poste (Roswell Park Memorial Institute, Bufflo, NY). Tetracaine HCl was purchased from K & K Laboratories, Inc. (Plainview, NY). Anhydrous calcium chloride, reagent grade, was purchased from Baker & Adamson (Morristown, NJ). $Na_2[^{51}Cr]O_4$ (sp. act. 380 mCi/mg) was purchased from New England Nuclear (Boston, MA). Eagle's minimum essential medium without calcium and magnesium was purchased from Flow Laboratories (Rockville, MD).

Animals. Inbred C57BL/6 mice were obtained from the Experimental Animal Production Area, Frederick Cancer Research Center, Frederick, MD.

Preparation and purification of macrophage cultures. Macrophages were obtained from normal C57BL/6 mice. Three ml of thioglycollate medium (Baltimore Biological, Cockeysville, MD) was injected intraperitoneally (i.p.) into each mouse and 5 days later the animals were killed by ether inhalation. Their peritoneal exudate cells (PEC) were harvested by lavage with Hanks' balanced salt solution (HBSS) (Grand Island Biological, Grand Island, NY) containing 2 units/ml of heparin (Schein, Flushing, NY). The PEC were centrifuged at 250 *g* and resuspended in Fagle's minimum essential medium supplemented with 10% fetal calf serum, sodium pyruvate, nonessential amino acids, penicillin-streptomycin, L-glutamine and twice the vitamins, designated CMEM (Flow Laboratories, Rockville, MD). PEC were plated into 16-mm dia. polystyrene culture dishes (Costar, Cambridge, MA) at a concentration of 5 10^5 PEC/dish in 1.0 ml. After a 30-min incubation at 37° in an atmosphere of 5% CO_2, the resultant monolayers were rinsed once and re-fed with an equal volume of CMEM to remove nonadherent cells. The remaining adherent cells were capable of phagocytizing carbon, had the typical macrophage morphology[24], and were used in the subsequent studies.

Preparation of opsonized ^{51}Cr-*labeled sheed red*

blood cells. SRBC were maintained in Alsever's solution (Grand Island Biological, Grand Island, NY) at 4°. Opsonization was accomplished by a 60-min incubation at 37° of 50 ml of a 2% v/v SRBC suspension with 0.1 ml of 1:10 dilution of mouse anti-SRBC-serum. The antiserum was prepared by injecting mice i.p. 7 days earlier with 0.25 ml of 10% v/v washed SRBC. The suspension was centrifuged, and the supernatant fraction was discarded. Radioactive labeling of the SRBC was accomplished by adding 200 μCi $Na_2[^{51}Cr]O_4$ (1 mCi/ml) for an incubation period of 2 hr at 37°. The opsonized SRBC were then washed three times in 50 ml of cold (4°) HBSS to remove excess ^{51}Cr, and the final volume was adjusted to yield a 2% v/v suspension of SRBC.

Quantitative assay for macrophage phagocytic uptake. Twenty-four hr after initial plating, macrophage cultures were rinsed and re-fed with either 1.0 ml CMEM or 1.0 ml CMEM containing various concentrations of the local anesthetics. After a 2-hr incubation, macrophage cultures were rinsed and re-fed with either 1.0 ml CMEM or 1.0 ml CMEM with local anesthetics. Two-tenths ml of 2% opsonized SRBC was added to each macrophage culture. The plates were swirled to ensure uniform distribution of the SRBC. The surface of the culture plate was completely covered with SRBC after the addition of 0.2 ml of a 2% suspension. After a 2-hr incubation at 37°, the cultures were washed three times with 1.0 ml HBSS prewarmed to 37° to remove nonphagocytized SRBC. The remaining adherent cells were lysed by incubating the monolayer with 1.0 ml of 1.0 N NaOH at 37°. The lysate was pipetted directly into 16 125 mm polystyrene test tubes (Falcon Plastics, Oxnard, CA) and radioactivity was determined using a Searle Analytic model 1185 counter with an NaI (T1) well-type detector.

Calculation of percentage inhibition.

$$\text{Per cent inhibition} = \left(1 - \frac{\text{cpm } ^{51}\text{Cr test macrophage}}{\text{cpm } ^{51}\text{Cr normal macrophage}}\right) 100$$

Statistical analysis. Results were analyzed for statistical significance by Student's t-test (two-tailed).

RESULTS

Local anesthetics have been reported to release cells attached to a substratum and have been used to harvest cultured macrophages[10, 25, 26]. It was, therefore, necessary to determine first the concentrations of the local anesthetics which did not cause detachment of the macrophages from the culture plate, and thus to establish an upper limit to usable concentrations. The upper limits were established to be 1.0 mM tetracaine, 1.0 mM dibucaine and 10 mM procaine over a 48-hr interval.

Effect of local anesthetics on phagocytosis of opsonized SRBC by macrophages. In the initial experiment, macrophage cultures were incubated for 2 hr with CMEM containing appropriate concentrations of dibucaine, tetracaine or procaine. The cultures were rinsed and challenged with the phagocytic stimulus in either the previous incubation concentration of the local anesthetic (Table 1) or CMEM alone (Table 2). Dibucaine treatment caused the greatest inhibition of macrophage phagocytosis, whereas procaine exerted the least effect; tetracaine was found to have an intermediate effect. The different potencies of the local anesthetics corresponded well with their lipophilic natures as measured by octanol–water partition coefficients[9].

A comparison of the levels of inhibition of macrophage phagocytosis in Table 1 with those in Table 2 suggested three possible explanations. First, when the cultures were washed free of the local anesthetics and phagocytosis was allowed to occur in CMEM (Table 2), a reversal of local anesthetic-induced inhibition of phagocytosis could have begun to occur. Second, when phagocytosis was allowed to occur in the pretreatment concentration of the local anesthetic (Table 1), an effect could also be exerted on the opsonized SRBC that

Table 1. Inhibition of macrophage phagocytic uptake of opsonized [^{51}Cr]SRBC by local anesthetics*

Treatment concn (mM)	Assay concn (mM)	Average ^{51}Cr activity in lysate at 2 hr† (cpm ± S.D.) Local anesthetic: Dibucaine	Tetracaine	Procaine
10.0	10.0	Not done	Not done	241 ± 74‡ (98)§
1.0	1.0	2 ± 1‡ (99)§	1 ± 1‡ (99)§	7225 ± 1101‖ (26)§
0.1	0.1	842 ± 123‡ (92)§	2365 ± 420‡ (76)§	8737 ± 779¶ (10)§
0.05	0.05	2340 ± 427‡ (76)§	4516 ± 360‡ (54)§	Not done
0.01	0.01	7407 ± 914‖ (24)§	6915 ± 958‖ (29)§	9379 ± 484¶ (3)§

*SRBC input gave an activity of 22,718 ± 247; SRBC and macrophage cultures incubated in CMEM for 2 hr gave an activity of 9667 ± 591.

†Macrophage cultures were incubated for 2 hr in the local anesthetic, after which the phagocytic assay was incubated in the same concentration. Two-tenths ml of a 2% opsonized SRBC suspension, labeled with 200 μCi $Na_2[^{51}Cr]O_4$ (1 mCi/ml), was added to each culture dish. Values are means of triplicate cultures.

‡Counts significantly reduced from control value ($P < 0.001$).

§Percentage inhibition calculated as described in Materials and Methods.

‖Counts significantly reduced from control value ($P < 0.05$).

¶No statistical significance.

Table 2. Inhibition of macrophage phagocytic uptake of opsonized [^{51}Cr]SRBC by local anesthetics after removal of the drugs*

Treatment concn (mM)	Assay concn	Average ^{51}Cr activity in lysate at 2 hr† (cpm ± S. D.) Local anesthetic: Dibucaine	Tetracaine	Procaine
10.0	None, CMEM	Not done	Not done	4966 ± 395‡ (49)§
1.0	None, CMEM	6 ± 6‡ (99)§	1 ± 1‡ (99)§	8437 ± 74‖ (13)§
0.1	None, CMEM	2731 ± 169‡ (72)§	5269 ± 224‡ (46)§	8972 ± 343¶ (8)§
0.05	None, CMEM	6065 ± 45‡ (38)§	7622 ± 543‖ (22)§	Not done
0.01	None, CMEM	8251 ± 532‖ (15)§	8608 ± 1494¶ (11)§	9903 ± 220¶ (−2)§

*SRBC input gave an activity of 22,718 ± 247; SRBC and macrophage cultures incubated in CMEM for 2 hr gave an activity of 9667 ± 591.

†Macrophage cultures were incubated for 2 hr in the local anesthetic after which the phagocytic assay was incubated in CMEM. Two-tenths ml of a 2% opsonized SRBC suspension, labeled with 200 μCi $Na_2[^{51}Cr]O_4$ (1 mCi/ml), was added to each culture dish. Values are means of triplicate cultures.

‡Counts significantly reduced from control value ($P < 0.001$).

§Percentage inhibition calculated as described in Materials and Methods.

‖Counts significantly reduced from control value ($P < 0.05$).

¶No statistical significance.

Table 3. Effect of local anesthetic-treated opsonized SRBC on macrophage phagocytic uptake*

SRBC treatment concn (mM)	Assay concn	Average ^{51}Cr activity in lysate at 2 hr† (cpm ± S. D.) Local anesthetic: Dibucaine	Tetracaine	Procaine
10.0	None, CMEM	Not done	Not done	1955 ± 194‡ (28)§
1.0	None, CMEM	1796 ± 121‡ (35)§	2094 ± 132‖ (25)§	2679 ± 541¶ (3)§
0.1	None, CMEM	3166 ± 140¶ (−14)§	2891 ± 318¶ (−4)§	2464 ± 196¶ (11)§
0.05	None, CMEM	2514 ± 96¶ (9)§	2603 ± 109¶ (6)§	Not done
0.01	None, CMEM	2717 ± 535¶ (2)§	2739 ± 475¶ (1)§	2592 ± 83¶ (6)§

*SRBC input gave an activity of 13,811 ± 336; SRBC incubated in CMEM for 2 hr and macrophage cultures gave an activity of 2756 ± 308.

†Suspensions of opsonized SRBC were incubated 2 hr in the local anesthetic, washed and resuspended in CMEM. The phagocytic assay was incubated in CMEM. Two-tenths ml of a 2% opsonized SRBC suspension, labeled with 200 μCi $Na_2[^{51}Cr]O_4$ (1 mCi/ml), was added to each culture dish. Values are means of triplicate cultures.

‡Counts significantly reduced from control value ($P < 0.025$).

§Percentage inhibition calculated as described in Materials and Methods.

‖Counts significantly reduced from control value ($P < 0.05$).

¶No statistical significance.

enabled them to avoid engulfment. Third, local anesthetic interaction with SRBC might have caused leakage of ^{51}Cr, which could be interpreted as an apparent inhibition of phagocytosis.

To distinguish between these possibilities, we performed the following study. Opsonized, ^{51}Cr-labeled suspensions of SRBC were incubated for 2 hr in the various local anesthetics. The phagocytosis assay was then carried out in CMEM. The data (Table 3) demonstrate: (1) treatment of SRBC with the local anesthetics did not result in ^{51}Cr leakage from the cells over the time interval studied; (2) at the highest concentrations only (1.0 mM dibucaine or tetracaine and 10 mM procaine), significant inhibition of phagocytosis by local anesthetics (35, 25 and 28% respectively) was observed; and (3) at all other concentrations of local anesthetics, macrophage phagocytosis was not inhibited.

The above data suggested that the inhibition of macrophage phagocytosis by the local anesthetics was reversible (Tables 1 and 2) and was due to a functional alteration of the macrophages. To determine the extent to which the macrophages could recover, cultures of macrophages were incubated with local anesthetics for 2 hr. The cultures were rinsed and re-fed with CMEM. Two hr later, opsonized, ^{51}Cr-labeled SRBC in CMEM were overlaid on the monolayer (Table 4). At 1.0 mM tetracaine and dibucaine, no recovery was observed. At all other concentrations, recovery of phagocytic function was observed. To compare the action of the three local anesthetics to each other, the concentrations required to give 50 per cent inhibition were determined graphically to be 0.08 mM for dibucaine, 0.2 mM for tetracaine and 10 mM for procaine (Table 5). Microscopic examination of the monolayers suggested that inhibition of phagocytosis was uniform from cell to cell; i.e. when phagocytosis was inhibited by approximately 50 per

Table 4. Reversibility to local anesthetic-induced inhibition of macrophage phagocytosis of opsonized [^{51}Cr]SRBC*

Treatment concn (mM)	Assay concn	Average ^{51}Cr activity in lysate at 2 hr† (cpm ± S. D.) Local anesthetic: Dibucaine	Tetracaine	Procaine
10.0	None, CMEM	Not done	Not done	6315 ± 652‡ (40)§
1.0	None, CMEM	69 ± 14‡ (99)§	64 ± 26‡ (99)§	9626 ± 260‖ (8)§
0.1	None, CMEM	4610 ± 1071‡ (56)§	8876 ± 311‖ (15)§	9324 ± 132‡ (11)§
0.05	None, CMEM	7546 ± 989‖ (18)§	9331 ± 572¶ (11)§	Not done
0.01	None, CMEM	9608 ± 62¶ (8)§	9865 ± 615¶ (6)§	9006 ± 184‡ (14)§

*SRBC input gave an activity of 22,730 ± 702; SRBC and macrophage cultures incubated in CMEM for 2 hr gave an activity of 10,397 ± 74.

†Macrophage cultures were incubated 2 hr in the local anesthetic and allowed to recover for 2 hr in CMEM. The phagocytic assay was incubated in CMEM. Two-tenths ml of a 2% opsonized SRBC suspension, labeled with 200 μCi $Na_2[^{51}Cr]O_4$ (1 mCi/ml), was added to each culture dish. Values are means of triplicate cultures.

‡Counts significantly reduced from control value ($P < 0.001$).

§Percentage inhibition calculated as described in Materials and Methods.

‖Counts significantly reduced from control value ($P < 0.01$).

¶No statistical significance.

Table 5. Per cent recovery of macrophage phagocytic uptake observed at 50 per cent inhibition dose for dibucaine, tetracaine and procaine after a 2-hr recovery incubation in CMEM

Local anesthetic	50% Inhibition dose (mM)	Percentage recovery of phagocytic uptake at 50% inhibition dose
Tetracaine	0.20	56
Dibucaine	0.08	38
Procaine	10.00	20

cent, it appeared that a reduced number of SRBC were ingested per macrophage rather than there being a reduction in the number of macrophages phagocytizing.

Effect of Ca^{2+} *on local anesthetic-induced inhibition of macrophage phagocytosis.* The antagonism between Ca^{2+} and local anesthetics is well documented [1, 3, 27–29] and was investigated here to determine its participation in phagocytosis inhibition. Macrophage cultures were incubated with the various local anesthetics in CMEM or in Ca^{2+}–Mg^{2+} free medium. The cultures were rinsed and opsonized; ^{51}Cr-labeled SRBC suspended in CMEM or in Ca^{2+}–Mg^{2+} free medium were added. A marked increase in inhibition of macrophage phagocytosis was observed after macrophage incubation with the local anesthetics in the absence of Ca^{2+} (Table 6). When the macrophage incubation was with 0.1 mM tetracaine in Ca^{2+}–Mg^{2+} free medium, the inhibition of phagocytosis was 70 per cent compared to 19 per cent inhibition after macrophage incubation with identical levels of tetracaine suspended in CMEM. This relationship is seen for dibucaine and procaine as well (Table 6). When macrophage cultures were incubated for 2 hr with local anesthetics in CMEM or Ca^{2+}–Mg^{2+} free medium, then presented with opsonized ^{51}Cr-labeled SRBC in the same medium, no significant variation in inhibition of phagocytosis was observed (data not shown); i.e. continuous incubation in the local anesthetic, regardless of the presence of Ca^{2+}, gave identical results.

The difference in the levels of inhibition observed between CMEM and Ca^{2+}–Mg^{2+} free medium in the assay (Table 6) suggested that Ca^{2+} antagonized the effects of local anesthetics. The assay was therefore performed by supplementing Ca^{2+}–Mg^{2+} free medium with different concentrations of Ca^{2+} to determine if the inhibition could be reversed.

Table 7 records the resulting activity and the corresponding percentage inhibition of phagocytosis when macrophage cultures were incubated with 0.1 mM tetracaine in Ca^{2+}–Mg^{2+} free medium supplemented with varying concentrations of Ca^{2+}. Because the action of all three drugs appears to be similar, only tetracaine was used in these experiments. Incubation of macrophage cultures in varying concentrations of Ca^{2+} without tetracaine had no significant effect on their phagocytosis of opsonized SRBC. Incubation of macrophage cultures with 0.1 mM tetracaine and 0.1 mM Ca^{2+} resulted in a 39 per cent inhibition of phagocytosis. By incubating the macrophage cultures in 0.1 mM tetracaine supplemented with Ca^{2+} at concentrations greater than or equal to 1.0 mM, no significant inhibition of phagocytosis was observed. Therefore, the inhibition was antagonized by Ca^{2+}, resulting in full restoration of macrophage capability.

DISCUSSION

The multitude of functions in which the macrophage participates intimately involves the cellular periphery. Phagocytosis, pinocytosis, antigen

Table 6. Effect of Ca^{2+} on local anesthetic-induced inhibition of macrophage phagocytic uptake*

Treatment conc (mM)	Assay concn	Average ^{51}Cr activity in lysate at 2 hr† (cpm ± S. D.)
None, CMEM	None, CMEM	16,380 ± 2176
None, Ca^{2+}–Mg^{2+} free	None, Ca^{2+}–Mg^{2+} free	15,639 ± 2707
Tetracaine (0.1) (CMEM)‡	None, CMEM	13,343 ± 2382§ (19)‖
Tetracaine (0.1) (Ca^{2+}–Mg^{2+} free)¶	None, Ca^{2+}–Mg^{2+} free	4765 ± 1333** (70)‖
Dibucaine (0.1) (CMEM)‡	None, CMEM	6539 ± 2160†† (61)‖
Dibucaine (0.1) (Ca^{2+}–Mg^{2+} free)¶	None, Ca^{2+}–Mg^{2+} free	3885 ± 279** (76)‖
Procaine (10.1) (CMEM)‡	None, CMEM	6761 ± 873** (59)‖
Procaine (10.1) (Ca^{2+}–Mg^{2+} free)¶	None, Ca^{2+}–Mg^{2+} free	3369 ± 130** (79)‖

*SRBC input gave an activity of 44,509 ± 423.

†Macrophage cultures were incubated 2 hr in the local anesthetic, after which the phagocytic assay was incubated in medium alone. Two-tenths ml of a 2% opsonized SRBC suspension, labeled with 200 μCi $Na_2[^{51}Cr]O_4$ (1 mCi/ml), was added to each culture dish. Values are means of triplicate cultures.

‡Appropriate concentration of the local anesthetic was prepared in CMEM.

§No statistical significance.

‖Percentage inhibition calculated as described in Materials and Methods.

¶Appropriate concentration of the local anesthetic was prepared in Ca^{2+}–Mg^{2+} free medium.

**Counts significantly reduced from control value ($P > 0.001$).

††Counts significantly reduced from control value ($P > 0.005$).

Table 7. Ca^{2+}-dependent reversibility of local anesthetic-induced inhibition of macrophage phagocytic uptake*

Treatment concn	Assay concn	Average ^{51}Cr activity in lysate at 2 hr† (cpm ± S. D.)
None, Ca^{2+}–Mg^{2+} free	None, Ca^{2+}–Mg^{2+} free	16,959 ± 2389
0.1 mM Tetracaine (Ca^{2+}–Mg^{2+} free)‡	None, Ca^{2+}–Mg^{2+} free	9188 ± 401§ (46)‖
None, 0.1 mM Ca^{2+}¶	None, Ca^{2+}–Mg^{2+} free	14,703 ± 599
0.1 mM Tetracaine + 0.1 mM Ca^{2+}‡	None, Ca^{2+}–Mg^{2+} free	8970 ± 584** (39)‖
None, 1.5 mM Ca^{2+}¶	None, Ca^{2+}–Mg^{2+} free	15,654 ± 486
0.1 mM Tetracaine + 1.0 mM Ca^{2+}‡	None, Ca^{2+}–Mg^{2+} free	13,950 ± 2232†† (11)‖
None, 1.5 mM Ca^{2+}¶	None, Ca^{2+}–Mg^{2+} free	15,467 ± 473
0.1 mM Tetracaine + 1.5 mM Ca^{2+}‡	None, Ca^{2+}–Mg^{2+} free	15,544 ± 814†† (0)‖
None, 2.0 mM Ca^{2+}¶	None, Ca^{2+}–Mg^{2+} free	15,809 ± 1644
0.1 mM Tetracaine + 2.0 mM Ca^{2+}‡	None, Ca^{2+}–Mg^{2+} free	16,741 ± 2121†† (−5)‖
None, 5.0 mM Ca^{2+}¶	None, Ca^{2+}–Mg^{2+} free	13,463 ± 1158
0.1 mM Tetracaine + 5.0 mM Ca^{2+}‡	None, Ca^{2+}–Mg^{2+} free	11,193 ± 1802†† (17)‖

*SRBC input gave an activity of 63,221 ± 853.

†Macrophage cultures were incubated 2 hr in the local anesthetic, after which the phagocytic assay was incubated in medium alone. Two-tenths ml of a 2% opsonized SRBC suspension, labeled with 200 μCi $Na_2[^{51}Cr]O_4$ (1 mCi/ml), was added to each culture dish. Values are means of triplicate cultures.

‡Appropriate concentration of the local anesthetic supplemented with Ca^{2+} was made up in Ca^{2+}–Mg^{2+} free medium.

§Counts significantly reduced from control value ($P > 0.001$).

‖Percentage inhibition calculated as described in Materials and Methods.

¶Appropriate concentration of Ca^{2+} made up in Ca^{2+}–Mg^{2+} free medium.

**Counts significantly reduced from control value ($P > 0.01$).

††No statistical significance.

binding, secretion and cell recognition are essentially surface phenomena[30]. A primary event of macrophage participation in immunological phenomena concerns the recognition of a foreign invader or antigen by the macrophage membrane and the subsequent transmission of such recognition into an appropriate response on the part of the cell. For instance, the major role of macrophages in host defense, phagocytosis, has been studied for many years. Nonetheless, the exact nature of this phenomenon is still unclear.

The present studies demonstrate that the tertiary amine local anesthetics, dibucaine, tetracaine and procaine, exert a concentration-dependent, reversible inhibition of SRBC phagocytosis by cultured mouse peritoneal macrophages. The different potencies of the local anesthetics correspond well with their octanol–water partition coefficients[9]: dibucaine > tetracaine > procaine; that is the extent to which phagocytosis was inhibited is proportional to the affinity of the local anesthetic for lipid. Furthermore, the action of tetracaine, in inhibiting macrophage phagocytosis, could be antagonized by Ca^{2+}.

The inhibition observed was not due to effects of the local anesthetics on the opsonized SRBC

but on the macrophage itself, since treatment of SRBC with local anesthetics did not result in inhibition of phagocytosis (Table 3). A comparison of the data in Tables 2 and 4 indicates that the degree of recovery depends on the concentration of the local anesthetics. Recovery was not observed after incubation with 1.0 mM tetracaine or dibucaine. It is not possible to determine from the present experiments whether this inhibition was irreversible or whether the recovery period was of insufficient duration. However, irreversible inhibition above a critical local anesthetic concentration has been observed for rapid axonal transport[31]. This may be related to the present studies because axonal transport presumably occurs via filaments similar or identical to cytoskeletal elements.

Data obtained from studies with phospholipid vesicles[1, 2, 32] suggest that tertiary amine local anesthetics exert their actions at the level of anionic groups of acidic phospholipids. In support of this, the effects of local anesthetics have been correlated with their lipophilic character[33]. A linear relationship was reported[9] between octanol–water partition coefficients and the 50 per cent inhibitory dose for macrophage spreading on glass by tetracaine, dibucaine and procaine. Our present studies also demonstrate such a linear relationship for 50 per cent inhibition of macrophage phagocytosis and the octanol–water partition coefficients.

The importance of these points becomes clear in view of the influence of Ca^{2+}. Certain effects of local anesthetics on membranes result from their ability to displace membrane-associated Ca^{2+} from the anionic groups of acidic phospholipids[1–3, 32]. In this respect, Ca^{2+} has been shown to antagonize local anesthetic effects in model membranes[3, 32] and natural membranes[7], presumably antagonism for acidic phospholipid domains[32].

The relationship between 50 per cent inhibition for phagocytosis and octanol–water partition coefficients for the three tertiary amines strongly supports the above model. This implies that the observed effects are proportional to the affinity of the anesthetics for lipid. In the absence of Ca^{2+}, pretreatments with the local anesthetics resulted in a pronounced enhancement of inhibition of phagocytosis (Table 6). Moreover, the inhibitory effect of 0.1 mM tetracaine could be reversed by increasing the Ca^{2+} concentration to 1.0 mM in the pretreatment medium (Table 7). However, the continuous interaction of the local anesthetics with the macrophages resulted in a similar inhibition of phagocytic uptake, regardless of the Ca^{2+} concentration. These data suggest that the Ca^{2+} does not readily displace the tertiary amine local anesthetic, which appears to have a greater affinity for the sites.

Data have been reported previously which demonstrate that the relative anesthetic potency obtained with frog sciatic nerve trunk and the relative inhibition of Ca^{2+} binding obtained with a two-phase extraction system is such that dibucaine > tetracaine > procaine[1]. This appears to support our data on the concentration of the three local anesthetics required to produce 50 per cent inhibition of macrophage phagocytosis (Table 5).

We suggest that the lipophilic character of local anesthetics is responsible for their partition from the aqueous environment to the membrane lipids of macrophages. Once this partition into the membrane lipids occurs, the postulated interaction of local anesthetics with acidic phospholipids would cause a depolarization by displacing previously held Ca^{2+}. It is possibly depolarization by Ca^{2+} displacement which results in the subsequent disruption of cytoskeletal integrity. Although the displacement of calcium by local anesthetic agents in terms of local anesthetic activity *in vivo* is still a subject of much dispute[34, 35], the properties which characterize the action of tertiary amines as anesthetics, namely a lipophilic nature and antagonism by Ca^{2+}, are those attributable to the inhibition of macrophage phagocytosis *in vitro* as well.

Both the ability of tertiary amine local anesthetics to disrupt the cytoskeletal system[6–8] and the participation of microtubules and microfilaments in phagocytizing macrophages[17] imply that the inhibition of phagocytosis resulted from disruption of the macrophage cytoskeletal system. Based on this observation, one could envisage two possible modes by which the observed inhibition is mediated—either at the level of recognition of the opsonized SRBC by the macrophage or post-recognition at the ingestion step. The present experiments cannot distinguish between these alternatives and this is the subject of further studies.

Acknowledgement—Research sponsored by the National Cancer Institute Contract No. NO1-CO-75380 with Litton Bionetics, Inc.

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