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Effects of Local Anesthetics on Phospholipid Topology and Dopamine Uptake and Release in Rat Brain Synaptosomes

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Summary. The effects of local anesthetics on the topology of aminophospholipids and on the release and uptake of dopamine in rat brain synaptosomes have been examined. A metabolically intact preparation of synaptosomes was prepared which maintains aminophospholipid asymmetry and the capacity for sodium-driven uptake and depolarization-dependent release of dopamine. Incubation of synaptosomes with local anesthetics at 37 °C induced perturbations in the topology of aminophospholipids as determined by their reactivities to the covalent probe trinitrobenzenesulfonic acid. The reaction of trinitrobenzenesulfonate with phosphatidylethanolamine and phosphatidylserine was inhibited 10–20% by low concentrations of tetracaine (1–100 μM) and enhanced by high concentrations (0.3–1.0 mM). Other local anesthetics showed a similar biphasic effect with a potency order of dibucaine > tetracaine > lidocaine ≧ procaine. K^+-stimulated, Ca^{2+}-dependent release of [^{3}H]dopamine was inhibited significantly at low concentrations of tetracaine (1–10 μM) but enhanced at higher concentrations (0.1–1.0 mM). Dibucaine and procaine had a similar biphasic effect on the dopamine release. For each of the local anesthetics tested, the inhibition of the reaction of phosphatidylethanolamine and phosphatidylserine with trinitrobenzenesulfonate occurred at concentrations which were shown also to inhibit the release of [^{3}H]dopamine. Local anesthetics were shown to inhibit uptake of [^{3}H]dopamine with a potency order which reflects their potency in producing anesthesia. The inhibition of dopamine uptake by dibucaine, tetracaine, lidocaine, or procaine was characterized by inhibitory constants (K_I) of 1.8 ± 0.4 μM, 27 ± 5 μM, 190 μM and 0.5 mM, respectively.

Key words local anesthetics · TNBS · synaptosomes · phosphatidylethanolamine · phosphatidylserine · dopamine

Introduction

The biochemical mechanism by which local anesthetics interact with membranes and block conduction in neurons is not resolved. It is likely that both closed channel block and frequency-dependent block contribute to the inhibition of ion conduction through sodium channels during anesthesia (Strichartz, 1973; Hille, 1977). Local anesthetics differ in their ability to inhibit by one or the other of the two modes (Courtney, 1980). It now seems probable that there are at least two separate sites on the sodium channel that can be affected by local anesthetics (Khodorov et al., 1976; Cahalan & Almers, 1979). Synergism between lidocaine or its derivatives and benzocaine in inhibiting sodium current has been demonstrated (Mrose & Ritchie, 1978; Huang & Ehrenstein, 1981). The open state of the sodium channel represents one binding site to which both the cationic form of amine anesthetics and perhaps the uncharged species can bind. The binding enhances inactivation and effectively inhibits impulse conduction. A separate site on the sodium channel seems to be affected by only the neutral forms of local anesthetics and the resulting inhibition of Na^+ flux has been likened to that seen with alcohols and volatile general anesthetics in being proportional to the lipid solubility of the drug (Mrose & Ritchie, 1978). Hypotheses have been suggested to explain how the interaction of anesthetics with the lipid phase of the cell membrane could affect sites on the sodium channel (Lee, 1976; Trudell, 1977). It is therefore profitable to study the effects that physiologic concentrations of local anesthetics have on the arrangement of membrane lipids and whether these changes may be correlated with altered function of membrane proteins.

We have examined the effects of local anesthetics on synaptosomes prepared from rat brain. Aminophospholipid topology was determined at different concentrations of anesthetics by quantitating the extent of reaction of PE[1] and PS with TNBS, a probe which is impermeable under the conditions employed. The effects of local anesthetics on dopamine uptake and release also were determined under the same conditions in order to correlate changes in dopamine transport with changes in aminophospholipid topology. These data on the effects of local anesthetics on synaptosomes have relevance both to the action of

[1] *Abbreviations:* TNBS = 2,4,6-trinitrobenzene sulfonate; PE = phosphatidylethanolamine; PS = phosphatidylserine; ESR = electron spin resonance; TLC = thin-layer chromatography; DA = dopamine.

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local anesthetics in nerves and to the normal physiology of neurotransmitter release and uptake.

Materials and Methods

Preparation of Synaptosomes

Synaptosomes were isolated by a modification of a method reported by Fontaine, Harris and Schroeder (1980). All procedures were carried out at 0 °C. Whole brains (minus the cerebellum) from 150–200 g male Sprague Dawley rats were homogenized in isotonic sucrose containing 2 mM Hepes, pH 7.4, using a 250 μm clearance Thomas Teflon to glass homogenizer. Nuclear and tissue debris were removed by a low speed spin (1,500 × *g*, 8 min). The supernatant was centrifuged at 17,000 × *g* for 15 min to yield the crude synaptosomal pellet. This pellet was washed with sucrose-Hepes buffer and then suspended in 4 ml of buffer. The suspension was layered onto a discontinuous Ficoll gradient (12, 7.5 and 0% Ficoll in isotonic sucrose Hepes buffer) and centrifuged at 70,000 × *g* for 60 min. Synaptosomes banded between the 12 and 7.5% Ficoll layers and were removed with a Pasteur pipette. Protein was determined by the method of Lowry et al. (1951). Phospholipid phosphorus was quantitated by a modification of the procedure of Skipski, Peterson and Barchaq (1964). Synaptosomes contained 0.5 μmol of lipid phosphorus per mg of protein.

Reaction of Synaptosomes with TNBS

To determine the asymmetry of PE and PS and the effects of local anesthetics on that asymmetry, synaptosomes were reacted with TNBS at 0 °C so that only those aminophospholipids on the external face of the membrane react. Three hundred μl of the synaptosomal suspension equivalent to 700–1200 nmol of phospholipid phosphate were suspended in 4.7 ml of a modified Hank's buffer pH 7.4, containing different concentrations of local anesthetic and the following components: (in mM) 137, NaCl; 5.4, KCl; 4.2, $NaHCO_3$; 0.6, Na_2HPO_4; 0.4, KH_2PO_4; 0.6, $MgSO_4$; 0.5, $MgCl_2$; 1.0, $CaCl_2$; and 5.6, glucose. The mixture was incubated at 37 °C for 10 min and then immersed in ice water for 6–10 min. Five ml of buffer at pH 8.5 containing 120 mM NaCl, 5 mM KCl, 35 mM $NaHCO_3$, 5 mM glucose, and TNBS at 0 °C were added to the synaptosomes. The final TNBS concentration was 2 mM. The reaction was carried out at 0 °C for 20 min, then 10 ml of the pH 8.5 buffer containing 20 mM glycine were added in order to stop further reaction of TNBS with aminophospholipids. The synaptosomes were centrifuged for 15 min at 9,000 × *g* and the pellet was transferred to a glass test tube by two 1-ml CH_3OH rinses. Two ml of $CHCl_3$ were added, and the mixture was sonicated for 10 sec and allowed to extract overnight at 4 °C. The samples were centrifuged at 2,000 rpm for 10 min, and the supernatants containing the lipids were rectified according to the method of Folch, Lees and Stanley (1957). The lower phase containing the lipids was concentrated to a volume of 100–200 μl and applied to 20 × 20 cm silica gel TLC plates (Merck-Darmstadt silica gel 60, 0.25 mm). A solvent system of $CHCl_3/CH_3OH$/concentrated NH_4OH (65:25:4) was used to develop the plates. Phospholipid species were separated from each other and from the trinitrophenyl derivatives of aminophospholipids and were quantitated as described previously (Crain, Marinetti & O'Brien, 1978). All data are reported as mean ± SD.

Synaptosomal Uptake of [^{3}H]Dopamine

Sixty μl of the synaptosomal suspension containing 100–200 μg of protein were added to 440 μl of Hank's buffer pH 7.4 containing 0.25 mM Ca^{2+} and different concentrations of local anesthetic. The samples were incubated at 37 °C for 15 min at which time 490 μl of the Hank's buffer pH 7.4 containing 500 μM ascorbate and 12.5 μM iproniazid were added along with [^{3}H]dopamine (New England Nuclear) to give a final dopamine concentration of 100–120 nM. The samples were incubated at 37 °C for 5 min, and the reaction was stopped by the addition of 5 ml ice-cold Hank's buffer. The mixtures were immediately filtered through Whatman GF/C glass fiber filters, and the test tubes and filters were rinsed with 15 ml ice-cold buffer. Whole filters were counted for radioactivity, and the counts recorded were taken as a measure of total [^{3}H]dopamine uptake by the synaptosomes. Nonspecific uptake or binding of [^{3}H]dopamine was determined by doing the assay at 0 °C. This was subtracted from all values and represented about 3% of the total uptake at 37 °C.

Potassium-Stimulated Release of [^{3}H]Dopamine

Two and one-half ml of the crude synaptosomal pellet containing about 30 mg of protein were added to an equal volume of Hank's buffer pH 7.4 without calcium and containing 20 mM mannitol, 500 μM ascorbate, and 12.5 μM iproniazid. After 5 min at 37 °C, [^{3}H]dopamine was added to give a final concentration of 190–200 nM, and the incubation was continued for 15 min. The uptake reaction was stopped by immersing the test tube in ice water. The mixture was layered onto a discontinuous Ficoll gradient and centrifuged at 70,000 × *g* for 45 min. The [^{3}H]dopamine-containing synaptosomes were removed from the gradient by pipette and were kept at 0 °C until used. Seventy microliters of the dopamine-loaded synaptosomes containing 150–200 μg protein were added to 430 μl of Hank's buffer pH 7.4 containing 1 mM calcium, 10 μM benztropine, and various concentrations of anesthetic. After a 4-min incubation at 37 °C, 1 ml of a Hank's buffer pH 7.4 containing 5 or 75 mM potassium was added. The high potassium buffer was maintained isosmotic by omitting an equivalent amount of sodium. The incubation was continued at 37 °C for 4 min, and the release of dopamine was terminated by adding 5 ml of ice-cold Hank's buffer containing no calcium and then filtering the sample through Whatman GF/C glass fiber filters. Radioactivity remaining in the synaptosomes was determined by liquid scintillation counting. The proportion of [^{3}H]dopamine which was released was determined by comparing the potassium-treated samples to samples which were incubated only in the 430 μl of Hank's calcium-free benztropine buffer.

Electron Microscopy

Synaptosomes were incubated at 37 °C for 10 min in a Hank's mannitol buffer pH 7.4 containing zero or 100 μM tetracaine. The synaptosomes were then centrifuged into pellets, fixed in 0.1% glutaraldehyde in 0.1 M phosphate buffer, post-fixed in 1% osmium tetroxide, dehydrated in increasing concentrations of ethanol, and embedded in Spurr low viscosity resin. Semi-thin and ultra-thin sections were cut on an ultramicrotome, mounted on copper-rhodium grids, and stained with uranyl acetate and lead citrate. Sections were examined with a Zeiss 10A electron microscope.

Results

Electron micrographs of synaptosomes fixed in 0.1% glutaraldehyde after a 10-min incubation at 37 °C in Hank's mannitol buffer pH 7.4 with or without 100 μM tetracaine are shown in Fig. 1. The micrographs are representative samples of numerous fields which were scanned. By morphological criteria the synaptosomes appear to be intact and contain mito-

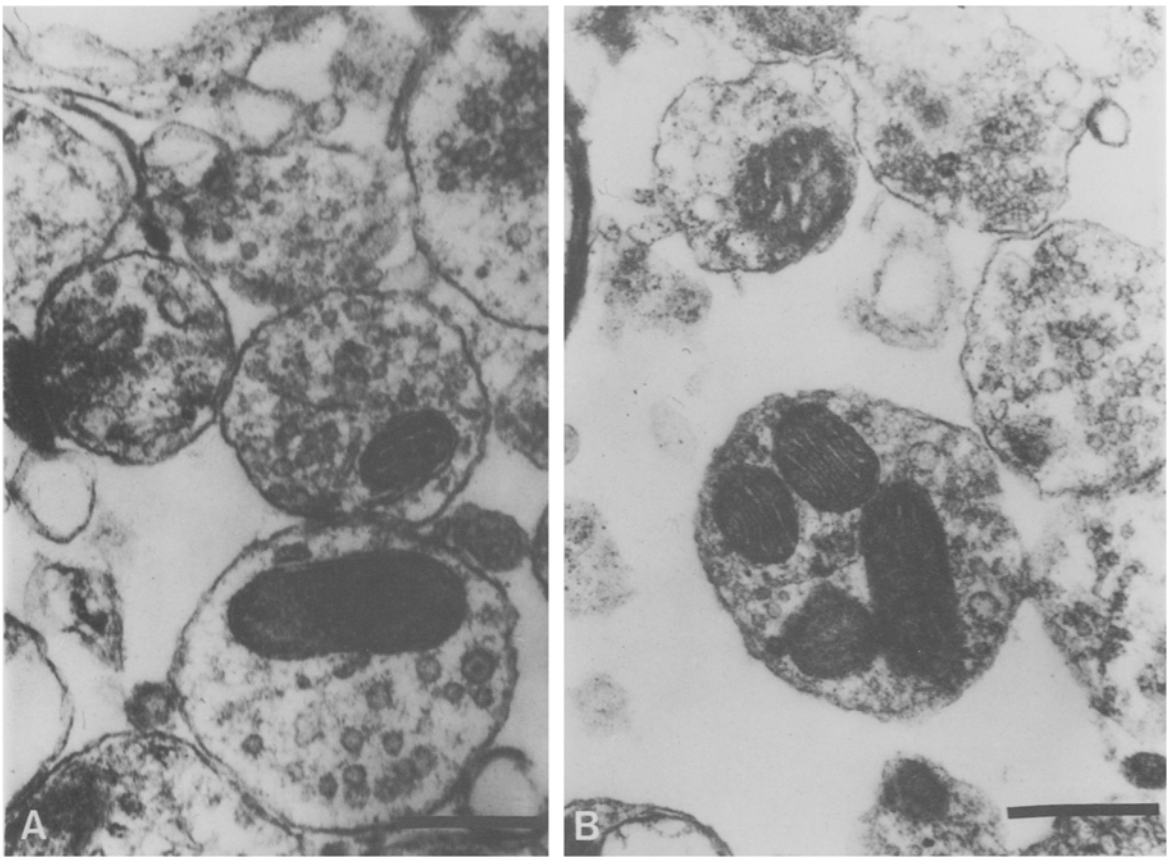

Fig. 1. Electron micrographs of rat brain synaptosomes incubated with (*B*) or without (*A*) tetracaine. Synaptosomes were incubated at 37 °C for 10 min in Hank's mannitol buffer pH 7.4 with or without 100 μM tetracaine. The synaptosomes were then fixed with 0.1% glutaraldehyde in 0.1 M phosphate buffer. Bar = 1 μm

chondria and synaptic vesicles as normally found in nerve endings. Incubation with tetracaine has no detectable effect on the synaptosome morphology. By other criteria, these preparations are intact and metabolically active. Thus synaptosomes maintain phospholipid asymmetry with $22.4 \pm 1.4\%$ of the PE and $9.8 \pm 0.7\%$ of the PS on the outside of the membrane as determined by reaction with TNBS at 0 °C. Moreover, ionic gradients are established and resting potential is maintained as evidenced by the capacity of the synaptosomes to accumulate [^{3}H]dopamine by sodium-driven transport and to release a fraction of this [^{3}H]dopamine by a K^+-stimulated, Ca^{2+}-dependent mechanism.

The effect of incubation of synaptosomes with tetracaine on the reaction of TNBS with aminophospholipids in shown in Figs. 2 and 3. The labeling reaction was carried out at 0 °C, and under these conditions TNBS is a nonpenetrating probe so only those aminophospholipids on the outside of the membrane react. Saturation of available PE and PS sites under control conditions occurs with 22% of the total PE and with 10% of the total PS. Incubation of synaptosomes with low concentrations of tetracaine (1–30 μM) results in fewer aminophospholipid molecules being able to react with TNBS when compared to control samples. At 3 μM tetracaine only $83 \pm 3\%$ of the control PE reaction is attained. However, 30 μM tetracaine is required to see an equivalent inhibition of reaction with PS ($83 \pm 5\%$). With greater concentrations of anesthetic the TNBS reaction is enhanced. At 300 μM tetracaine the extent of the TNBS reaction is $133 \pm 11\%$ for PE and $123 \pm 12\%$ for PS when compared to control samples. This biphasic effect on the reactivity of PE and PS towards TNBS was also observed with dibucaine, procaine, and lidocaine. The profiles are not shown, but the values for maximal inhibition of the reaction of PE and PS with TNBS are given in Table 1. For each of these drugs, low concentrations inhibited and high concentrations enhanced the reaction of PE and PS with TNBS. At high anesthetic concentrations, where membrane perturbation is the greatest, it is possible that part of the enhanced reaction may be due to penetration of TNBS, enhanced rate of phospholipid "flip-flop" onto the outer surface or lysis of some synaptosomes.

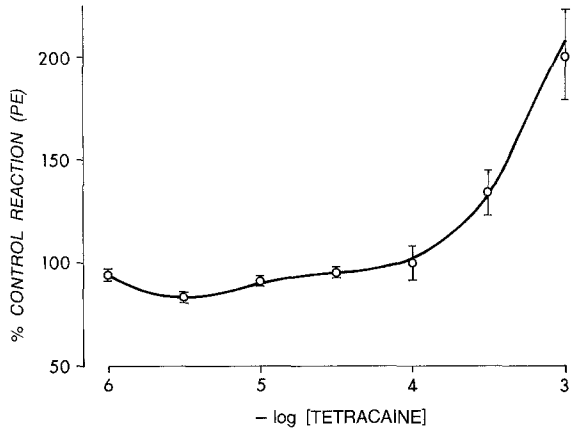

Fig. 2. Effect of different concentrations of tetracaine on the labeling of synaptosomal PE with TNBS. Synaptosomes were incubated at 37 °C for 15 min with tetracaine at the indicated concentrations and then at 0 °C for 20 min with 2 mM TNBS. Synaptosomal lipids were extracted and analyzed as described in the text. One hundred percent reaction represents reaction of 22% of the total PE. Bars indicate standard deviations of quadruplicate determinations

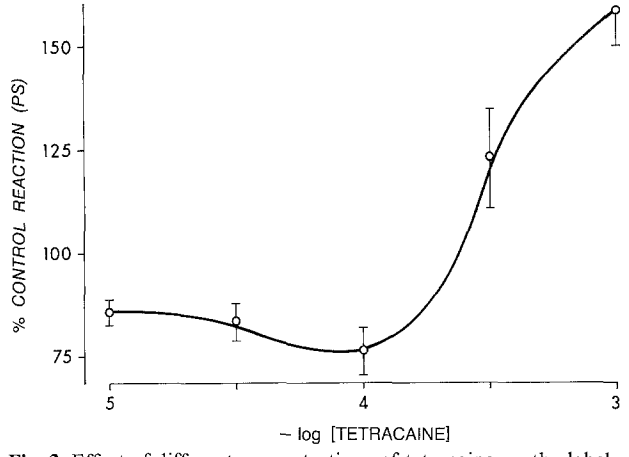

Fig. 3. Effect of different concentrations of tetracaine on the labeling of synaptosomal PS with TNBS. Conditions are the same as in Fig. 2. One hundred percent reaction represents reaction of 10% of the total PS. Bars indicate standard deviations of quadruplicate determinations

Table 1. Effects of anesthetics on the reaction of PE and PS with TNBS and on dopamine release and uptake[a]

	Concentrations giving maximal inhibition			K_I for [^{3}H]DA uptake	MBC
	TNBS reaction		[^{3}H]DA release		
	PE	PS			
Dibucaine	1(14)	10(7)	3(44)	1.8	2–3
Tetracaine	3(17)	30(20)	1–10(19)	27	3–5
Lidocaine	100(13)	100(11)	ND	186	ND
Procaine	100 (7)	1000(7)	100(32)	500	2000–3000

[a] Experimental conditions are given in the text. Values represent μM concentrations of anesthetic. Numbers in parentheses represent percent inhibition.
ND = not determined.
MBC = minimum concentration for sciatic nerve block (taken from Skou, 1954).

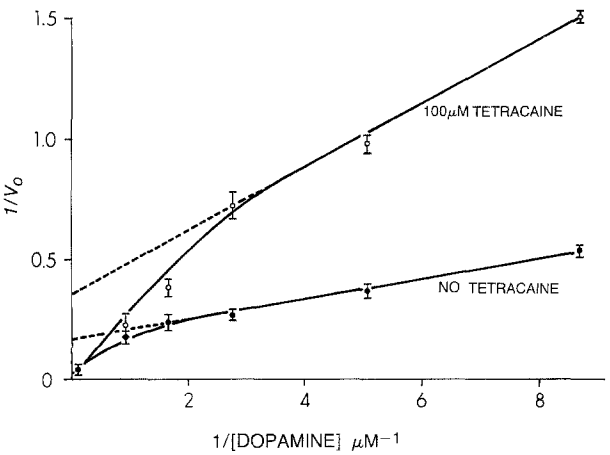

Fig. 4. Lineweaver-Burk kinetic analysis of dopamine uptake by synaptosomes in the presence and absence of tetracaine. Synaptosomes were incubated for 15 min at 37 °C in Hank's buffer with or without 100 μM tetracaine. [^{3}H]Dopamine and unlabeled dopamine were added along with buffer containing iproniazid and ascorbate to yield the indicated concentrations. Uptake was terminated after 5 min by dilution and filtration through glass fiber filters. Bars represent standard deviations of triplicate assay. V_0 is reported as pmol dopamine accumulated per mg protein per min.

In Table 1 are listed the concentrations of local anesthetics at which the greatest inhibition of the reaction of TNBS with PE and PS is observed. The order of potency for producing this inhibition is dibucaine > tetracaine > lidocaine > procaine for PS and is dibucaine > tetracaine > lidocaine = procaine for PE. It is noteworthy that for a given local anesthetic the greatest inhibition of labeling of PS was seen at anesthetic concentrations an order of magnitude greater than that observed for PE.

Purified synaptosomes retain the capacity for sodium-driven [^{3}H]dopamine uptake. Under the conditions employed, the system displays Michaelis-Menton kinetics for dopamine concentrations less than 1 μM with $K_M = 140$ nM and an extrapolated $V_{max} = 5.1$ pmol per mg protein per min. This K_M is comparable to the value of 130 nM reported by Holz and Coyle (1974) for purified striatal synaptosomes, although the V_{max}, as reported here for whole brain synaptosomes, is considerably less than the value of 125 pmol per mg protein per min determined by these workers for dopamine-rich striatal tissue.

The effect of local anesthetics upon the high affinity dopamine uptake process was examined. In Fig. 4 is shown a Lineweaver-Burk kinetic analysis of the control and the tetracaine-treated reactions. There are

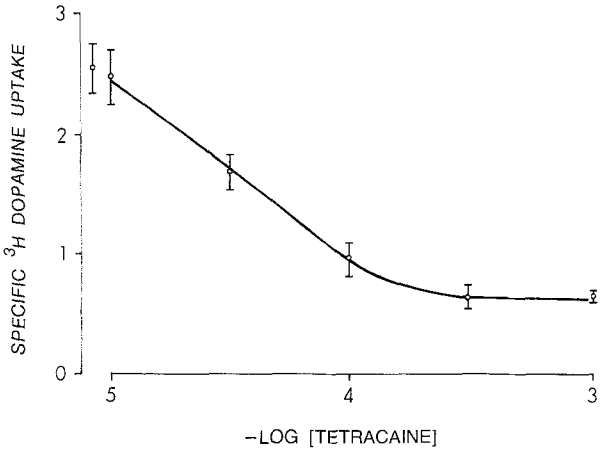

Fig. 5. Effects of different concentrations of tetracaine on the uptake of dopamine by synaptosomes. Uptake assay is the same as in Fig. 4 except the tetracaine concentration was varied and the dopamine concentration was kept constant at 120 nM. Bars represent standard deviations of triplicate assays. Unit of uptake is pmol dopamine accumulated per mg protein per min

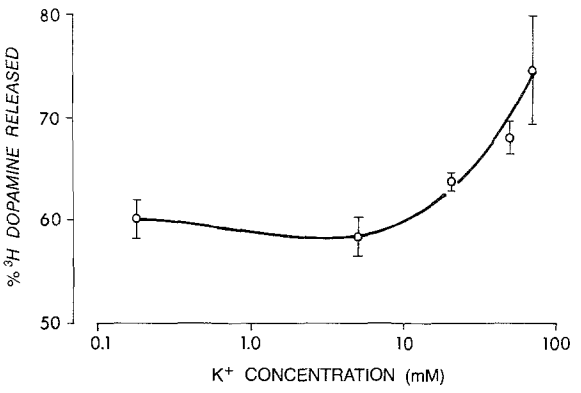

Fig. 6. Effect of external K^+ concentration on the release of [^{3}H]dopamine from synaptosomes. Crude synaptosomes were allowed to accumulate added [^{3}H]dopamine as described. [^{3}H]Dopamine-containing synaptosomes were then isolated and resuspended in Hank's mannitol buffer pH 7.4 with the indicated concentration of K^+. NaCl concentration was adjusted to maintain constant osmotic strength. Release at 37 °C was terminated after 12 min by dilution and filtration through glass fiber filters. Ca^{2+} concentration was 1.28 mM. Bars represent standard deviations of triplicate determinations

two components of the dopamine uptake with a break at about 0.5 μM dopamine. The second component probably represents a diffusion process, adding to the uptake at high dopamine levels (Clouet & Williams, 1974). The effects of local anesthetics on the uptake of low concentrations of dopamine were determined, representing effects on the uptake system at physiologic concentrations of dopamine. Tetracaine acts as a noncompetitive inhibitor in this system by decreasing the extrapolated V_{max} to 2.8 pmol per mg protein per min and by raising the K_m to 380 nM. Because the inhibition is noncompetitive, it is thought that tetracaine is acting at a site removed from the dopamine binding site, most likely in the lipid phase surrounding the protein uptake site. If the interaction of tetracaine with the surrounding lipid causes the transport protein to adopt a new conformation, then it might be expected that the binding of dopamine and the subsequent translocation of the complex would be suboptimal.

The inhibition of [^{3}H]dopamine uptake as a function of the concentration of tetracaine is shown in Fig. 5. From the concentration of inhibitor, which causes 50% inhibition of uptake, values of the inhibitory constant, K_I, can be determined using the Cheng-Prusoff procedure (1973). These constants for the four local anesthetics examined are given in Table 1. The potency order of the anesthetics for inhibiting the uptake of dopamine is the same as that which inhibits labeling of PS with TNBS and corresponds to the potency order of the anesthetics for producing nerve block. This potency order is: dibucaine > tetracaine > lidocaine > procaine (Blaustein & Goldman, 1966).

Synaptosomes maintain resting membrane potential and the capacity for calcium-dependent neurotransmitter release following depolarization by high external potassium concentrations. The dependence on potassium for the release of [^{3}H]dopamine from synaptosomes which had been allowed to take up the neurotransmitter, is shown in Fig. 6. In the presence of calcium and benztropine, an uptake inhibitor, a large increase in the release of [^{3}H]dopamine is observed with increasing potassium concentrations from 5 to 75 mM. The release of neurotransmitter which is enhanced by potassium and which is dependent upon calcium is believed to reflect the physiological process whereby vesicular bound dopamine is extruded from the nerve ending. This increase represents only a fraction (10–20%) of the previously accumulated [^{3}H]dopamine which is released. There is therefore a substantial, spontaneous release of [^{3}H]dopamine which is calcium-independent and may represent efflux of nonvesicular transmitter (Mulder, Van den Berg & Stoof, 1975) or leakage of dopamine from damaged synaptosomes.

The effect of a short incubation of synaptosomes with different concentrations of procaine on the potassium-elicited release of previously accumulated [^{3}H]dopamine is shown in Fig. 7. Relatively low concentrations (30–100 μM) of this anesthetic significantly inhibit the stimulated release of dopamine when compared to untreated preparations. However, at greater concentrations an enhancement of release is observed. The effects of tetracaine and dibucaine were similar

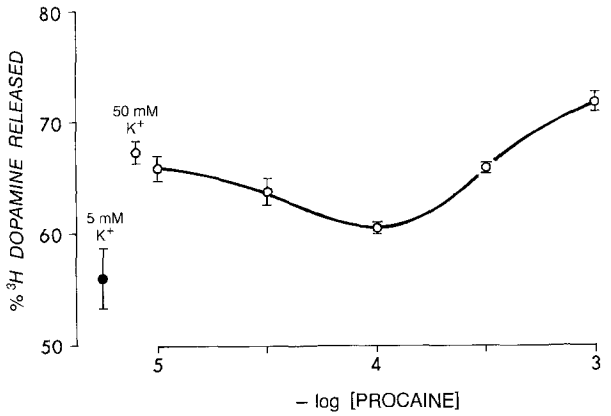

Fig. 7. Effect of procaine on the K^+-stimulated, Ca^{2+}-dependent release of [^{3}H]dopamine from synaptosomes. Assay was performed as described in the text. Bars represent standard deviation of quadruplicate determinations

Table 2. Effect of dibucaine and tetracaine on K^+-stimulated, Ca^{2+}-dependent release of [^{3}H]dopamine[a]

Anesthetic conc.	% of Control	
	Dibucaine	Tetracaine
None	100.0±3.3	100.0±6.6
100 nM	100.0±5.7	104.7±14.4
1 μM	107.6±6.7	80.9±4.0
3	56.1±3.2	–
10	87.5±6.1	83.9±6.1
30	81.0±4.0	–
100	109.3±3.9	299.5±4.0
1 mM	–	460.3±2.2

[a] One hundred percent release is defined as the amount of [^{3}H]-dopamine released from synaptosomes incubated in 50 mM K^+, 1 mM Ca^{2+} Hank's buffer pH 7.4 minus that released in 5 mM K^+, zero Ca^{2+} Hank's buffer pH 7.4. Values are the mean and standard deviations of four determinations.

to those of procaine and are reported in Tables 1 and 2. The inhibition of release at low concentrations of local anesthetics and enhancement at high concentrations are concordant with their effects on aminophospholipid (in particular PS) reactivity towards TNBS.

Discussion

Evidence has been presented which indicates that local anesthetics interact with synaptosomes and affect both phospholipid topology and neurotransmitter release and uptake. Low concentrations of these drugs cause an inhibition of labeling of PE and PS with TNBS. Under the experimental conditions TNBS is unable to permeate the synaptosome (Fontaine et al., 1980), so that only those effects of local anesthetics in the outer half of the bilayer are being detected. Greater concentrations of local anesthetics enhance the reaction, as has been reported with erythrocytes (Bradford & Marinetti, 1981). With synaptosomes, high concentrations of the anesthetic may become sufficiently disruptive to the membrane to allow penetration of TNBS. Thus, the effects of high concentrations of local anesthetics are difficult to interpret. Biphasic effects of local anesthetics on synaptosomal membrane fluidity have been reported (Rosenberg, 1980). At low concentrations the inhibition of labeling of PE and PS with TNBS is particularly interesting because less drug is required to inhibit the reaction of TNBS with PE than that needed to observe an equivalent inhibition of reaction with PS. This indicates that the anesthetic agent more readily perturbs the topology of PE than PS. Our previous studies have shown that PS is more tightly bound to membrane proteins than is PE (Crain et al., 1978).

It has been reported that relatively low concentrations of ethanol can enhance the binding of Ca^{2+} to erythrocyte membranes, perhaps by increasing the separation distance of critical negatively charged sites between adjacent protein or phospholipid components of a Ca^{2+} attachment site (Low, Lloyd, Stein & Rogers, 1979). The inhibition of reaction of aminophospholipids with TNBS caused by low concentrations of local anesthetics, as reported here, could be explained in part along these lines. At low concentrations of anesthetics the uncharged, free base form will partition preferentially into the lipid phase of the membrane (Ueda et al., 1980). Small amounts of anesthetics will separate protein and phospholipid components of Ca^{2+} attachment sites allowing optimal binding of the cation. Aminophospholipids involved in Ca^{2+} binding would be less reactive towards TNBS. Greater concentrations of anesthetic may further separate PE and PS molecules, displacing bound Ca^{2+} and an enhanced reaction with TNBS might be expected. Since the energy of a PE-Ca^{2+} linkage is much less than that of a PS-Ca^{2+} linkage (Seimiya & Ohki, 1973), lower concentrations of local anesthetics may be required for the anesthetic to affect the PE sites.

K^+-stimulated, Ca^{2+}-dependent release and sodium-driven uptake of dopamine by synaptosomes are affected differentially by local anesthetics. Like the effects on the labeling of aminophospholipids with TNBS, the release of dopamine is inhibited by low concentrations and stimulated by high concentrations of local anesthetics. These results may be interpreted

in light of the current theories for the requirement of fluid phospholipid domains or lateral phase separations for vesicular fusion (Papahadjopoulos et al., 1977; van der Bosch & McConnell, 1975). If low concentrations of these drug molecules stabilize areas of the plasma and/or vesicular membranes which are involved in the exocytotic process, then an inhibition of stimulated release might be expected. Conversely, high concentrations of local anesthetics may fluidize these regions of the membrane and thus potentiate fusion and release of neurotransmitter. To support this, it has been shown that low concentrations of lidocaine increased the order parameter of an ESR spin label in synaptic plasma membranes, indicating a decrease in fluidity (Rosenberg, 1980). However, high concentrations fluidized the membranes.

The biphasic effects of anesthetics on synaptosomal aminophospholipid topology, on ESR order parameters, and on the K^+-stimulated, Ca^{2+}-dependent release of dopamine suggest that a fluid membrane is required for the exocytotic process and that anesthetics affect the release by altering the lipid fluidity. The presence of the drug in the membrane may in itself directly affect membrane fluidity or the change may be secondary to anesthetic effects on phospholipases and acylating enzymes which may regulate the membrane fluidity.

The dose-dependent inhibition by local anesthetics of the sodium driven uptake of dopamine indicates that a specific lipid milieu is necessary for the uptake protein to remain functional. The dopamine-uptake process may be similar to intestinal sugar transport in that binding of the ligand and sodium ion(s) causes a conformational change of the transport protein which results in the ligand and sodium ion(s) being released at the opposite surface of the membrane. For dopamine transport, the intercalation into the membrane of even small amounts of anesthetic may make transport and presumably the conformational change of the protein unfavorable. Like the closed channel block of the sodium channel caused by local anesthetics in nerves, an interaction of anesthetics with the synaptosomal membrane lipids may prevent the dopamine-uptake protein from adopting an active conformation. In this way the dopamine-uptake protein and the sodium channel may be similar if both require changes in their conformation in order to function properly, and these changes are regulated by the physical state of the surrounding lipids.

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