MOLECULAR PHARMACOLOGY, 24:55–59

MOL PHARM

Inhibition of [^{3}H]Acetylcholine Active Transport by Tetraphenylborate and Other Anions

D. C. ANDERSON,[1] STEVEN C. KING,[2] AND STANLEY M. PARSONS

Department of Chemistry and the Marine Science Institute, University of California, Santa Barbara, California 93106

Received November 1, 1982; Accepted March 16, 1983

SUMMARY

The effects of tetraphenylborate and other anions on the active uptake of [^{3}H]acetylcholine by synaptic vesicles isolated from *Torpedo californica* electric organ were studied. Tetraphenylborate completely inhibits active uptake with a half-inhibitory concentration of 0.3 μM. Dipicrylaminate also half-inhibits at 0.3 μM, phenyldicarbaundecaborane at 14 μM, fluoride at 2 mM, thiocyanate at 3 mM, and azide at 16 mM. Tetraphenylborate had no effect on the vesicle ATPase activity or the transmembrane electric potential at low concentrations where it inhibits [^{3}H]acetylcholine active transport. The mechanism for tetraphenylborate inhibition is uncertain, but it might be similar to that of its action as a mitochondrial uncoupler. Solubility products for the acetylcholine, choline, and potassium salts of the tetraphenylborate and dipicrylaminate anions also were measured. The inhibition results confirm the hypothesis of Marshall and Parsons [*Br. J. Pharmacol.* 54:333–338 (1975)] that tetraphenylborate acts on intact neuromuscular preparations to inhibit transmitter storage, and constitute new pharmacological evidence that evoked release of acetylcholine is mediated by synaptic vesicles.

INTRODUCTION

The organic anion TPB[3] exhibits several presynaptic actions on neuromuscular transmission (1, 2). After an initial increase in the frequency of miniature end-plate potentials and quantal content of evoked AcCh release, neurotransmission fails due to a drastic reduction in quantal size (3). This biphasic action was attributed to two effects of TPB. The initial enhanced release of AcCh was assigned to a lesion in removal of intraterminal calcium ion, whereas the secondary failure was assigned to inhibition of AcCh storage by synaptic vesicles (4). Only after depletion of preexisting stores of AcCh does the drug become inhibitory. It appears not to have significant effects on other cholinergic components (1, 3–5).

Active transport of [^{3}H]AcCh by cholinergic vesicles isolated from the electric organ of *Torpedo* has been demonstrated recently. It is linked via a proton gradient to a bicarbonate-stimulated Ca^{2+}- or Mg^{2+}-ATPase (6–8), which has some kinetic similarities to the mitochondrial ATPase (9). Neither the ATPase nor transport of [^{3}H]AcCh requires other specific ions such as Na^+, K^+, or Cl^- (10).

Here we test Marshall and Parsons' hypothesis regarding the failure of neuromuscular transmission in the presence of TPB. We have studied the effects of it and some other anions on active transport or [^{3}H]AcCh by purified electric organ synaptic vesicles. An attempt to define the mechanisms of action of TPB was made by studying its effects on the ATPase and the transmembrane electric potential as well.

MATERIALS AND METHODS

Synaptic vesicles were isolated from *Torpedo californica* electric organ as described (10). Sodium TPB and Triton X-100 were obtained from Sigma Chemical Company (St. Louis, Mo.). Trimethylammonium PCB was a generous gift from Dr. Fred Hawthorne, Department of Chemistry, University of California, Los Angeles. It was converted to the potassium salt using ion exchange chromatography as described (11). Dipicrylamine was obtained from Aldrich Chemical Company (Milwaukee, Wisc.) and was neutralized with KOH to produce DPA. [^{14}C]Thiocyanate (11 mCi/mmole) was from ICN (Cleveland, Ohio), and [6,6′(*N*)-^{3}H]sucrose (5 Ci/mmole) was from Amersham (Arlington Heights, Ill.). All other chemicals were of the highest grade commercially available.

[^{3}H]AcCh uptake experiments were carried out by two methods. PCB, thiocyanate, fluoride, and azide inhibition experiments were conducted using [^{14}C]mannitol to determine the uptake ratio as described (8). The uptake ratio gives the ratio of the concentration of [^{3}H]AcCh inside the vesicles as compared with the concentration outside. TPB and DPA inhibition experiments were conducted in the

This research was supported by Grant NS 15074 from the National Institute of Neurological and Communicative Disorders and Stroke and by a grant from the Muscular Dystrophy Association (to S.M.P.).

[1] Recipient of a United States Public Health Service National Research Service Award.

[2] Present address, Department of Pharmacology, Harvard Medical School, Boston, Mass. 02115.

[3] The abbreviations used are: TPB, tetraphenylborate; AcCh, acetylcholine; Ch, choline; PCB, phenyldicarbaundecaborane; DPA, dipicrylaminate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; AH 5183, 2-(4-phenylpiperidino)cyclohexanol.

0026-895X/83/040055-05$02.00/0
Copyright © 1983 by The American Society for Pharmacology and Experimental Therapeutics.
All rights of reproduction in any form reserved.

MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012

indicated buffer. Active uptake was terminated at 30 min by vacuum filtration of the vesicle suspension through Millipore filters (Type HAWP-013 00). The filter was immediately washed three times by vacuum filtration with 1-ml volumes of buffer at 0°.

[^{14}C]Thiocyanate uptake was determined using [^{3}H]sucrose to measure the amount of buffer solution occluded in vesicle pellets after centrifugation. Vesicle suspensions containing TPB were equilibrated at 25° for 30 min with 0.9 mM [^{14}C]SCN^- and centrifuged at 4° for 10 min at 95,000 rpm on a Beckman Airfuge in an A-100/18 rotor. The concentration gradient for [^{14}C]SCN^- partitioning into the vesicles was calculated as the uptake ratio = $(T/A - S/B)/P \times V$, where T is total disintegrations per minute of [^{14}C]SCN^- in the 3% Triton X-100-solubilized pellet fraction, A is disintegrations per minute per microliter of [^{14}C]SCN^- in the supernatant, B is disintegrations per minute per microliter of [^{3}H]sucrose in the supernatant, S is disintegrations per minute of [^{3}H]sucrose in the pellet, P is milligrams of protein in the pellet, and V is the internal vesicular solvent volume per milligram of protein and is 5.3 μl/mg (12). Typical values for T, A, S, B, and P, respectively, were 1.2×10^5 dpm, 2×10^4 dpm/μl, 4×10^4 dpm, 2.4×10^4 dpm/μl, and 0.07 mg. Pellet protein was determined after solubilization in 3% Triton X-100.

Solubility products (K_{sp}) were determined by one of two methods. For TPB salts, the K_{sp} was preliminarily estimated as below. AcCh, Ch, or potassium chloride solutions in water were made at a concentration of $K_{sp}^{1/2}$ and titrated with 100-fold concentrated sodium TPB in water. The conductivity increase was determined with a Radiometer 2e conductivity meter, and the breakpoint was taken as the solubility limit for TPB. For DPA, saturated solutions of the pure salts of AcCh and Ch made by crystallization were prepared in water, and their concentrations were determined using the extinction coefficient, which was determined as 2.28×10^4 M^{-1} cm^{-1}.

The ATPase was assayed in the presence of TPB by measuring $^{32}P_i$ liberated from [γ-^{32}P]ATP in a manner similar to that described (13). Assays were linear to 10% hydrolysis of ATP. Protein was determined by the method of Bradford (14).

RESULTS

Inhibition of [^{3}H]AcCh active transport by TPB and other anions. Because some are unusual, the structures of the large organic anions studied here are shown in Fig. 1. Figure 2 shows the effects that the tested anions had on active uptake of [^{3}H]AcCh by purified *Torpedo* electric organ synaptic vesicles. TPB is a potent, concentration-dependent inhibitor exhibiting an IC_{50} of about 0.3 μM. DPA is as potent, whereas PCB, fluoride, thiocyanate, and azide inhibited with IC_{50} values of 14 μM, 2 mM, 3 mM, and 16 mM, respectively. Thus, all of these anions inhibit active transport, but with widely varying potencies. In the concentration range shown in Fig. 2, TPB had no effect on the passive uptake of [^{3}H]AcCh.

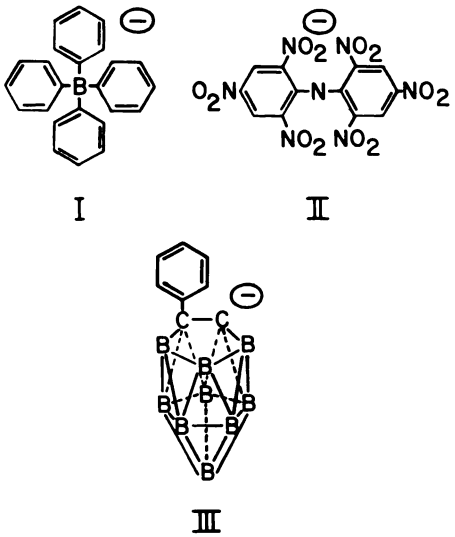

FIG. 1. *Structures of TPB (I), DPA (II), and PCB (III) anions*

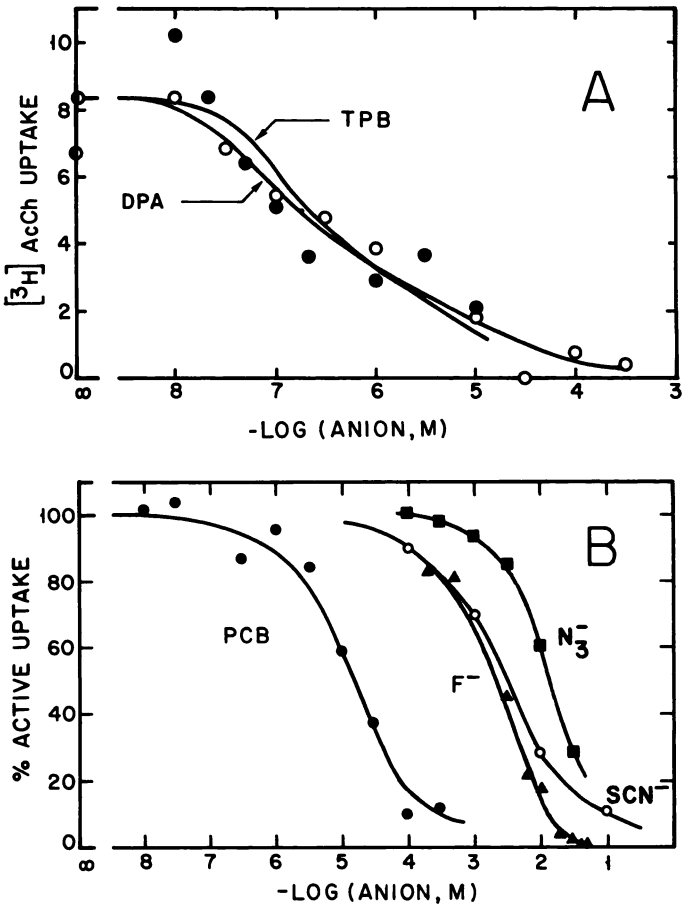

FIG. 2. *Inhibition of active [^{3}H]AcCh uptake by anions*

A. Synaptic vesicles (160 μl for each point, 1.6 mg of protein per milliliter), in 0.2 M Hepes, 0.6 M glycine, 1 mM EDTA, 1 mM EGTA, and 0.02% (w/v) NaN_3 (adjusted to pH 7.40 with 0.8 M NaOH), were added at 22° to 160 μl of 100 μM [^{3}H]AcCh, 10 mM MgATP, 4 mM $MgCl_2$, 80 mM $NaHCO_3$, and TPB or DPA in the same buffer. After 30 min, 250 μl of the solution were filtered and washed. AcCh uptake is presented as ^{3}H disintegrations per minute $\times 10^{-3}$. At concentrations of TPB above those shown, apparent uptake of [^{3}H]AcCh by vesicles increased dramatically, but the increase was not dependent on whether active or passive transport conditions were maintained. It may have been due to ion pairing of the lipophilic anion with [^{3}H]AcCh followed by subsquent nonspecific permeation into the vesicle, and is considered artifactual. Similar results were obtained with the centrifugation-gel filtration assay.

B. Synaptic vesicles suspended in 160 μl (for each point) of the above buffer for the SCN^- and N_3^- experiments or the same buffer, with K^+ replacing Na^+ for the PCB and F^- experiments, were added to 160 μl of 100 μM [^{3}H]AcCh, 10 mM MgATP, 4 mM $MgCl_2$, 80 mM $NaHCO_3$, or $KHCO_3$ (PCB, F^-) in the same buffer, and the inhibitors, giving the final inhibitor concentrations shown. Uptake at 22° was terminated after 30 min by centrifugation-gel filtration of 250 μl of the combined solutions. Final protein concentration was 0.8 mg/ml. Uptake of [^{3}H]AcCh is presented as percentage of active uptake = (uptake ratio with inhibitor-passive uptake ratio) × 100/(uptake ratio with no inhibitor-passive uptake ratio). The uptake ratios in the absence of SCN^-, PCB, F^-, and N_3^- were 16.8, 5.9, 34, and 24, respectively.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012

Effect of TPB on ATPase activity. The mechanism of TPB inhibition of [^{3}H]AcCh active transport was investigated. TPB possibly could act on the ATPase to inhibit or to uncouple it. Classic uncoupling would stimulate the ATPase (7, 8). Figure 3 shows that there was no effect on the ATPase up to micromolar concentrations of TPB. Above this concentration, there was only a small but still not significant increase in ATPase activity. Thus, there was no evidence that TPB inhibits active transport of [^{3}H]AcCh by acting directly or indirectly on the ATPase in a classical manner.

Effect of TPB on transmembrane electric potential. The action of TPB on the transmembrane electrical potential of the vesicles was investigated, since this large hydrophobic anion potentially could permeate the membrane. The potential was measured by determining the transmembrane distribution of [^{14}C]SCN^-. For these experiments, it was shown that [^{14}C]SCN^- rapidly equilibrates across the vesicle membrane at 22°. Under active conditions, [^{14}C]SCN^- was taken up by vesicles with an uptake ratio of 1.6 (Fig. 4), which corresponds to a transmembrane electric potential of 12 mV. TPB above 10 μM decreased the uptake of [^{14}C]SCN^- by vesicles (Fig. 4), suggesting that it made the vesicle interior more electrically negative as a result of uptake. However, at micromolar concentration, where TPB is significantly inhibitory toward active transport of [^{3}H]AcCh, there was no effect on the transmembrane electric potential.

Solubility products for TPB and DPA salts. TPB and DPA form insoluble salts with a number of cations of importance to neuromuscular transmission. Precipitation of the salts constrains allowed experimental protocols and could be of importance to the mechanism of their physiological effects. Thus, the K_{sp} values for these salts were determined conductimetrically or spectrophotometrically and are presented in Table 1. TPB precipitates AcCh, Ch, and K^+ at low concentrations. It does not precipitate Mg^{2+} or Ca^{2+}. From the K_{sp} we estimate that 10 mM AcCh will form a precipitate in TPB concentrations higher than 3 μM.

DPA is about as effective as TPB in precipitating AcCh, but it is much more selective in that it precipitates

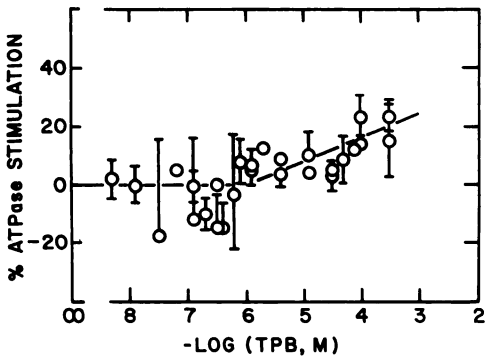

FIG. 3. *Effect of TPB on ATPase activity*

Synaptic vesicles (0.4 mg of protein per milliliter) were incubated at 25° in 0.7 M glycine, 0.1 M Hepes, 0.02% (w/v) NaN_3 (adjusted to pH 7.40 with 0.8 M NaOH), containing 3 mM [γ-^{32}P]ATP, 5 mM $MgCl_2$ and the indicated concentrations of TPB, for 5 min before quenching the ATPase reaction and analyzing for liberated phosphate. Data are presented as the mean ± 1 SD of three determinations.

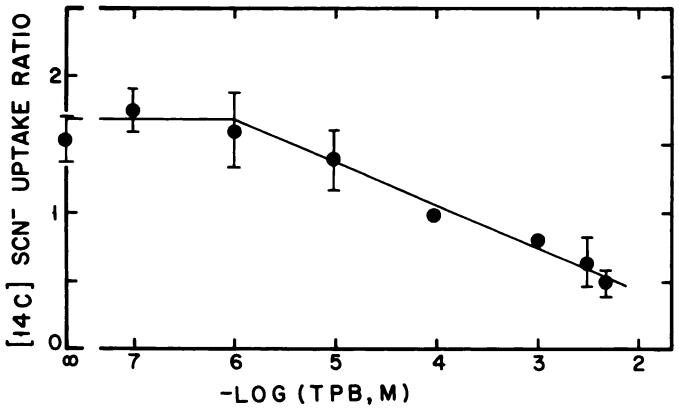

FIG. 4. *Effect of TPB on [^{14}C]SCN^- uptake*

Synaptic vesicles (0.8 mg of protein per milliliter), in 0.2 M Hepes, 0.6 M glycine, 1 mM EDTA, 1 mM EGTA, and 0.02% NaN_3 (adjusted to pH 7.40 with 0.8 M NaOH), were added to an equivalent volume of the same buffer containing 10 mM MgATP, 4 mM $MgCl_2$, 0.9 mM [^{14}C]SCN^-, and TPB at twice the indicated concentrations. After a 30-min equilibration, the vesicles were centrifuged in a Beckman Airfuge. Each point shown is an average of three experimental points ± 1 SD. The uptake ratio represents the concentration of [^{14}C]SCN^- inside the synaptic vesicles compared with the concentration outside.

Ch and K^+ only at 27- and 10^3-fold higher concentrations, respectively. It also does not precipitate Mg^{2+} or Ca^{2+}. Although not quantitatively studied here, PCB is a good precipitant of AcCh and Ch, whereas the K^+, Mg^{2+} and Ca^{2+} salts are very soluble.

In all of the vesicle experiments reported above, care was taken to keep ionic concentrations below solubility limits. The inhibition of active transport shown in Fig. 2 could not have been due to precipitation of [^{3}H]AcCh. Because of the nature of either assay method, this effect actually would have increased the amount of [^{3}H]AcCh detected.

DISCUSSION

TPB is a potent inhibitor of [^{3}H]AcCh active transport by purified synaptic vesicles. Our biochemical study confirms the hypothesis of Marshall and Parsons (4) that TPB can act on the motor nerve terminal to inhibit AcCh storage. In their work they suggested that a primary action of TPB might be to uncouple nerve terminal mitochondria, thus decreasing the availability of ATP necessary for vesicular storage. However, 100-fold higher concentrations of TPB are required to uncouple intact mitochondria as compared with those required to inhibit [^{3}H]AcCh active transport (15, 16). The maintenance of hyperpolarization in the neuromuscular preparations studied by Marshall and Parsons suggests that TPB did not drastically compromise the cellular energy supplies. Thus, it is not necessary to invoke an indirect mechanism

TABLE 1

Solubility products for TPB and DPA salts[a]

Cation	TPB	DPA
AcCh	3.0×10^{-8}	2.3×10^{-8}
Ch	1.2×10^{-7}	6.1×10^{-7}
K^+	1.4×10^{-7}	$>2.5 \times 10^{-5}$

[a] In water at 22° in units of M^2.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012

to explain inhibition of AcCh storage *in vivo* and, indeed, it seems unlikely.

What the direct vesicular mechanism might be is perplexing. We found no indication that TPB inhibits the vesicle ATPase or that it stimulates the ATPase as classic uncouplers do, consistent with its inability to bind protons (17). Also, it does not alter the transmembrane electric potential at low concentrations where it is effective in inhibiting active transport of [^{3}H]AcCh. A purely electrical mode of inhibition by TPB is unlikely also, because thiocyanate, which is freely permeant and would certainly have an electrical effect, does not cause inhibition below 100 μM. It is unlikely that TPB or the other anions bind to the AcCh transport active site to inhibit it, since the charge is inappropriate and TPB at low concentrations did not alter the passive uptake of [^{3}H] AcCh. Over-all, there is no evidence that TPB acts on synaptic vesicles in a classic manner to inhibit active transport of [^{3}H]AcCh.

TPB must act on a limited number of sites per vesicle. The synaptic vesicle molarity in the experiment shown in Fig. 2 was about 40 nM (8). At the IC_{50} for TPB, only about 10 TPB ions were present per synaptic vesicle. Thus, a nonspecific medium effect by TPB, such as detergent-like action, is unlikely. Because other large organic anions of quite different structures, namely DPA and PCB, also were potent, the vesicle target site is relatively nondiscriminatory.

The perplexing nature of TPB action on synaptic vesicles also applies to its mitochondrial action (18). It is a much more potent uncoupler of submitochondrial particles than of intact mitochondria (19). The difference presumably is due to an accessibility effect arising from the membrane eversion. Preliminary evidence suggests that the vesicle ATPase is mitochondrial-like (9) and outwardly oriented (20). Thus, the vectorial nature of synaptic vesicle transport processes is more similar to that of the submitochondrial particle, and it is possible that the mechanism of action of TPB in the two systems is similar. For submitochondrial particles, it has been suggested that TPB is an atypical site-specific uncoupler, which does not necessarily stimulate the ATPase (19). Perhaps a similar target site exists in synaptic vesicles. An anion binding site which interacts with a wide range of anions, including bicarbonate, is located on the vesicle ATPase (9). The relationship of this site to the TPB target site is unknown. It seems likely that DPA and PCB act in a manner similar to that of TPB because of their high potencies and grossly similar structures.

Whether fluoride, thiocyanate, and azide inhibit [^{3}H] AcCh active uptake in a manner similar to that of TPB is unknown. These anions inhibit many enzymes in the millimolar concentration range. Fluoride has been shown to be a good reactivator of AcCh esterase inhibited by organophosphorus reagents (21). This would result in hydrolysis of [^{3}H]AcCh to [^{3}H]acetate and loss of radioisotope uptake by the vesicles. Since esterase reactivation depends on the nucleophilic character of fluoride, it is possible that thiocyanate and azide could behave similarly. In any case, it is clear that care must be exercised in the use of these anions to inhibit phosphatases and microbial growth and to measure electrical potentials.

Although the AcCh salt of TPB is of limited solubility, it is not likely that TPB inhibits AcCh storage and release in intact preparations by precipitating AcCh in the nerve terminal cytoplasm. A concentration as low as 1 μM bathing TPB produced characteristic effects on AcCh release (4). If the nerve terminal cytoplasm contains 2–10 mM AcCh (22, 23), the K_{sp} would not be exceeded in 1 μM TPB, especially considering the probable lower cytoplasmic concentration of TPB due to diffusion and electrical barriers. It also is unlikely that TPB acts to precipitate precursor Ch, since the nerve terminal Ch concentration is estimated to be significantly lower than the AcCh concentration (24), and the Ch salt is more soluble.

The other potent anions studied here might be more desirable to use in further physiological studies. Original work with TPB was conducted in K^+-free Ringer's solution (2–4) to avoid precipitation of the potassium salt, but this significantly hyperpolarized the neuromuscular preparation. Potassium salts of both DPA and PCB are fully soluble, and they should be compatible with normal Ringer's solution.

The concordance between our observations on TPB and those of Marshall and Parsons (3, 4) constitute new (although indirect) pharmacological evidence that the source of evoked AcCh release is vesicular and not cytoplasmic, as some workers have held. Marshall (25) also predicted that a quite different type of drug, AH5183, which is a tertiary amine, blocks AcCh storage by vesicles *in vivo*. We have confirmed this hypothesis as well (13). Of more than 80 drugs which we have screened, TPB and AH5183 are two of the most potent inhibitors of [^{3}H] AcCh active transport by purified vesicles. Because of their opposite charges and different structures, the two drugs probably act on the vesicles by different mechanisms. This suggests that there are at least two critical targets in cholinergic synaptic vesicles which are susceptible to dysfunction. The possibility that drugs exhibiting presynaptic anticholinergic activity do so by directly compromising AcCh storage should be considered more generally.

ACKNOWLEDGMENTS

We thank Dr. Fred M. Hawthorne for his generous gift of trimethylammonium PCB, and Marianne Henry for preparation of synaptic vesicles. We also thank Professor Jordan Warnick for his review of the manuscipt before submission.

REFERENCES

1. Marshall, I. G. Actions of tetraphenylboron on neuromuscular transmission in the biventer cervicus muscle of the chick. *Eur. J. Pharmacol.* **123**:51–54 (1970).
2. Marshall, I. G., R. L. Parsons, and W. K. Paull. Depletion of synaptic vesicles at the frog (*Rana pipiens*) neuromuscular junction by tetraphenylboron. *Experienta* (*Basel*) **32**:1423–1426 (1976).
3. Marshall, I. G., and R. L. Parsons. The effects of tetraphenylboron on neuromuscular transmission in the frog. *Br. J. Pharmacol.* **54**:325–332 (1975).
4. Marshall, I. G., and R. L. Parsons. The effects of tetraphenylboron on spontaneous transmitter release at the frog neuromuscular junction. *Br. J. Pharmacol.* **54**:333–338 (1975).
5. Guider, G., E. Seifter, and J. Seifter. Effects of a negative quaternary ion-sodium tetraphenylboron (TPB) on the rat phrenic nerve-diaphragm preparation. *Eur. J. Pharmacol.* **7**:253–258 (1972).
6. Parsons, S. M., R. S. Carpenter, R. Koenigsberger, and J. E. Rothlein. Transport in the cholinergic synaptic vesicle. *Fed. Proc.* **41**:2765–2768 (1982).
7. Anderson, D. C., S. C. King, and S. M. Parsons. Uncoupling of acetylcholine

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012

uptake from the *Torpedo* cholinergic synaptic vesicle ATPase. *Biochem Biophys. Res. Commun.* **103**:422–428 (1981).
8. Anderson, D. C., S. C. King, and S. M. Parsons. Proton gradient linkage to active uptake of [^{3}H]acetylcholine by *Torpedo* electric organ synaptic vesicles. *Biochemistry* **21**:3037–3043 (1982).
9. Rothlein, J. E., and S. M. Parsons. Origin of the bicarbonate stimulation of *Torpedo* electric organ synaptic vesicle ATPase. *J. Neurochem.* **39**:1660–1668 (1982).
10. Koenigsberger, R., and S. M. Parsons. Bicarbonate and magnesium ion-ATP dependent stimulation of acetylcholine uptake by *Torpedo* electric organ synaptic vesicles. *Biochem. Biophys. Res. Commun.* **94**:305–312 (1980).
11. Hawthorne, M. F., D. C. Young, P. M. Garrett, D. A. Owen, S. G. Schwerin, F. N. Tebbe, and P. A. Wegner. The preparation and characterization of the (3)-1,2- and (3)-1,7-dicarbododecahydroundecaborate (-1) ions. *J. Am. Chem. Soc.* **90**:862–868 (1968).
12. Carpenter, R. S., R. Koenigsberger, and S. M. Parsons. Passive uptake of acetylcholine and other organic cations by synaptic vesicles from *Torpedo* electric organ. *Biochemistry* **19**:4373–4379 (1980).
13. Anderson, D. C., S. C. King, and S. M. Parsons. Pharmacological characterization of the acetylcholine transport system in purified *Torpedo* electric organ synaptic vesicles. *Mol. Pharmacol.* **24**:48–54 (1983).
14. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
15. Utsumi, K., and L. Packer. Uncoupling of energy transfer reactions in mitochondria by tetraphenyl boron. *Arch. Biochem. Biophys.* **122**:509–515 (1967).
16. Cunarro, J., and M. W. Weiner. Mechanism of action of agents which uncouple oxidative phosphorylation: direct correlation between proton-carrying and respiratory-releasing properties using rat liver mitocondria. *Biochim. Biophys. Acta* **387**:234–240 (1975).
17. Cooper, J. N., and R. E. Powell. On the purported tetraphenylboric acid. *J. Am. Chem. Soc.* **85**:1590–1592 (1963).
18. Cockrell, R. S. The influence of permeant anions upon mitochondrial energy conservation by means of a K^+ gradient. *Biochem. Biophys. Res. Commun.* **46**:1991–1998 (1972).
19. Phelps, D. C., and W. G. Hanstein. The effect of tetraphenylborate on mitochondrial energy transduction. *Biochem. Biophys. Res. Commun.* **79**:1245–1254 (1977).
20. Michaelson, D. M., and I. Ophir. Sidedness of (calcium, magnesium) adenosine triphosphatase of purified *Torpedo* synaptic vesicles. J. Neurochem. **34**:1483–1490 (1980).
21. Karczmar, A. G. *International Encyclopedia of Pharmacology and Therapeutics*, Sect. 13, Vol. I: *Anticholinergic Agents*. Pergamon Press, Oxford, 237–238 (1970).
22. Morel, N., M. Israel, and R. Manaranche. Determination of ACh concentration in *Torpedo* synaptosomes. *J. Neurochem.* **30**:1553–1557 (1978).
23. Weiler, M., I. S. Roed, and V. P. Whittaker. The kinetics of acetylcholine turnover in a resting cholinergic nerve terminal and the magnitude of the cytoplasmic compartment. *J. Neurochem.* **38**:1187–1191 (1982).
24. Tuček, S. In *Acetylcholine Synthesis in Neurons*. Chapman and Hall, London, 153–162 (1978).
25. Marshall, I. G. A comparison between the blocking actions of 2-(4-phenylpiperidino)cyclohexanol (AH 5183) and its *N*-methyl quaternary analogue (AH5954). *Br. J. Pharmacol.* **40**:68–77 (1970).

Send reprint requests to: Dr. D. C. Anderson, Department of Chemistry and the Marine Science Institute, University of California, Santa Barbara, Calif. 93106.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012