

AD-A153 046

EFFECTS OF ORGANOPHOSPHATES ON TRANSMITTER CONTROLLED
IONIC CHANNELS(U) STATE UNIV OF NEW YORK AT ALBANY
C EDWARDS FEB 85 ARO-18518. 4-LS DAAG29-82-K-0065

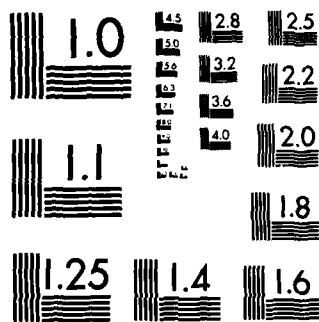
1/1

UNCLASSIFIED

F/G 6/20

NL

						END								
						FORMED								
						DTIC								



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM	
REPORT NUMBER ARO 18518.4-LS	2. GOVT ACCESSION NO. N/A	3. RECIPIENT'S CATALOG NUMBER N/A	
TITLE (and Subtitle) Effects of Organophosphates on Transmitter Controlled Ionic Channels		5. TYPE OF REPORT & PERIOD COVERED 22 Feb 82 - 31 Dec 84 Final Report	
AUTHOR(s) Charles Edwards		6. PERFORMING ORG. REPORT NUMBER	
PERFORMING ORGANIZATION NAME AND ADDRESS State University of New York at Albany		8. CONTRACT OR GRANT NUMBER(s) DAAG29-82-K-0065	
CONTROLLING OFFICE NAME AND ADDRESS U. S. Army Research Office Post Office Box 12211 Research Triangle Park, NC 27709		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE Feb 85	
		13. NUMBER OF PAGES 31	
		15. SECURITY CLASS. (of this report) Unclassified	
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited.			
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) NA			
18. SUPPLEMENTARY NOTES The view, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.			
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Drugs, Neuromuscular Junctions, Acetylcholine Thiamine Guanethidine; Phenethylguanidine, Hycanthone, Memantine			
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) We have examined the postsynaptic blocking effects of thiamine (Vitamin B1), guanethidine, phenethylguanidine, hycanthone and memantine on the neuromuscular junction, using voltage clamp and noise analysis. L-... pertains to...			

DTIC FILE COPY

DTIC
ELECTE
APR 30 1985

DD FORM 1473

1 JAN 73

EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ARO 18518.4-LS

EFFECTS OF ORGANOPHOSPHATES ON TRANSMITTER CONTROLLED IONIC CHANNELS

FINAL REPORT

31 DECEMBER, 1984

U. S. ARMY RESEARCH OFFICE

CONTRACT DAAG 2982K0065

STATE UNIVERSITY OF NEW YORK AT ALBANY

ALBANY, NEW YORK, 12222

APPROVED FOR PUBLIC RELEASE;

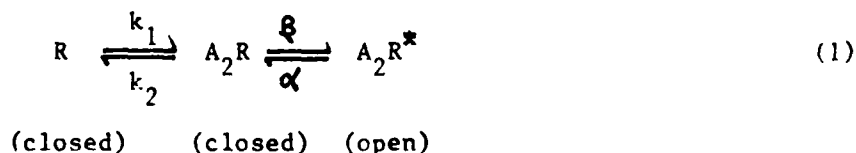
DISTRIBUTION UNLIMITED.



Accession For	
NTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

1. POSTSYNAPTIC EFFECTS OF SOME DRUGS ON THE FROG NEUROMUSCULAR JUNCTION.

At the neuromuscular junction, invasion of the presynaptic nerve terminal by the nerve impulse, leads to an increase of the probability of the release of acetylcholine (ACh) from the nerve terminal. The ACh diffuses across the synaptic cleft between the nerve terminal and muscle membrane, and reacts with a specific protein, called the ACh receptor-ion channel complex. The binding of ACh to the ACh receptor changes the conformation of this ion channel from closed to open. The ensuing movements of ions through the open channel is responsible for the endplate potential. The properties of this reaction at the neuromuscular junction have been well studied (e.g. Peper et al., 1982). The Hill coefficient of the dose-response curve of ACh is around 2, and so the reaction scheme for ACh binding needs two steps. Thus, the scheme of the reaction will be:



where R is ACh receptor-ion channel complex, A is ACh, k_1 , k_2 , α and β are rate constants.

There are many drugs which inhibit neuromuscular transmission. Though some of them are clinically important, especially in the field of anaesthesiology, the mechanisms of actions of these drugs are not fully understood.

The postsynaptic blockers, which bind with the ACh receptor-ion channel complex and block it, fall into three categories, depending on the affinity for the states, R , $A_n R$ and $A_n R^*$. (1) drugs which bind R are 'ground state blockers'; α -bungarotoxin and d-tubocurarine are in this category. (2) drugs which bind to $A_n R$ are 'transient state blockers'; some amitriptyline compounds and meproadifen are in this class. (3) drugs which bind to $A_n R^*$ are 'open channel blockers'; many local anaesthetics are in this category.

We have examined the postsynaptic blocking effects of thiamine (Vitamin B1), guanethidine, phenethylguanidine, hycanthone and memantin on the neuromuscular junction, using voltage clamp and noise analysis.

Material and Methods

The sartorius nerve-muscle preparation of the frog, Rana pipiens was used. Superficial muscle fibres were voltage-clamped by a conventional two microelectrode procedure. The positions of the microelectrodes were monitored carefully by requiring that the rise time of the evoked endplate current (EPC) was no more than about 0.5 msec and that the clamp speed was faster than 100 μ sec. Therefore, the error of the clamp on the voltage trace was smaller than 1% of the motive force of the EPC. To prevent muscle contraction, either the cut-muscle method or $MgCl_2$ + Na Dantrolene Ringer were used. The mean quantal content ($\bar{m}$)

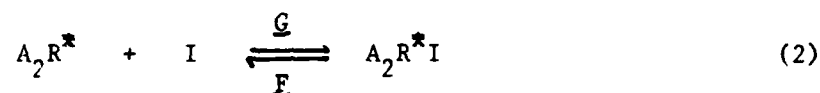
and the quantal height (q) were usually measured by statistical analysis since many of the drugs used decreased the peak height of the miniature endplate current (MEPC). In some cases, in which the MEPC height was unaffected in the presence of drugs, m was estimated directly. The effects of drugs on the peak height of the evoked EPC and the decay time constant/membrane potential relationships were determined.

The single channel conductance and the mean channel lifetime were measured by analysis of the ACh-induced noise spectrum by methods which have been described elsewhere (e.g. Anderson and Stevens, 1972). In noise analysis, it was very hard to work with frequencies over 500 Hz, since the ACh noise decreased at those frequencies, while the control noise increased dramatically. The power spectrum could not be measured much below 5 Hz since, as was pointed out by Katz and Miledi (1975), there are artifacts due to slow vibrations of the floor, fluctuations in local ACh concentration and drifts in membrane sensitivity. These constraints limit the method of noise analysis, and the lack of higher and lower frequency components in the analysis may lead some error in the value of the single channel conductance. In fact, the single channel conductances of some agonists measured with patch clamp and noise analysis are different (Gardner et al, 1984).

Thiamine

In the presence of high concentration of thiamine (1-2 mM), the decay phase of the EPC remained exponential but the decay time constant increased dramatically. The EPC height/membrane potential relationships changed from linear to highly nonlinear. The mean channel lifetime

increased while the single channel conductance decreased. It is concluded that thiamine modifies the kinetics of ACh receptor-ion channel complex and that this effect is dependent on the membrane voltage. In the presence of thiamine, the time course of the single channel opening-closing reaction may change from single square pulse to high frequency bursts. The main target of thiamine was thought to be A_nR^* . The possible reaction is:



where I is the inhibitor (open channel blocker in this case), and $\underline{F}$ and $\underline{G}$ are rate constants.

Guanethidine and Phenethylguanidine

Guanethidine and phenethylguanidine are derivatives of guanidine. Guanethidine is used for the treatment of hypertension. Both compounds (100 μ M) decreased the EPC height. Since $\underline{m}$ was unaffected by both drugs, it was concluded that the target of both drugs is the post-synaptic ACh receptor-ion channel complex. Both drugs changed the time course of the EPC from a single exponential to biphasic, and the peak EPC/membrane potential relations from linear to highly nonlinear. Guanethidine changed the power spectrum from a single to a double Lorentzian curve, so it is likely that guanethidine is an open channel blocker. The decrease of the single channel conductance and the form of its voltage dependence in the presence of guanethidine supported this idea. Phenethylguanidine shifted the cutoff frequency of the power spectrum to the right, but the power spectrum remained a single

Lorentzian. These unexpected phenomena can be explained if phenethylguanidine has two actions: one is irreversible block of $A_n R^x$ (i.e. F in scheme 2 is negligibly small) and the other is reversible block of $A_n R$.

Hycanthone

In the presence of 1-20 μM of the antischistosomal drug, hycanthone, m was slightly but significantly increased while the peak height of the MEPC remained unaffected. Consistently 1 μM hycanthone increased the decay time constant of the EPC and the channel lifetime measured by noise analysis. However, even in the presence of 2 μM hycanthone, the response for the ionophoretically applied ACh was dramatically decreased. It was concluded that the primary site of hycanthone block is $A_n R$, and that hycanthone is a 'transient state blocker'. However, this drug also has other sites of action. One is the presynaptic nerve terminal, since hycanthone increased the nerve evoked transmitter release. Also, hycanthone changed the kinetics of $A_n R^*$ since the channel lifetime was transiently increased by about 1 μM hycanthone, but decreased by higher doses of hycanthone.

Memantin

Analogues of amantadines, which were once used as antiviral drugs, especially for the treatment of influenza A2, are useful also for Parkinsonism. Recent studies of analogues of amantadine revealed that many of them block neuromuscular transmission (Warnick et al., 1983).

Memantin is a derivative of amantadine, and 2-50 μ M memantin decreased the peak height of the EPC. Statistical analysis revealed that memantin decreased g whereas m remained unaffected. Thus, it is clear that memantin inhibits neuromuscular transmission postsynaptically. The decay time constant of the EPC was decreased but the decay phase remained a single exponential. Noise analysis showed that the power spectrum was shifted to the right but that the shape of the power spectrum remained a single Lorentzian. The single channel conductance was unaffected. It is concluded that memantin is an open channel blocker but the rate constant F in scheme (2) is negligibly small.

References

- Anderson, C.R. & C.F. Stevens, J. Physiol (Lond). 235, 655, 1973.
Gardner, P., D.C. Ogden & D. Colquhoun, Nature 309, 160, 1984.
Peper, K. R.J. Bradley & F. Dreyer, Physiol Rev. 62, 1271, 1982.
Warnick, J.E., M.A. Maleque & E.X. Albuquerque, J. Pharmacol. Exp. Ther. 228, 73, 1983.

CONT'D
→ Experiment II pertains to the
MODE OF ACTION OF ORGANOPHOSPHATES AT THE FROG NEUROMUSCULAR
JUNCTION.

→ The goal of the research was to investigate the kinetics of organophosphate block of the acetylcholine receptor-channel complex (AChR). A wide variety of organophosphorus compounds have been synthesized over the last fifty years for use as pesticides (Eto, 1974) or chemical warfare agents (Meselson and Robinson, 1980) and their

toxicity is usually attributed to the inhibition of acetylcholinesterase (AChE) (Taylor, 1980). Inhibition of AChE slows the removal of acetylcholine (ACh) from the synaptic cleft and this is said to cause receptor desensitization and, eventually, failure of synaptic transmission.

Direct block of AChR by organophosphates was also observed early in the history of research on these compounds. Eccles and MacFarlane (1949) found that 1 mM diisopropyl fluorophosphonate (DFP) reversibly reduced the amplitude of the endplate potential at the frog neuromuscular junction. Since then a number of other investigators have reported AChR block by organophosphates (Bartels and Nachmansohn, 1969; Eldefrawi et al., 1971 and 1982; Kuba et al., 1974; Dekin et al., 1978; Henderson et al., 1982; Pascuzzo et al., 1984). Therefore, direct block of AChR, rather than desensitization, may be the principal cause of death in acute organophosphate poisoning.

The kinetics of the blocking reaction have been examined for only one compound, namely DFP (Kuba et al., 1974; Pascuzzo et al., 1984) and even in this case the concentration dependence of the rate constants has not been determined. The goal of the present research was to investigate further organophosphate-AChR binding kinetics using two organophosphate pesticides, 2,2 dichlorovinyl dimethylphosphate (DDVP) and 3-hydroxy-n,n-dimethyl-cis-crotonamide dimethylphosphate (dicrotophos), which have been reported to block the depolarization of frog muscle by carbamylcholine (Dekin et al., 1978) and ACh binding to isolated AChR from eel electroplax (Eldefrawi et al., 1982). DDVP is one of the few organophosphate pesticides in common use today and is the

→ Originator supplied to the...

DD1473 (Field 19)

active ingredient in flea collars for pets. Dicrotophos is no longer in common use because of its relatively high toxicity to mammals.

METHODS

The effects of DDVP, dicrotophos, and methanesulfonyl fluoride (MSF) on postsynaptic ACh receptors at the frog neuromuscular junction were studied using a two microelectrode voltage clamp (Takeuchi and Takeuchi, 1958). Both neurally evoked endplate currents (EPCs) and ACh-induced current noise were used to study the kinetics of drug binding. MSF is an irreversible anticholinesterase which is thought not to have any receptor effects (Kordas et al., 1975; Kordas, 1977) and it provided a control for the anticholinesterase effects of the organophosphates.

Individual cutaneous pectorus muscles were dissected out from the frog (Rana pipiens) and bathed in low calcium frog ringer (116 mM NaCl, 2.5 mM KCl, 0.9 mM CaCl₂, and 5 mM PIPES, pH=7.2) at 10° C. The low bath temperature and anticholinesterase effects of MSF pretreatment made it difficult to detect small differences in EPC rise time. Therefore, endplates were localized by ionophoretic rather than electrical mapping. After impaling a muscle fibre with the voltage electrode, a 1 M Ω ACh-filled microelectrode was moved along its length until the largest and fastest voltage response was found. The voltage electrode was then re-inserted at this spot. Voltage electrodes were filled with 3 M KCl and had resistances ranging from 5-8 M Ω . Current electrodes were filled with a mixture of 90% 2M KCitrate and 10% 3M KCl in the EPC experiments and 3 M KCl in the noise experiments. The resistances of the citrate filled electrodes were 5-10 M Ω .

Injection of hyperpolarizing current with KCl filled electrodes can result in DC current artifacts (Adams, 1975), but KCl filled current electrodes proved necessary in the noise experiments because their low resistance reduced background current noise. The magnitude of the injected current in the noise experiments was kept as low as possible to minimize such artifacts.

Different current monitors were used for the EPC and noise experiments. A virtual ground current monitor (Burr-Brown 3523L amplifier with a $100\text{ K}\Omega$ feedback resistor) was used for the EPC experiments because it had a large bandwidth (30 KHz) and allowed the use of a high voltage source ($\pm 110\text{ V}$) for current injection. The voltage drop across a $1\text{ M}\Omega$ series resistance was used to measure current in the noise experiments to allow bath grounding and eliminate noise from the perfusion system.

Muscles were transected at both ends to prevent contraction in EPC experiments (Lambert et al., 1981) while osmotic shock with 1.5 M formamide was used to block contraction in noise experiments (Del Castillo and De Motta, 1978). The formamide treatment not only blocked the contraction, but also reduced the membrane capacitance (Argiro, 1981). The drop in membrane capacitance increased the clamp speed (time to 90% of final membrane potential after a step change in the command voltage) and reduced the background noise of the cell under voltage clamp. However, the formamide treatment did not completely block contraction without also blocking ACh release from most nerve terminals. It also did not prevent twitching in response to direct fibre depolarization. Therefore, it was not used to prevent contraction in EPC experiments. The cut muscle preparation was suitable for the EPC

experiments, but not for the noise experiments because the low input impedance and resting potential of the cut muscle fibre increased the background noise of the cell under voltage clamp. The clamp speed averaged between 200-300 μ sec in the cut muscles and 50-100 μ sec in the formamide-treated muscles. The increased clamp speed in the formamide-treated fibres was due to a drop in current electrode resistance as well as membrane capacitance. The efficacy of the space clamp was checked in several cut muscle fibres with a third electrode placed close to the endplate region. Membrane potential during the FPC was maintained to within 5% of the resting potential even at greatly hyperpolarized potentials.

All data were acquired and analyzed with the aid of a PDP-11/23 computer (Digital Equipment Corporation (D.E.C.)) equipped with an A/D converter (Data Translation, model # DT2782), programmable clock (D.E.C., model # KWF-11A), and Basic-23[®] software. High frequencies were filtered out with a low pass, 8-pole Butterworth filter (Frequency Devices, model # 901F) above 500 Hz in the noise experiments and above 5 KHz in the EPC experiments to avoid aliasing. Frequencies below 1 Hz were filtered out of the noise data with a high pass, 6-pole Butterworth filter (Frequency Devices, model #723H6B-1) to avoid biasing the Fourier transform. Estimates of EPC time constants (T_{epc}) were obtained from the slope of the log transform of EPC decay using linear least squares regression. Noise data were collected in 1 sec samples and the power spectrum for each sample was computed using a fast Fourier transform after cosine tapering (Cooley and Tukey, 1965). The ACh-induced noise spectrum was obtained as the difference between the average spectrum of 14 sec of noise taken during ACh ionophoresis and of 14 sec of

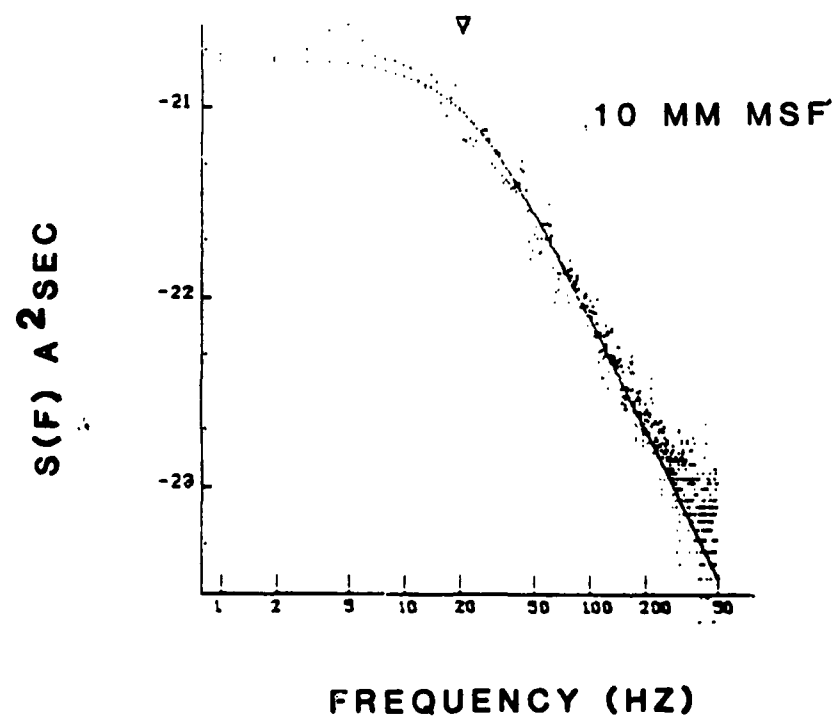
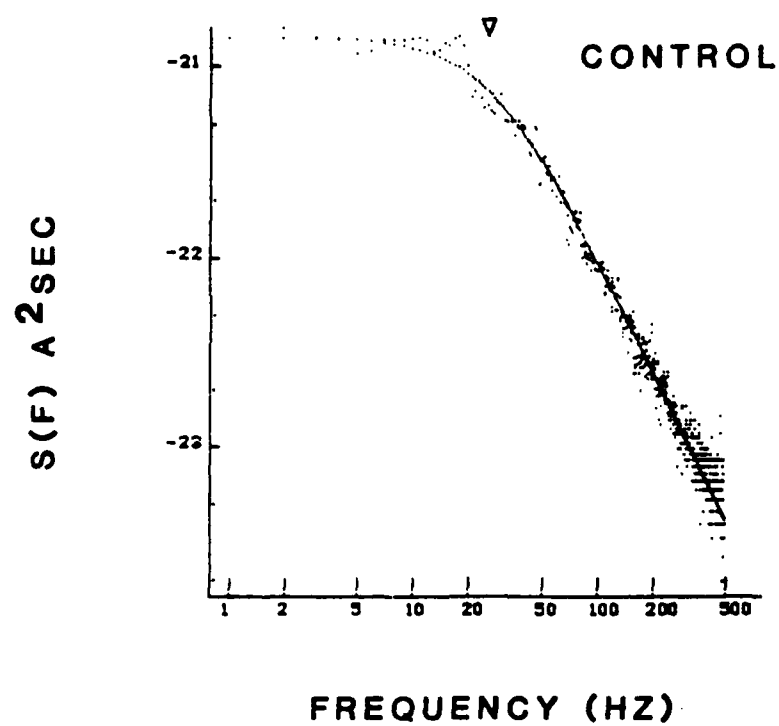


Figure 1

increases linearly with drug concentration (119 Hz at 0.5 mM DDVP to 225 Hz at 1 mM). Holding potential = -80 mV. Temp. = 10⁰ C.

Fig. 5 EPCs in 3 mM dicotophos. Three millimolar dicotophos (Bidrin) has no effect on EPCs without prior inhibition of cholinesterase. Each trace is the average of 20 EPCs. The average time constant (τ) and peak amplitude (I_p) of the EPCs are given on the left. Holding potential = -80 mV. Temp. = 10⁰ C. (Note scale change in bottom panel.)

Fig. 6 EPCs in 3 mM dicotophos after MSF pretreatment. MSF potentiates the effect of dicotophos (Bidrin) on EPCs. EPC amplitude (I_p) is drastically reduced in 3 mM dicotophos after cholinesterase inhibition but the decay remains single exponential with only a slight reduction in the time constant (τ). Other information same as in fig. 6.

Fig. 7 Effect of dicotophos on ACh noise. Dicotophos (Bidrin) concentrations up to 1 mM had no effect on noise. Holding potential and temperature same as in fig. 6.

Fig. 1 Effect of MSF on ACh Noise. Ten millimolar MSF has no effect on the ACh noise spectrum. The cutoff frequency is indicated by an inverted triangle. Lorentzian functions have been fit to the data as described in METHODS. Data are plotted on a log-log scale. Ordinate. Spectral density ($S(F)$). Holding potential = -80 mV. Temp. = 10° C.

Fig. 2 EPC decay in 1 mM DDVP. EPC decay was double exponential in 1 mM DDVP. Top. EPC decay at holding potentials between -100 and +10 mV. Bottom. Semilogarithmic plots of EPC decay. Each trace is the average of 20 individual EPCs. Temp. = 10° C.

Fig. 3 Rate constants of EPC decay as a function of DDVP concentration. The sum of the two rate constants of EPC decay (SUM) in DDVP is linearly dependent on drug concentration. A straight line (solid) was fit to the data using linear least squares regression (slope = $0.23 \text{ (msec} \cdot \text{mV)}^{-1}$, incpt. = 1.6 msec^{-1}). Inner set of dashed lines gives the 95% confidence interval for the slope of the line and outer set gives the 95% confidence interval for the points. The 0 mM value was estimated from the sum of the control rate constant (0.08 msec^{-1}) and drug dissociation rate constant (0.09 msec^{-1}) estimated from the control, 1 mM, and 2 mM time constants. Holding potential = -80 mV. Temp. = 10° C.

Fig. 4 Effect of DDVP on ACh noise. The cutoff frequency (inverted triangles) of the fast component of the spectra in DDVP

- Takeuchi, A. and Takeuchi, N. Active phase of frog's end plate potential. J. Neurophys. 22: 395-411, 1959
- Taylor, P. Anticholinesterase agents, in: The Pharmacological Basis of Therapeutics (Gilman, A. G., Goodman, L. S., and Gilman, A., eds.) Macmillan Publishing Co., Inc. pp.100-119, 1980
- Vyskocil, F., Nikolsky, E., and Edwards, C. An analysis of the mechanisms underlying non-quantal release of acetylcholine at the mouse neuromuscular junction. Neurosci. 9: 429-435, 1983
- Wray, D. Noise analysis and channels at the postsynaptic membrane of skeletal muscle. Prog. Drug Res. 24: 9-56, 1980

- currents in frog sartorius muscle. J. Pharm. Exp. Ther. 189: 499-512, 1974
- Lambert, J. J., Durant, N. N., Reynolds, L. S., Volle, R. L., and Henderson, E. G. Characterization of end-plate conductance in transected frog muscle: modification by drugs. J. Pharm. Exp. Ther. 216: 62-69, 1981
- Magleby, K. L. and Stevens, C. F. The effect of voltage on the time course of end-plate current. J. Physiol. (Lond.) 223: 151-171, 1972
- Magleby, K. L. and Stevens, C. F. A quantitative description of end-plate currents. J. Physiol. (Lond.) 223: 173-197, 1972
- Menzer, R. E. and Casida, J. E. Nature of toxic metabolites formed in mammals, insects, and plants from 3-(dimethoxy-phosphovinyloxy)-n,n-dimethyl-cis-crotonamide and its n-methyl analog. J. Agri. Food Chem. 13: 102-112, 1965
- Meselson, M. and Robinson, J. P. Chemical warfare and chemical disarmament. Sci. Am. 242: 37-47, 1980
- Pascuzzo, G. J., Akaike, A., Maleque, M. A., Shaw, K., Aronstam, R. S., Rickett, D. L., and Albuquerque, E. X. The nature of the interactions of pyridostigmine with the nicotinic acetylcholine receptor-ionic channel complex. I. Agonist desensitizing and binding properties. Mol. Pharm. 25: 92-101, 1984
- Sachs, F. Automated analysis of single channel records, in: Single-Channel Recording (Sakmann, B. and Neher, E., eds.) Plenum Press. pp. 265-286, 1983
- Stevens, C. F. Inferences about membrane properties from electrical noise measurements. Biophys. J. 12: 1028-1047, 1972

- Eldefrawi, M. E., Britten, A. G., and O'Brien, R. D. Actions of organophosphates on binding of cholinergic ligands. Pest. Biochem. Physio. 1: 101-108, 1971
- Eto, M. Organophosphorus Pesticides: Organic and Biological Chemistry. C. R. C. Press, Cleveland, Ohio, 1974
- Gaines, T. B. Acute toxicity of pesticides. Toxicol. Appl. Pharm. 14: 515-534, 1969
- Henderson, E. G., Volle, R. L., Reynolds, L. S., Lambert, J. J., and Epstein, P. M. Ecothiophate (217MI) and its tertiary amine analogue (217A0) decrease voltage dependence of endplate current (E. P. C.) decay in frog skeletal muscle. Fed. Proc. 42: abstr. 4092, 1983
- Katz, B. and Miledi, R. The binding of acetylcholine to receptors and its removal from the synaptic cleft. J. Physiol. (Lond.) 231: 549-574, 1973
- Katz, B. and Miledi, R. The nature of the prolonged endplate depolarization in anti-esterase treated muscle. Proc. R. Soc. Lond B. 192: 27-38, 1975
- Kordas, M. On the role of junctional cholinesterase in determining the time course of the endplate current. J. Physiol. (Lond.) 270: 133-150, 1977
- Kordas, M., Brzin, M., and Majcen, Z. A comparison of the effects of cholinesterase inhibitors on end-plate current and on cholinesterase activity in frog muscle. Neuropharm. 14: 791-800, 1975
- Kuba, K., Albuquerque, E. X., Daly, J., and Barnard, E. A. A study of the irreversible cholinesterase inhibitor, diisopropyl fluorophosphonate, on the time course of end-plate

REFERENCES

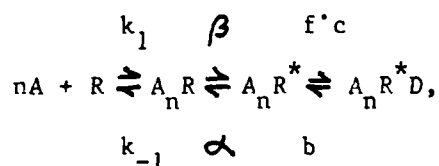
- Adams, P. R. A study of desensitization using voltage clamp. Pflugers Arch. 360: 135-144, 1975
- Adams, P. R. Drug blockade of open end-plate channels. J. Physiol. (Lond.) 260: 531-552, 1976
- Adams, P. R. Quinacrine (mepacrine) action at frog end-plate. J. Physiol. (Lond.) 306: 261-281, 1980
- Anderson, C. R. and Stevens, C. F. Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. J. Physiol. (Lond.) 235: 655-691, 1973
- Argiro, V. Excitation-contraction uncoupling of striated muscle fibres by formamide treatment: evidence of detubulation. J. Muscle Res. Cell Mot. 2: 283-294, 1981
- Bartels, E. and Nachmansohn, D. Organophosphate inhibitors of acetylcholine-receptor and -esterase tested on the electroplax. Arch. Biochem. Biophys. 133: 1-10, 1969
- Cooley, J. W. and Tukey, J. W. An algorithm for the machine caculation of Fourier series. Math. of Computn. 19: 297-301, 1965
- Dekin, M. S., Guy, H. R., and Edwards, C. Effects of organophosphate insecticides on the cholinergic receptors of frog skeletal muscle. J. Pharm. Exp. Ther. 205: 319-325, 1978
- Del Castillo, J. and De Motta, G. E. A new method for excitation-contraction uncoupling in frog skeletal muscle. J. Cell. Bio. 78: 782-784, 1978
- Eldefrawi, A. T., Mansour, N. A., and Eldefrawi, M. E. Insecticides affecting acetylcholine receptor interactions. Pharm. Ther. 16: 45-65, 1982

the anticholinesterase properties of the drug and do not indicate receptor binding.

One of the surprising results of the study was that millimolar dicrotophos concentrations did not prolong EPC decay despite a previous report of anticholinesterase activity in the micromolar range (Menzer and Casida, 1965) and a mammalian toxicity 3 to 4 times greater than DDVP (Gaines, 1969) which is an effective anticholinesterase. There are a number of possible explanations for this apparent anomaly in the data including species differences in AChE (Menzer and Casida used fly head and human blood cholinesterase while frog muscles were used in this study) and differences in the temperatures at which the two studies were conducted (10° in this study versus $20-23^{\circ}$ C in the Menzer and Casida study). Nonetheless, it does raise the possibility that the mammalian toxicity of these organophosphates is not due to their inhibition of AChE. The data do not support the hypothesis that channel block is the primary cause of toxicity in the case of dicrotophos since it had no effect on neuromuscular transmission by itself.

The second unexpected result was the great difference between the channel binding rate constants for dicrotophos and DDVP. The rate constants for DDVP are in the millisecond range and for dicrotophos in the second range. The noise spectrum with 1 mM DDVP at 10° C is very similar to a previously published spectrum with 1 mM DFP at 22° C (figure 8b, Pascuzzo et al., 1984). Thus, the rate constants for DDVP and DFP binding to AChR are similar. Dicrotophos is much more soluble and stable in water than either DDVP or DFP. The difference in the rate constants for channel binding between dicrotophos, DDVP, and DFP may be due to differences in their solubility and/or reactivity.

some drugs bind to and block the open state of the ACh channel ($A_n R^*$) according to the following scheme:



where $f \cdot c$ is the linearized forward rate constant for drug binding, c is the drug concentration, b is the drug dissociation rate constant, and $A_n R^* D$ is the drug-bound receptor state. This scheme will result in biphasic EPC decay and double Lorentzian noise spectra if $f \cdot c$ and b are in the millisecond range because α will no longer be the rate limiting step in the reaction. The sum of the rate constants for the fast and slow components of the EPC decay and noise spectrum will be linearly dependent on the drug concentration (Adams, 1980). If $f \cdot c$ and b are much smaller than α , then the channels will be blocked only if they are open often enough for the drug to bind to them. A drug that binds and unbinds very slowly to the channel will block AChR during long ionophoretic pulses but not the very brief release of ACh during the EPC. DDVP binds and unbinds to the channel fast enough to produce biphasic EPC decay and double Lorentzian noise spectra while dicotophos binds to the channel so slowly that it can block AChR only during long ionophoretic pulses or slow, nonquantal release of ACh.

MSF had no effect on noise, EPC reversal potential, or the voltage sensitivity of EPC decay but it did irreversibly prolong EPC decay and increase MEPC amplitude. Its effects on the EPC can be attributed to

presence of 3 mM dicotophos after a 1 hr pretreatment with 10 mM MSF. EPC amplitude was reduced by a factor of 40 while decay remained monoexponential with only a slight decrease in T_{epc} .

In 0.5 mM dicotophos the noise spectrum was unchanged (figure 7). It proved difficult to obtain noise spectra in dicotophos concentrations higher than 1 mM because steady DC currents in response to ACh ionophoresis could not be obtained. At higher concentrations, a low frequency hump appeared in the spectra because the current response to ACh declined exponentially with a time constant of several seconds after the onset of ionophoresis. A second ionophoretic response could not be evoked for several minutes once the initial response had declined to zero.

Dicotophos seems to block AChR very slowly. It blocks AChR during long ionophoretic pulses but not during EPCs without prior MSF treatment. It does not affect the EPC by itself because the ACh channels are not open long enough during the EPC for dicotophos to bind to them. Once the muscle has been treated with an anticholinesterase such as MSF, nonquantal release of ACh from the nerve terminals may keep enough ACh channels open (Vyskocil et al., 1983) for dicotophos to bind to them and to block the EPC. One surprising result of these experiments is that dicotophos itself does not seem to be a very effective anticholinesterase.

DISCUSSION

The actions of organophosphates on AChR can be explained by the open channel blocking model (Adams, 1976). According to this model,

The sum of two rate constants for double exponential EPC decay was linearly dependent on DDVP concentration in the 1-3 mM range (figure 2). In 1 mM DDVP, EPC decay remained biphasic throughout the range of holding potentials looked at (from -150 to +40 mV). Figure 3 shows the results of an experiment with holding potentials between -100 and +10 mV. The average magnitude of H (see eq. 1) for both the fast and slow components of EPC decay in 1 mM DDVP ($H_{fast} = -0.0034 \pm 0.0011 \text{ mV}^{-1}$, $H_{slow} = -0.0024 \pm 0.0033 \text{ mV}^{-1}$, $N = 9$) was less than either the control ($-0.0015 \pm 0.0002 \text{ mV}^{-1}$, $N = 9$) or washout ($-0.0092 \pm 0.0024 \text{ mV}^{-1}$, $N = 9$) values. The size of the MEPCs was also reduced in mM DDVP concentrations. Pretreatment of the muscles with 10 mM MSF for 1 hr before the experiment did not seem to change the effects of DDVP on the EPC.

The noise data were consistent with the EPC data. In 0.5 and 1 mM DDVP, the ACh-induced noise spectra were double Lorentzians (figure 4). Above 1 mM the amplitude of the noise was too small and the cutoff frequencies too close to the limits of the signal bandwidth for the spectra to be measured accurately. From 0.5 to 1 mM DDVP, F_c for the fast component increased linearly with concentration from 119 Hz at 0.5 mM DDVP to 225 Hz at 1 mM. After washout of DDVP, the ACh-induced noise spectra returned to a single Lorentzian with no change in F_c .

DICROTOPHOS

Concentrations of dicrotophos as high as 3 mM did not significantly affect EPCs (figure 5). However, pretreatment of the muscle with 10 mM MSF dramatically potentiated the effect of dicrotophos (figure 6). In the experiment shown in figure 6, EPCs were reversibly blocked in the

The average reversal potential (E_r) of the EPC for 26 fibres was -3.2 ± 4.2 mV and was close to the -6.6 mV average value previously reported by Lambert *et al.* (1981) for transected frog muscles. Resting potentials of the cut fibres ranged from -15 to -40 mV.

MSF

MSF prolonged EPC decay but did not affect the reversal potential, the voltage sensitivity of decay, single channel conductance, or the noise cutoff frequency. Treatment of cut muscles with 10 mM MSF for 1 hr at -80 mV holding potential and 10° C prolonged T_{epc} twofold on the average and doubled the size of the miniature EPCs (MEPCs) but EPC decay remained monoexponential. The noise spectra at -80 mV and 10° C before and after a 1 hr treatment with 10 mM MSF show there was no change in F_c with MSF (figure 1). The absence of a change in F_c and the voltage dependence of EPC decay mean that MSF has no effect on channel lifetime. The EPC decay was prolonged by MSF because of its inhibition of acetylcholinesterase. When AChE is inhibited, there is an increase in the average number of times an ACh molecule released by the nerve terminal binds to a receptor and this repetitive binding prolongs the EPC decay (Katz and Miledi, 1973 and 1975).

DDVP

At concentrations greater than 0.5 mM DDVP reversibly blocked ACh channels. In 0.5-4 mM DDVP, the EPC decay became biphasic and the peak amplitude was reduced. Neuromuscular transmission was completely blocked in 4-8 mM DDVP. DDVP also irreversibly prolonged EPC decay, but the decay again became a single exponential after washout of the drug.

The average F_c for these fibres was 23 ± 4.2 Hz and was within the range that could be expected from previous work on frog and toad muscle (29.6 ± 7.5 Hz, at holding potentials between -90 and -60 mV and temperatures between 6° and 8° C, (Wray, 1980)).

The open channel lifetime of the ACh channel is exponentially dependent on membrane potential (Magleby and Stevens, 1972a and b; Anderson and Stevens, 1973). Magleby and Stevens found that the rate constant of EPC decay (α) was an exponential function of transmembrane voltage (V):

$$\alpha(V) = Be^{AV},$$

where A and B are constants, and A gives the voltage sensitivity of α . Since $\alpha = 1/T_{epc}$ and $B = 1/T_{epc}$ at 0 mV (T_0), the relationship between T_{epc} and V is:

$$T_{epc}(V) = T_0 e^{HV}, \quad (1)$$

where $H = -A$. The average value of H for 19 cut muscle fibres was -0.0117 mV^{-1} at 10° C and closely agreed with the value of -0.0125 mV^{-1} reported by Magleby and Stevens (1972b) for a glycerol-treated fibre at 10° C. The average T_0 was about twice that reported by Magleby and Stevens. Thus, EPC decay was slower than in Magleby and Stevens' preparation. Since Magleby and Stevens reported data from only one fibre and T_0 was somewhat variable in the present study it is impossible to say whether or not this difference was significant.

background noise. The average spectrum was smoothed using the 3-point method (Sachs, 1983) and the data were transformed to linearize the spectrum by taking the reciprocal of the spectral density and squaring the corresponding frequency. The cutoff frequency was obtained from the slope of a straight line fit to the transformed spectrum using linear least squares regression. The low frequency data had to be heavily weighted to obtain an adequate fit for single Lorentzian spectra with low frequency cutoffs. The single channel conductance (γ) was obtained from the mean and variance of the ACh current noise (Stevens, 1972).

RESULTS

Estimates of ACh open channel lifetime (T_{ACh}) and conductance from the control EPC and noise data were consistent with each other and with previously published results (Anderson and Stevens, 1973). At low temperatures, estimates of T_{ACh} from EPC and noise data should coincide (Anderson and Stevens, 1973) thus,

$$T_{epc} = 1/2\pi F_c.$$

At -80 mV and 10-12° C, the average T_{epc} for 12 cut muscle fibres was 7.9 ± 1.9 msec while the average value of $1/2\pi F_c$ for 16 formamide treated fibres was 6.9 ± 1.3 msec. Thus, estimates of T_{ACh} using these two methods were reasonably close. The average single channel conductance for 16 fibres at the same holding potential and temperature was 17.8 ± 3.2 pS and was close to the average value of 20.5 pS reported by Anderson and Stevens (1973) for glycerol-treated sartorius muscles.

CONTROL

1 MM DDVP

WASHOUT

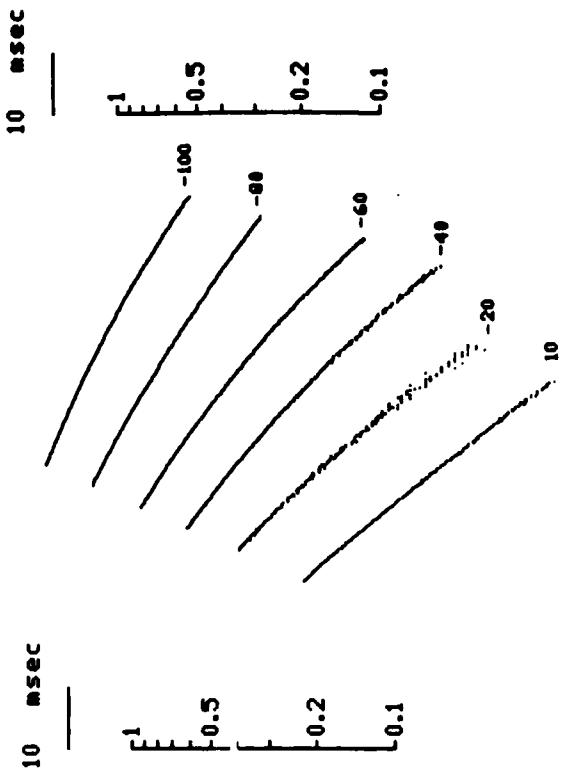
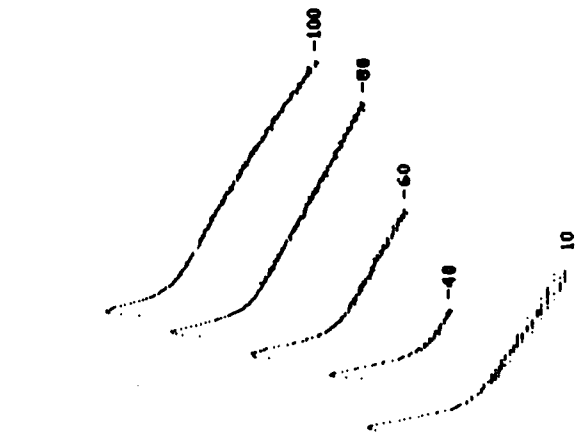
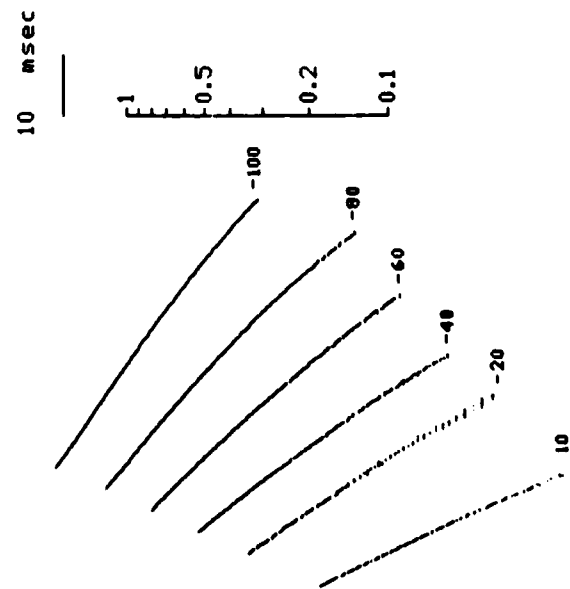
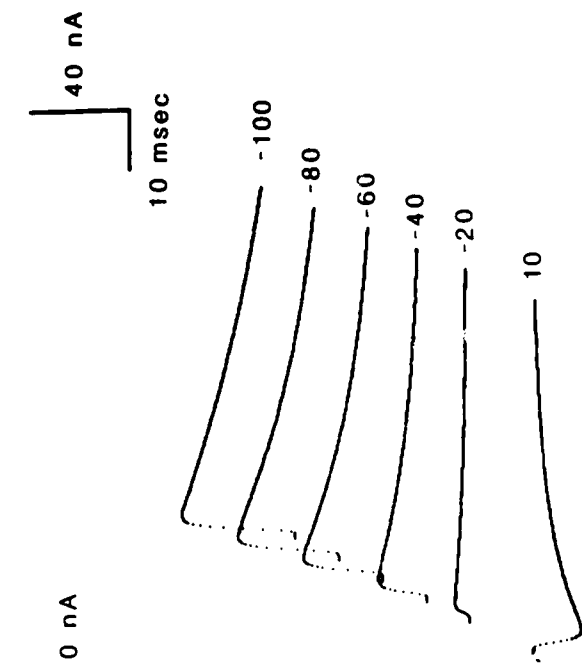
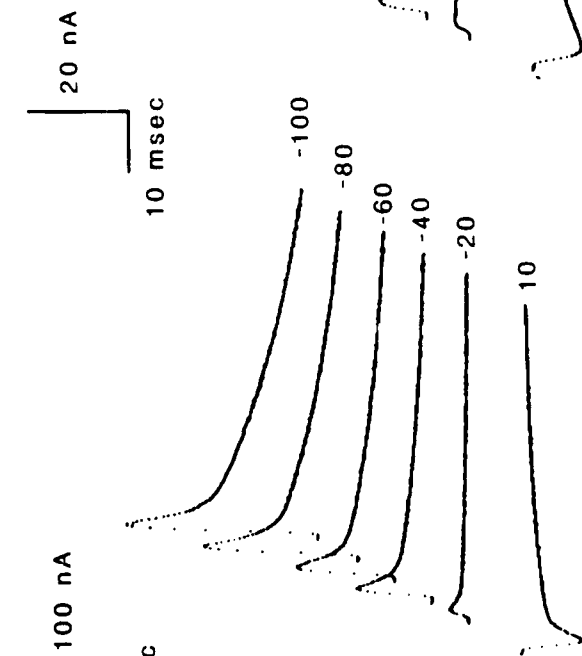
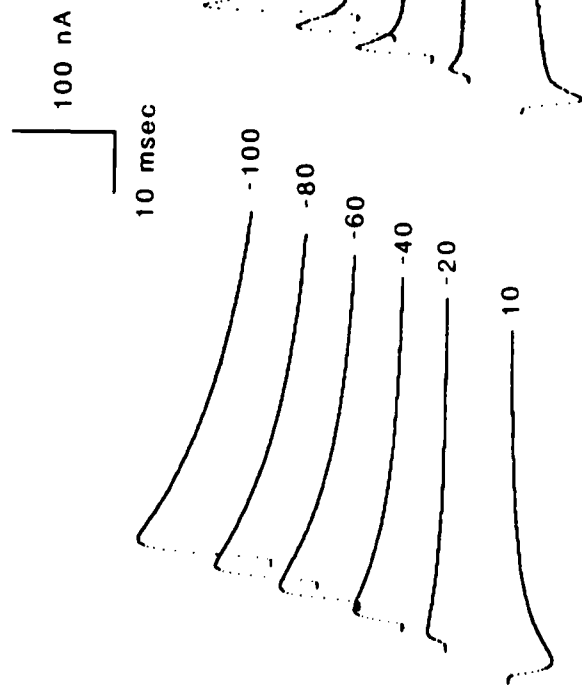


Figure 2

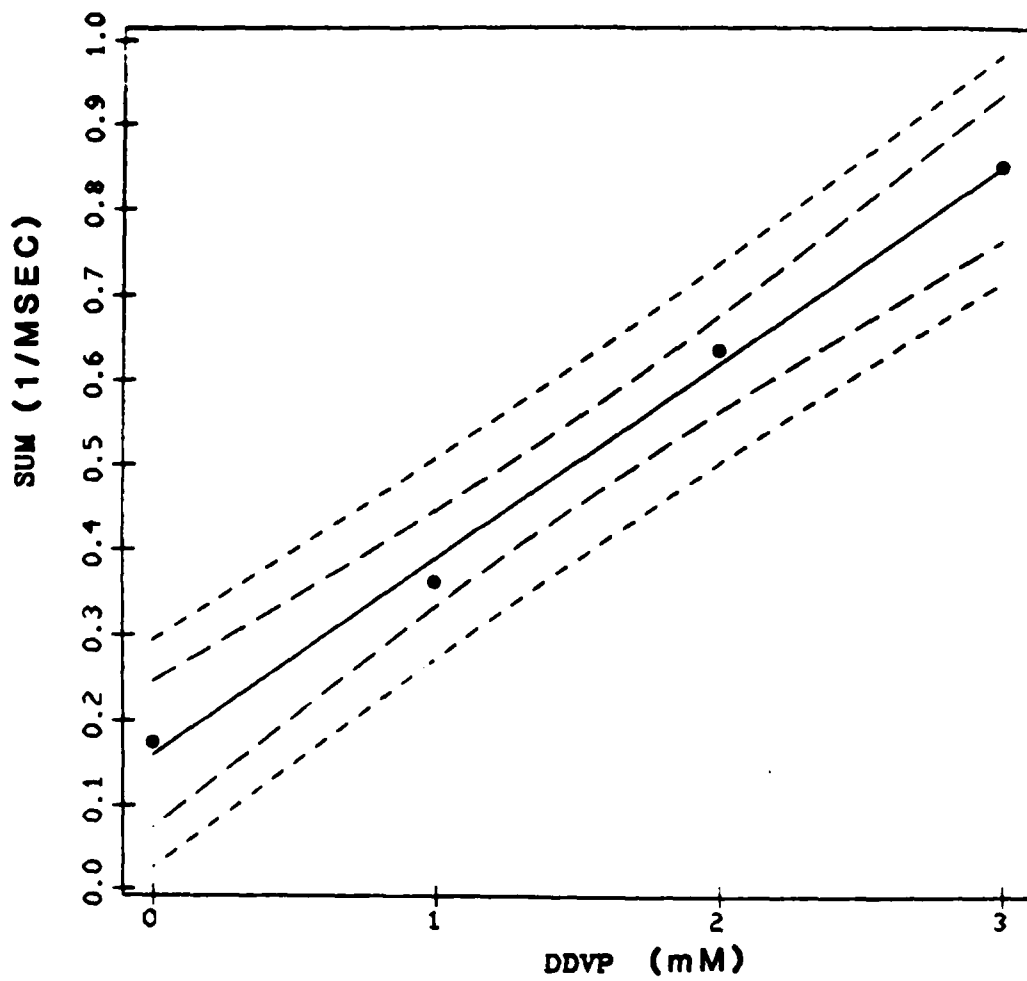


Figure 3

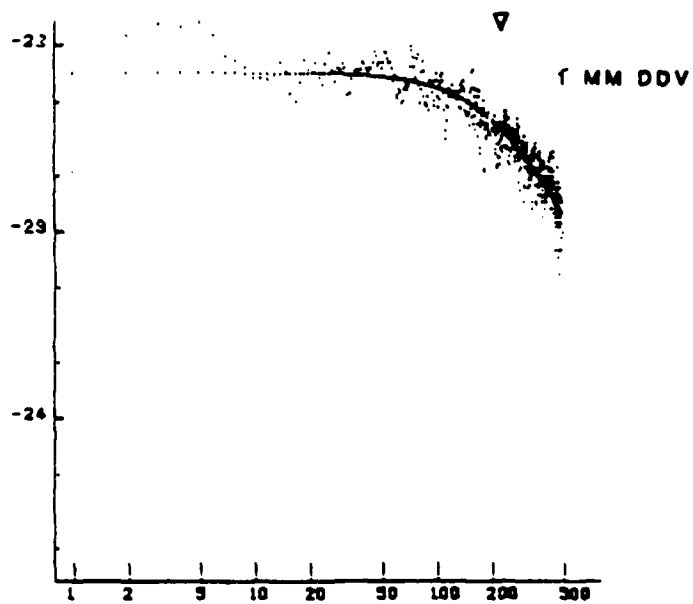
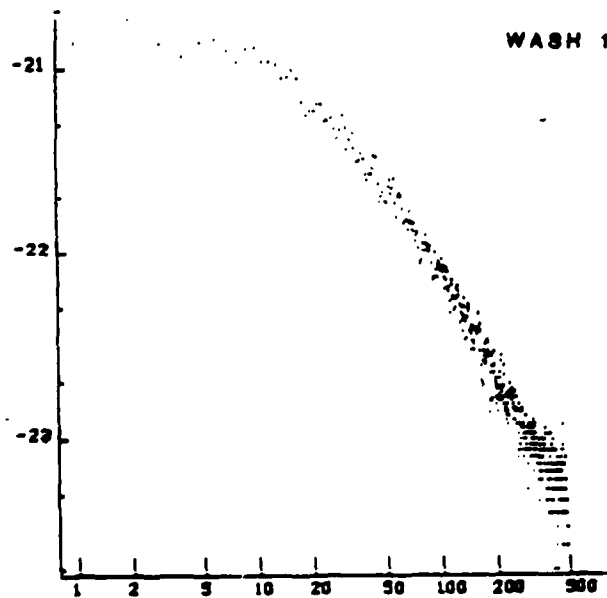
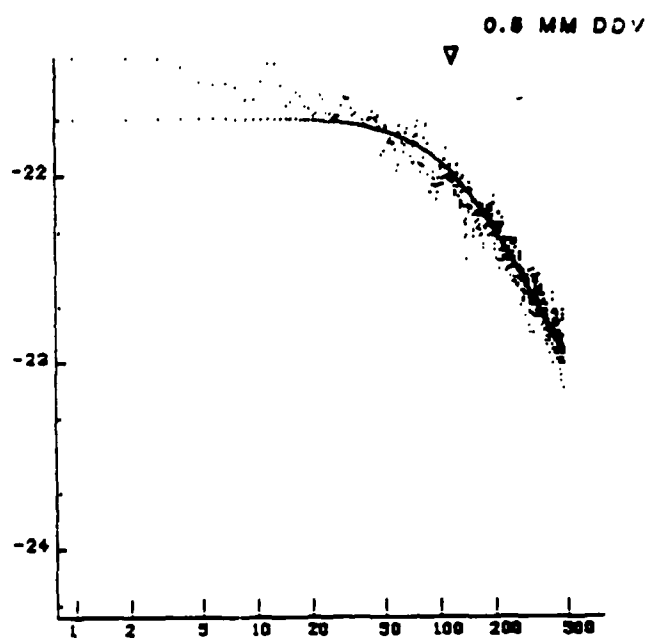
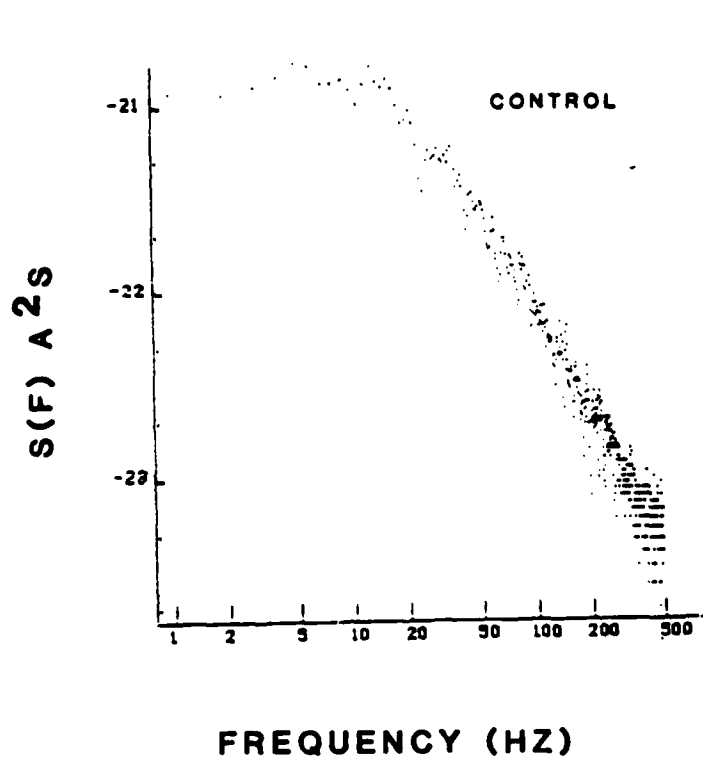


Figure 4

CONTROL

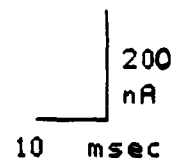
NO MSF TREATMENT

$$\tau = 9.9 \text{ msec}$$

$$I_p = 703 \text{ nA}$$

Hold -80 mV

N= 20



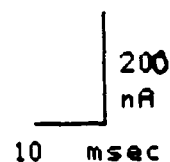
3 MM BIDRIN

$$\tau = 11.9 \text{ msec}$$

$$I_p = 683 \text{ nA}$$

Hold -80 mV

N= 20



WASH

$$\tau = 9.0 \text{ msec}$$

$$I_p = 1020 \text{ nA}$$

Hold -80 mV

N= 20

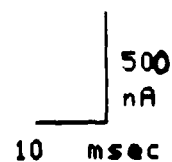


Figure 5

CONTROL

MSF PRETREATMENT

$\tau = 14 \text{ msec}$

$I_p = 593 \text{ nA}$

Hold -80 mV

N = 20

200
nA
15 msec

3 MM BIDRIN

$\tau = 12 \text{ msec}$

$I_p = 14 \text{ nA}$

Hold -80 mV

N = 20

10
nA
15 msec

WASH

$\tau = 13.7 \text{ msec}$

$I_p = 656 \text{ nA}$

Hold -80 mV

N = 20

200
nA
15 msec

Figure 6

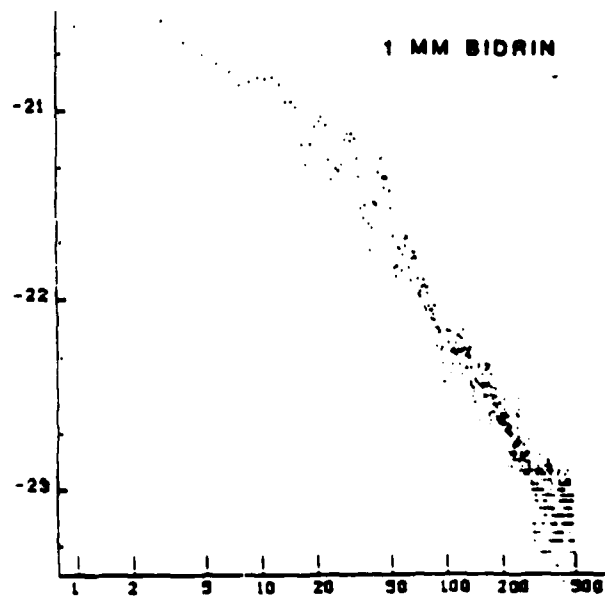
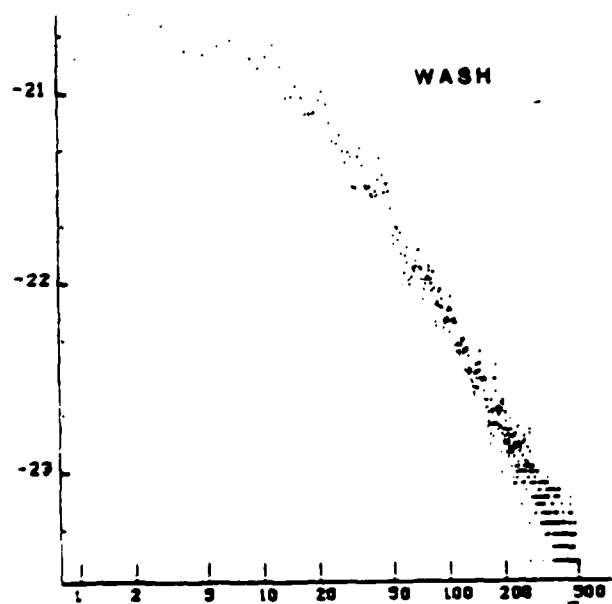
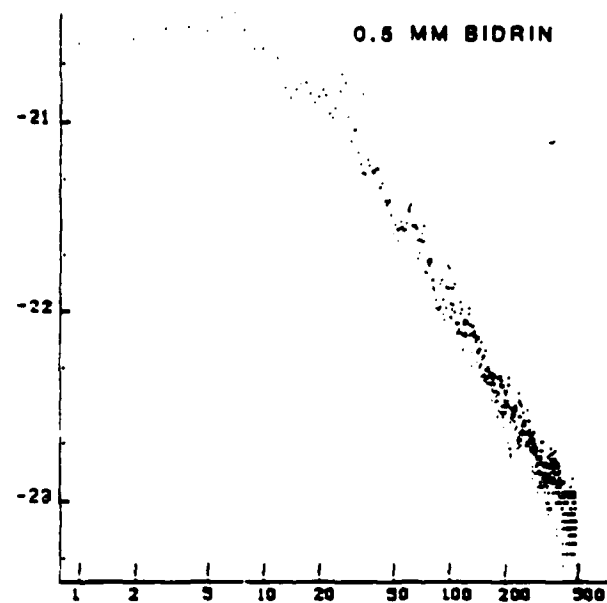
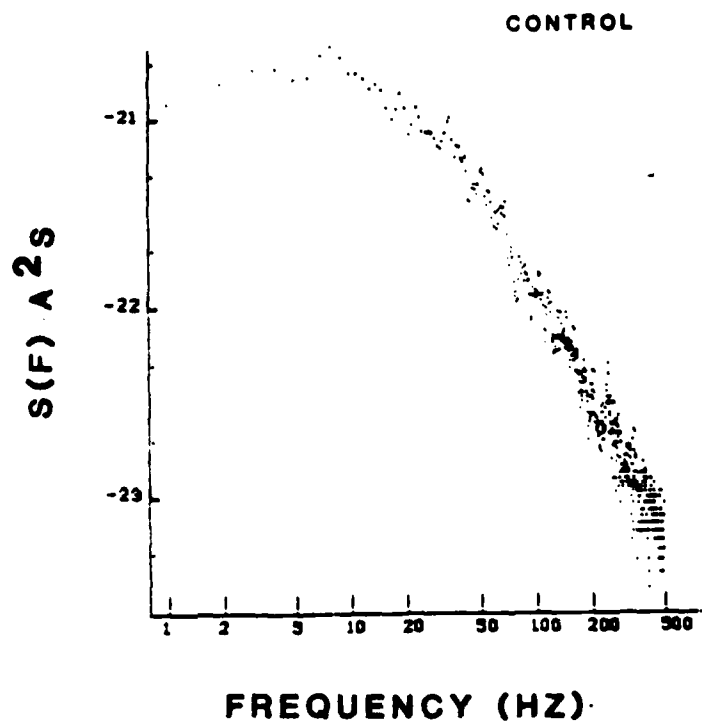


Figure 7

END

FILMED

5-85

DTIC