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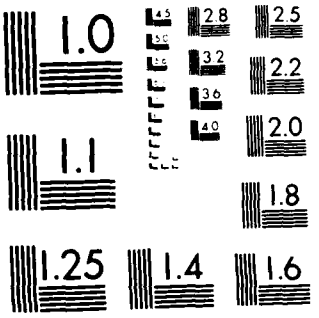

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REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER: AFOSR-TR- 83-0435	2. GOVT ACCESSION NO.: AD A127434
	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle): ACUTE EFFECTS OF ANTICHOLINESTERASE AGENTS ON PUPILLARY FUNCTION	5. TYPE OF REPORT & PERIOD COVERED: FINAL REPORT 6-15-81/12-31-82
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Ezio Giacobini	8. CONTRACT OR GRANT NUMBER(s): AFOSR-81-0229
9. PERFORMING ORGANIZATION NAME AND ADDRESS: University of Connecticut, Dept. Biobehavioral Sciences, Storrs, CT 06268	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 2312/K1, 61102F
11. CONTROLLING OFFICE NAME AND ADDRESS: Air Force Office of Scientific Research/NI, Bolling AFB, DC 20332	12. REPORT DATE: 3/29/83
	13. NUMBER OF PAGES: 15
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	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)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
anticholinesterases; pupillary function; vision; DFP

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The effect of anticholinesterase agents on pupillary function and parameters of cholinergic activity were investigated both in vitro and in vivo following topical administration. The study describes changes in three different aspects of cholinergic function: 1) uptake of choline, 2) release of acetylcholine and 3) AChE activity and pupil size. Our results are consistent with the concept of existence of a presynaptic muscarinic autoreceptor which is affected (DFP directly or through acetylcholine). DFP exerts multiple effects on various cholinergic parameters.

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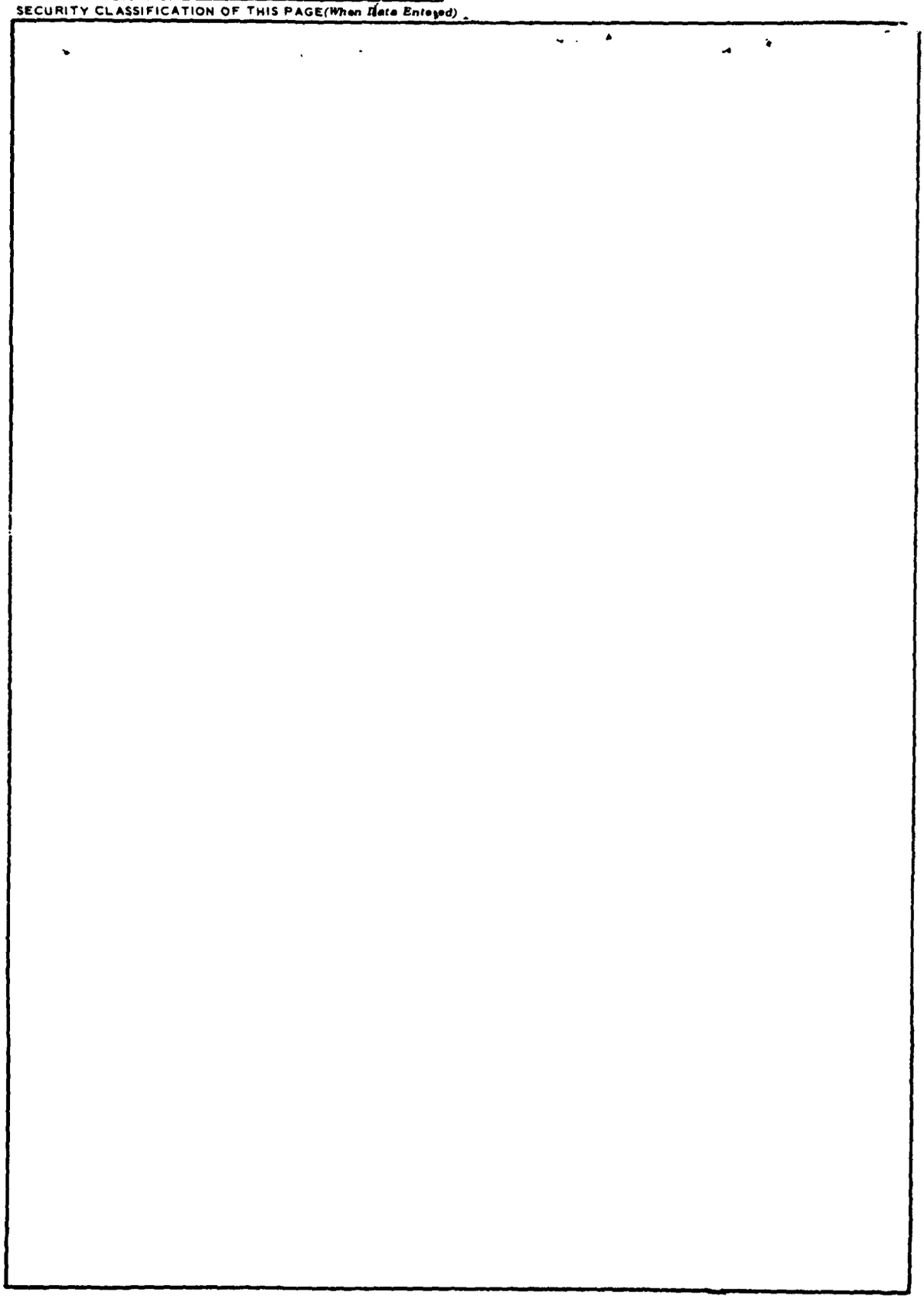

AFOSR Grant Number 81-0229
Final Technical Report
March 29, 1983

GIACOBINI, Ezio
472-50-2140

ACUTE EFFECTS OF ANTICHOLINESTERASE AGENTS ON PUPILLARY FUNCTION

UNIVERSITY OF CONNECTICUT
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Dr. Ezio Giacobini

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GIACOBINI, Ezio
472-50-2140

FINAL REPORT

(through December 31, 1982)

The strategy of this investigation is to correlate closely any impairment of pupillary function to parameters of cholinergic function in vitro and in vivo, following topical or systemic administration of antiChEs. The study focuses upon three aspects of cholinergic function: the synthesis and turnover of ACh, the cholinergic receptor and the release of ACh (Fig. 1a). A schematic diagram of the various steps involved in the analysis of multiple parameters of acetylcholine metabolism in a single isolated iris is reported in Fig. 1b.

The characteristics of the high and low affinity Ch uptake system which have been previously described by us for the developing and aging avian iris (Marchi et al., Dev. Neurosci. 3, 185, 1980 and Brain Res. 195, 423, 1980) have now been determined for the adult rat iris as well (Fig. 2).

Uptake of Choline

The uptake of choline by rat iris is linear over at least six concentrations of choline ranging from 0.1 μM to 10 μM (linear regression coefficient = 0.99). The rat iris exhibits two distinct Ch uptake systems. One component (Fig. 2), a Na^+ dependent, temperature sensitive, high affinity system which is blocked by ouabain and hemicholinium, is most likely confined to cholinergic nerve terminals. A second component, probably localized in the iris muscle cells, is Na^+ independent and shows low affinity. The substitution of 80% Na with lithium decreased uptake by 55%.

The kinetic curves show a low affinity and high affinity uptake system with a K_{m1} = 100 μM and K_{m2} + 6.67 μM, respectively (Fig. 2).

The effect of various drugs on choline uptake is reported in Figure 3 as a % control. Choline uptake, as expected, could be inhibited by ouabain which is consistent with Na^+ dependent uptake, and by hemicholinium. However, the iris preparation seems to be less sensitive to hemicholinium than brain preparations as a 100 μM concentration was necessary in order to produce a 35% inhibition of uptake. Of particular interest is the effect of DFP which at 1 mM concentration inhibits choline uptake by approximately 30% (Fig. 3). Scopolamine (1 mM) showed a 25% inhibition effect. This effect at higher concentrations may relate to its effect on release as seen in the following experiments.

Release of Acetylcholine

Acetylcholine was released from iris preloaded by incubation with 1 μM ^{3}H-Ch by 20 mA, 5 ms, 100 Hz bipolar nearly square waves (Fig. 4). The release is reported in Figure 5 as a percent of the total tissue radioactivity due to ACh at each individual time. Four to six subsequential stimulations were performed starting after spontaneous release had levelled off. Released tritiated ACh and Ch were separated and counted at each point. The evoked release ratio (ERR) was calculated from the areas under the curve (Fig. 5) representing released radioactivity and compared with corresponding non-stimulated controls. Electrical stimulation evokes 1- to 2-fold increase in the

release of ^{3}H-ACh over the spontaneous release during prestimulation baseline. The stimulated release is frequency dependent, tetrodotoxin sensitive and Ca^{++} dependent.

Scopolamine 10 nM increased evoked ACh release by at least 2-fold while 1 μM scopolamine increased spontaneous release only. These effects support the hypothesis of the presence in the iris terminals of a presynaptic muscarinic autoreceptor which controls release (Fig. 5).

DFP (1 μM) reduced both the spontaneous and the evoked release by 30% (Fig. 5). The effect was stronger following the first stimulations. This concentration of DFP inhibits AChE by approximately 80% and corresponds to the concentration used in the following in vivo experiments. This increase was blocked by scopolamine.

Acute Effects of DFP on the Iris

In this series of experiments the acute effects (up to 120 min) of DFP (5 mM) on the pupil were studied (Fig. 6).

5 μl of a .1% solution of DFP (corresponding to 5 μg of a 5 mM concentration) was instilled in the conjunctival sac of rats and its effect on the pupil area or diameter was recorded.

1. AChE activity was inhibited by 65% at 1 min and 95% at the following times (up to 1 hr). At 2 hrs the AChE activity was still inhibited by 75%.

2. Pupil size was unchanged at 1 min but decreased by more than 50% at successive times up to 2 hrs.

3. ACh levels were unchanged at 1 min, were increased by 40% at 5 min and by 50 min and were still increased by 30% at 120 min.

4. Choline levels were decreased at 5 min, showing a tendency toward a slight increase after 5 min with an increase of 25% at 120 min. In part, this reflects the low rate of hydrolysis of ACh in the presence of DFP. It is interesting to note that the pupil was still constricted at a time (120 min) when ACh had almost returned to baseline values. This may be due to a prolonged effect of ACh maintaining constantly high levels at the postsynaptic site.

In conclusion, it can be said that our results are consistent with the concept of existence of presynaptic muscarinic autoreceptors that control release of ACh from the cholinergic nerve terminals in the rat iris. With regard to the mechanism of action of DFP on the iris, we propose that the drug may exert multiple effects (see Fig. 7), such as:

a. inhibition of AChE with following increase in ACh levels both intra- and extrasynaptically,

b. inhibition of Ch uptake, and

c. reduction of both spontaneous and stimulated release.

Points b and c need further examination.

PUBLICATIONS OR COMMUNICATIONS SUPPORTED BY THE PRESENT GRANT

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Giacobini, E., J.S. Richardson and T.G. Mattio, Mechanisms of choline uptake and acetylcholine release in peripheral cholinergic synapses. Europ. Symp. on Cholinergic Transmission Presynaptic Aspects, Strasbourg, France, 1982, Abst.

Mussini, I. and E. Giacobini, Axonal and synaptic changes in the iris of aging chicks. Intl. Soc. for Develop. Neurosci., Patras, Greece, July 4-8, 1982, Abst.

Richardson, J.S., T.G. Mattio, H.L. Bernstein-Goral and E. Giacobini, Effects of DFP and other drugs on cholinergic nerve function in the rat iris. Soc. for Neurosci., Minneapolis, MN, 1982, Abst.

Mattio, T.G., E. Giacobini and J.S. Richardson, Effect of DFP on acetylcholine metabolism in the rat iris. 6th Ann. Meeting Midwest Neurobiologists, Monticello, IL, March 25-27, 1983, Abst.

Mattio, T.G., E. Giacobini and J.S. Richardson, Effect of DFP on acetylcholine metabolism in the rat iris. 9th Intl. Soc. Neurochem., Vancouver, CAN, 1983, Abst.

Mattio, T.G., E. Giaccobini and J.S. Richardson, Effect of DFP on acetylcholine metabolism in the rat iris. Amer. Soc. Neurochem., Salt Lake City, UT, 1983, Abst. 112.

Richardson, J.S., T.G. Mattio and E. Giacobini, Effext of drugs on uptake and release in rat iris parasympathetic neurons. Amer. Soc. Neurochem., Salt Lake City, UT, 1983, Abst. 113.

EFFECT OF DFP ON ACETYLCHOLINE METABOLISM IN THE RAT IRIS.
Mattio, T.G., Giacobini, E. and J.S. Richardson. Dept. Pharm., Southern Ill. Univ. School of Medicine, Springfield, IL 62708

The iris contains cholinergic nerve endings whose cell bodies are located in the ciliary ganglion. This makes this structure a good model of nerve terminal function free from contamination by cell body and glia effects. Following the characterization of the uptake system for choline (Ch) and the release of acetylcholine (ACh) in the isolated rat iris we have studied, the effect of the increase in ACh concentration following local administration of the irreversible cholinesterase inhibitor diisopropyl fluorophosphate (DFP). At various times after the topical administration of 0.1% DFP in sesame oil onto the corneal surface, the rats were sacrificed and the irises were removed. Pupil diameter was measured, ACh as well as Ch levels were determined and acetylcholinesterase (AChE) activity measured in segments of the same iris. One minute after DFP, no changes were found in pupil diameter and ACh levels, but AChE activity was decreased by 65%. At 5 minutes, pupil diameter was reduced by 60% (and remained at this level for the duration of the experiment), Ch by 30%, AChE by 92%, and ACh was increased by 38%. At 15 minutes ACh was increased by 28%, and Ch was still reduced (10%) but continued to recover reaching control levels at 60 minutes. Acetylcholine levels were still increased at 60 and 120 minutes. AChE activity was still inhibited 86% and 74% at 60 and 120 minutes, respectively. Our results show that in peripheral cholinergic terminals, in spite of the continual inhibition of AChE activity and the functional pupillary paralysis following a single exposure to antiChE agents, ACh and Ch tend to return toward normal levels. (Supported by GRANT AFOSR-81-0229 to E.G.)

EFFECT OF DFP ON ACETYLCHOLINE METABOLISM IN THE RAT IRIS.
Mattio, T.G.*, Giacobini, E. and J.S. Richardson*. Dept. Pharm., Southern Ill. Univ. School of Medicine, Springfield, IL 62708

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109

LIPID ABNORMALITIES DURING TREMBLER MOUSE DEVELOPMENT

Yao, J.K. and Bourre, J.M.
Mayo Clinic, Rochester, MN 55905 and INSERM U.134, Paris, France

In vitro incorporation of [^{14}C]acetate into desheathed sciatic nerve (endonurium) was studied in developing normal and mutant Trembler mice. The total uptake of [^{14}C]acetate peaked at 6 days after birth for both normal and Trembler mice and decreased thereafter. A substantial decrease of [^{14}C]acetate incorporation was found in Trembler mouse (~50% of normal) as early as 3 days after birth. The profile of radiolabeled lipids was also markedly different from normal. Proportionately less [^{14}C]acetate was incorporated into phospholipids and more into cholesterol in 3 and 6 days old Trembler mice. A decreased incorporation of cholesterol was found to be present in Trembler mice 9 days after birth and continued thereafter. On the other hand, at later times (13 to 55 days), the relative incorporation of phospholipids was increased above that of controls. In developing normal nerve, the relative incorporation of free fatty acids from [^{14}C]acetate increased (4 to 25%) progressively with age. By contrast, this incorporation in Trembler nerves remained constantly low (<7%) during development. The relative incorporation of triacylglycerol and cholesteryl esters were about two times higher in Trembler than in normal mice. Morphologically speaking, the nerve of Trembler mouse is markedly hypomyelinated. Thus, the biochemical profile of extended active but perturbed lipid metabolism, i.e., continued active incorporation of phospholipids but reduced cholesterol biosynthesis, may provide a biochemical basis for the morphologic findings of continued active but ineffective myelination in Trembler peripheral nerve.

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IN VITRO SYNTHESIS OF THE MYELIN BASIC PROTEINS IN NORMAL AND MUTANT MICE

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[a]Hopital de la Salpetriere, Paris and [b]Univ. of Md., College Park, MD.

Total polyribosomes were isolated from the brains of 16-20 day C57BL/6J mice, 4 neurological mutants (Qk/Qk, shi/shi, mld/mld and jp/Y) and 4 heterozygote or littermate controls (Qk/+, shi/+, mld and jp litterrates) and translated in a homologous, cell-free system. No significant differences were observed among the 9 genotypes in either the yield of polysomes (32.2±0.6 A_{260}/g brain) or in the incorporation of ^{35}S-methionine into TCA-precipitable protein. However when the 4 myelin basic proteins (MBPs) were isolated from the translation mixtures by immunoprecipitation followed by SDS-PAGE and fluorography, little incorporation of ^{35}S-methionine into the MBPs was noted in those assays directed by polysomes from mld/mld or from shi/shi animals. Compared with C57BL/6J polysomes, mld littermate and shi/+ polysomes incorporated approximately half the levels of label into the 4 MBPs while Qk/+ and Qk/Qk incorporated normal and close to normal levels. Polysomes from jp littermates and jp/Y brains synthesized 66% and <20% of the levels of the 14K MBP compared to C57BL/6J polysomes. Incorporation of label into the other 3 MBPs was normal with jp littermate polysomes and about half the control levels with jp/Y polysomes. The data indicate that shi/shi and mld/mld mutants either produce altered MBPs not recognized by our antibody or synthesize very low levels of MBP. The data provide additional support for the notion that the Qk/Qk mutant synthesizes much higher levels of MBP than are incorporated into myelin. They also indicate that in the jp mutant the synthesis of the 4 MBPs are affected to different extents and, therefore, the mutation cannot be easily characterized as either an "assembly" or "synthesis" defect. Supported by I.N.S.E.R.M., France, and PHS Grant NS15469, U.S.A.

111 EFFECTS OF SOMAN ON HIGH AFFINITY CHOLINE UPTAKE BY RAT BRAIN SYNAPTOSOMES

Whalley, C.E.* and Shih, T.-M. (SPON: B.E. Hackley, Jr.)
USA Med. Res. Inst. Chem. Def., Aberdeen Proving Ground, MD 21010

We have previously reported (Psychopharmacology, 78, 170, 1982) the regional variation in the effects of soman, a potent organophosphorus cholinesterase inhibitor, on brain levels of acetylcholine and choline. Available evidence (Kuhar et al., J. Neurochem., 30, 15, 1978) suggests that alterations of activity of cholinergic neurons in vivo parallel changes in sodium-dependent high affinity choline uptake (SDHACU) measured in vitro. In the present study, we examined the changes of SDHACU in synaptosomes isolated from rat striatum (S), hippocampus (H), cortex (C), midbrain (M), brainstem (B), cerebellum (R) and whole brain, following soman treatment. Incubation of synaptosomes with various concentrations (10^{-5}-10^{-8}M) of soman in vitro or subcutaneous administration of 0.9 LD50 (120 µg/kg) of soman in vivo was utilized. In the latter experiment, rats were killed 1, 4, 24 hrs and 7 days after soman administration. Incubation with various concentrations of soman in vitro did not affect synaptosomal SDHACU in the whole brain or in any of the brain regions. On the other hand, SDHACU following in vivo soman treatment showed in S an increase at 4 and 24 hrs (40 and 71%, respectively); in C a decrease at 1 and 4 hrs (54 and 45%, respectively); and in H a decrease only at 1 hr (41%). In B or M no significant change occurred in synaptosomal SDHACU at any time studied, while in R, SDHACU was not detectable in either control or soman treated animals. These data indicate that brain synaptosomal SDHACU does not appear to be affected directly by soman at concentrations studied in vitro and it is postulated that the regional differences observed in H and C or S following in vivo soman administration reflect changes in activity of cholinergic neurons during acute intoxication.

112 EFFECT OF DFP ON ACETYLCHOLINE METABOLISM IN THE RAT IRIS

Mattio, T. G.*, E. Giacobini, and J. S. Richardson
Lab Neuropsychopharm. Dept. Biob. Sc. Univ. Conn., Storrs, CT 06268

The iris contains cholinergic nerve endings whose cell bodies are located in the ciliary ganglion. This makes this structure a good model of nerve terminal function free from contamination by cell body and glia effects. Following the characterization of the uptake system for choline (Ch) and the release of acetylcholine (ACh) in the isolated rat iris we have studied, the effect of the increase in ACh concentration following local administration of the irreversible cholinesterase inhibitor diisopropyl fluorophosphate (DFP). At various times after the topical administration of 0.1% DFP in sesame oil onto the corneal surface, the rats were sacrificed and the irises were removed. Pupil diameter was measured, ACh as well as Ch levels were determined and acetylcholinesterase (AChE) activity measured in segment of the same iris. One minute after DFP, no changes were found in pupil diameter and ACh levels, but AChE activity was decreased by 65%. At 5 minutes, pupil diameter was reduced by 60% (and remained at this level for the duration of the experiment), Ch by 30%, AChE by 92%, and ACh was increased by 38%. At 15 minutes ACh was increased by 28%, and Ch was still reduced (10%) but continued to recover reaching control levels at 60 minutes. Acetylcholine levels were still increased at 60 and 120 minutes. AChE activity was still inhibited 86% and 74% at 60 and 120 minutes respectively. Our results show that in peripheral cholinergic terminals, in spite of the continual inhibition of AChE activity and the functional pupillary paralysis following a single exposure to antiChE agents, ACh and Ch tend to return toward normal levels.

(Supported by GRANT AFOSR-81-0229 to E. G.)

113.

Effects of drugs on uptake and release in rat iris parasympathetic neurons.
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The isolated iris of the rat accumulates choline (Ch) by high affinity (K_D = 6.6 µM) and low affinity (K_D = 100 µM) processes. The uptake of 1 µM Ch at 37°C is inhibited in a dose-dependent manner by hemicholinium (IC_{50} is 70 µM), by ouabain (IC_{50} is 1 mM) and by substituting equi-molar lithium for the sodium in the buffer (IC_{50} when lithium has replaced 60% of the sodium). At 0°C, Ch uptake is reduced by over 85% and this 0°C uptake is not sensitive to hemicholinium. This suggests that the high affinity process is active transport into cholinergic nerve terminals while the low affinity process may be diffusion into other tissue. The uptake of 1 µM Ch at 37°C is inhibited by 1 mM scopolamine and is reduced in a dose dependent manner by the irreversable anticholinesterase diisopropylfluorophosfate (DFP) and by pyridostigmine, an anticholinesterase with agonist properties. Physostigmine, an anticholinesterase lacking agonist actions, has a biphasic effect with Ch uptake being increased by 1 and 10 µM concentrations but not altered by 0.1 or 1000 µM concentrations. The release of labeled acetylcholine (ACh) induced by electrical stimulation of the iris, increases as stimulation frequency is increased up to 50 Hz. In the absence of an esterase inhibitor, the radioactivity in the perfusate was about 30% Ch and 70% ACh. The release of ACh evoked by electrical stimulation (20 mA, 5 ms, 50 Hz, at 35°C) was reduced by choline, DFP, and the norepinephrine uptake blockers imipramine and amitriptyline. ACh release was increased in a dose-dependent way by scopolamine. These results are consistant with the existance on the parasympathetic nerve terminal of presynaptic sympathetic receptors and muscarinic autoreceptors that inhibit the release of ACh. Supported in part by Grant USAFOSR-81-0229 to Dr. Giacobini.

114.

MONOCLONAL ANTIBODIES TO CHOLINE ACETYTRANSFERASE FROM RAT BRAIN
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We have recently described the production and partial characterization of five monoclonal antibodies reacting with rat brain choline acetyltransferase. Antibodies purified from ascites fluids were found to recognize a cluster of determinants restricted to a small portion of the enzyme surface. (Crawford et al, PNAS in press). One of the antibodies recognizes a determinant not destroyed by glutaraldehyde fixation and is presently being applied in a number of immunocytochemical investigations of choline acetyltransferase distribution in rat brain. Titration curves of the antibodies have been analyzed by Scatchard's method. Each antibody displays a single affinity for ChAT in the range of K_A= 10^6 to 10^9 M^{-1}. Cross reaction studies indicate that several antibodies react with enzyme present in other rodent brains, such as mouse and guinea pig, primate brain enzyme from babboon and human as well as enzyme from human placenta. One of the antibodies also cross reacts with enzyme from chicken brain, frog spinal cord, and Aplysia ganglion. The determinants thus appear to be not only clustered in a small region of the enzyme surface but also highly conserved. (Supported by NS 18858).

Monday, March 21, 1983

115 PLASTICITY OF NICOTINIC SYNAPTIC TRANSMISSION
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Previous studies have demonstrated long term potentiation (LTP) of synaptic transmission in the superior cervical ganglion of the rat (Brown and McAfee, 1982, Science 215:1411-1413). We obtained these results by incubating the ganglion in curare to reduce excitability, and then repetitively stimulated the preganglionic nerve at 20 Hz for only 20 seconds. While measuring the compound action potential in response to single preganglionic stimuli once every minute, we observed a 2-fold increase in the response amplitude which decayed as a double exponential with time constants 1-3 minutes (PTP) and 30-230 minutes (LTP). These findings, based on extracellular measurements, have now been confirmed using intracellular techniques.

In 21 of 41 cells, stimulation of the preganglionic nerve at 20 Hz for 20 sec induced an increase in the nicotinic excitatory postsynaptic potential (EPSP) or an increase in the ability of synaptic stimulation to generate an action potential in the postsynaptic neuron. The EPSP's were frequently obscured by synaptically driven action potentials which appeared after the tetanic stimulation. These effects lasted for 30 minutes to several hours, as long as the recording could be maintained. The potentiation was not accompanied by measurable changes in resting membrane potential or input resistance. Direct nonsynaptic stimulation of the postsynaptic neuron (20 Hz for 20 sec) failed to induce any increase in synaptic transmission in cells. Thus, LTP in the ganglion appears to be due to an increase in the efficacy of nicotinic synaptic transmission. We have hypothesized that LTP is accompanied by an increase in acetylcholine release but this awaits direct measurement. Supported NSF BNS-12414.

116 L(-)SULPIRIDE ENHANCEMENT OF STRIATAL ACETYLCHOLINE RELEASE IN VITRO
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The effect of L(-)sulpiride, a specific dopamine-2(D-2;non-adenylate cyclase-linked)receptor antagonist virtually devoid of muscarinic activity (SPANO et al 1979), on the release of acetylcholine(ACh) from rat striatal tissue slices was examined. Male Sprague-Dawley rats(250-350 Gm) were decapitated and striatal slices were prepared. Slices were gassed(100% O_2) continuously during a 20 min pre-incubation in Krebs-phosphate buffer containing glucose (5mM) and choline (50µM), and were immediately aliquoted to oxygenated tubes containing no drug(control) or L(-)sulpiride(10^{-5},10^{-4} or 10^{-3}M)in either 5 or 30mM K^+ Krebs-phosphate(glucose,5mM;choline,50µM; paraoxon,40µM) for a further 10 min. ACh in incubation medium was measured immediately by a radioreceptor method (EHLERT et al,Life Sci.31:347-54, 1982). Tissue protein(0.9±.03mg) was measured by the biuret method.

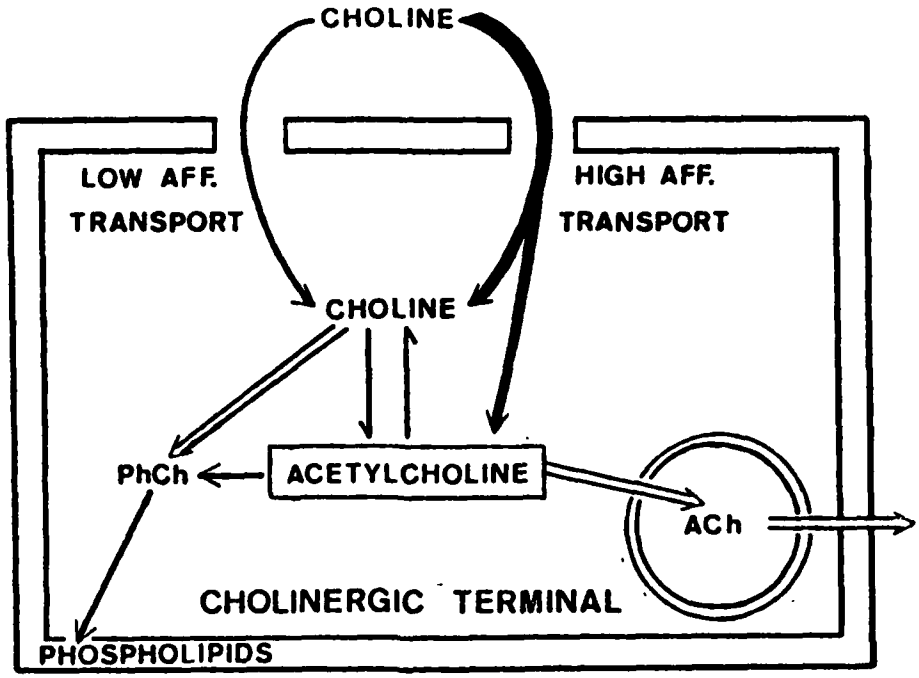
Incubation Condition	Control	L(-)Sulpiride 10^{-5}M	10^{-4}M	10^{-3}M
5mM K^+	452 ± 42(4)	497 ± 27(3)	482 ± 18(3)	408 ± 25(3)
30mM K^+	813 ± 25(4)+	946 ± 44(3)**	976 ± 44(3)**	746 ± 20(3)*

Each value is the mean of ACh released mg protein 10 min)±SEM of the sample number in parentheses, each based on triplicate assays. (*)and(**) indicate $P<.01$ or $<.001$, respectively, vs. appropriate control values. (+) indicates a $p<.05$ difference between 30 and 5mM K^+ control conditions.

These data show a significant enhancement of ACh release under depolarizing(30mM K^+) conditions and a lack of effect of L(-)sulpiride on resting (5mM K^+) ACh release from rat striatum. The results suggest that D-2 [illegible] (DA) receptors are important in mediating DA control of striatal [illegible], and emphasize that stimulated transmitter release must be present for L(-)sulpiride's effect to be apparent. [Supported by the Fund for Basic Research in Psychiatry.]

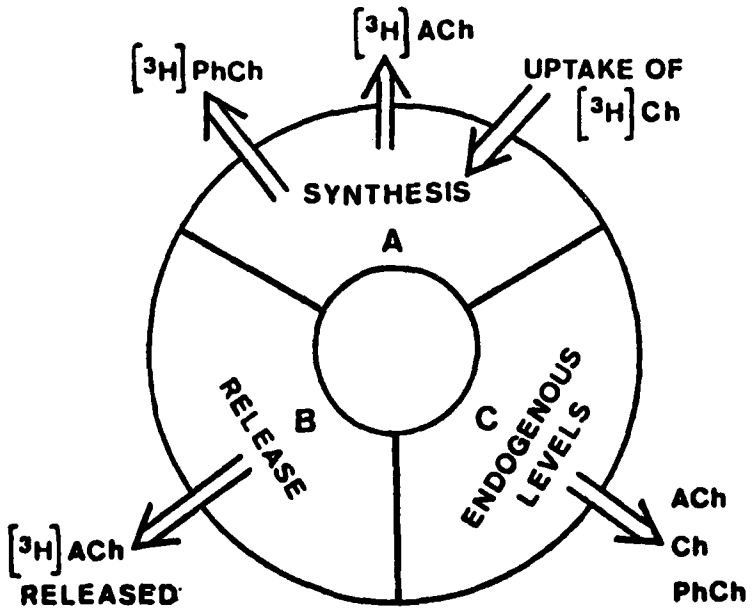
Figure 1

a)



ACh = acetylcholine, PhCh = phosphorylcholine, Ch = choline

b)



IRIS PREPARATION

a) Schematic diagram showing various steps involved in the regulation of acetylcholine synthesis in a cholinergic terminal.
b) Scheme of analysis of multiple parameters of acetylcholine metabolism in three segments (A, B, and C) of a single isolated iris.

Figure 2

KINETIC CURVES FOR Ch UPTAKE IN THE RAT IRIS

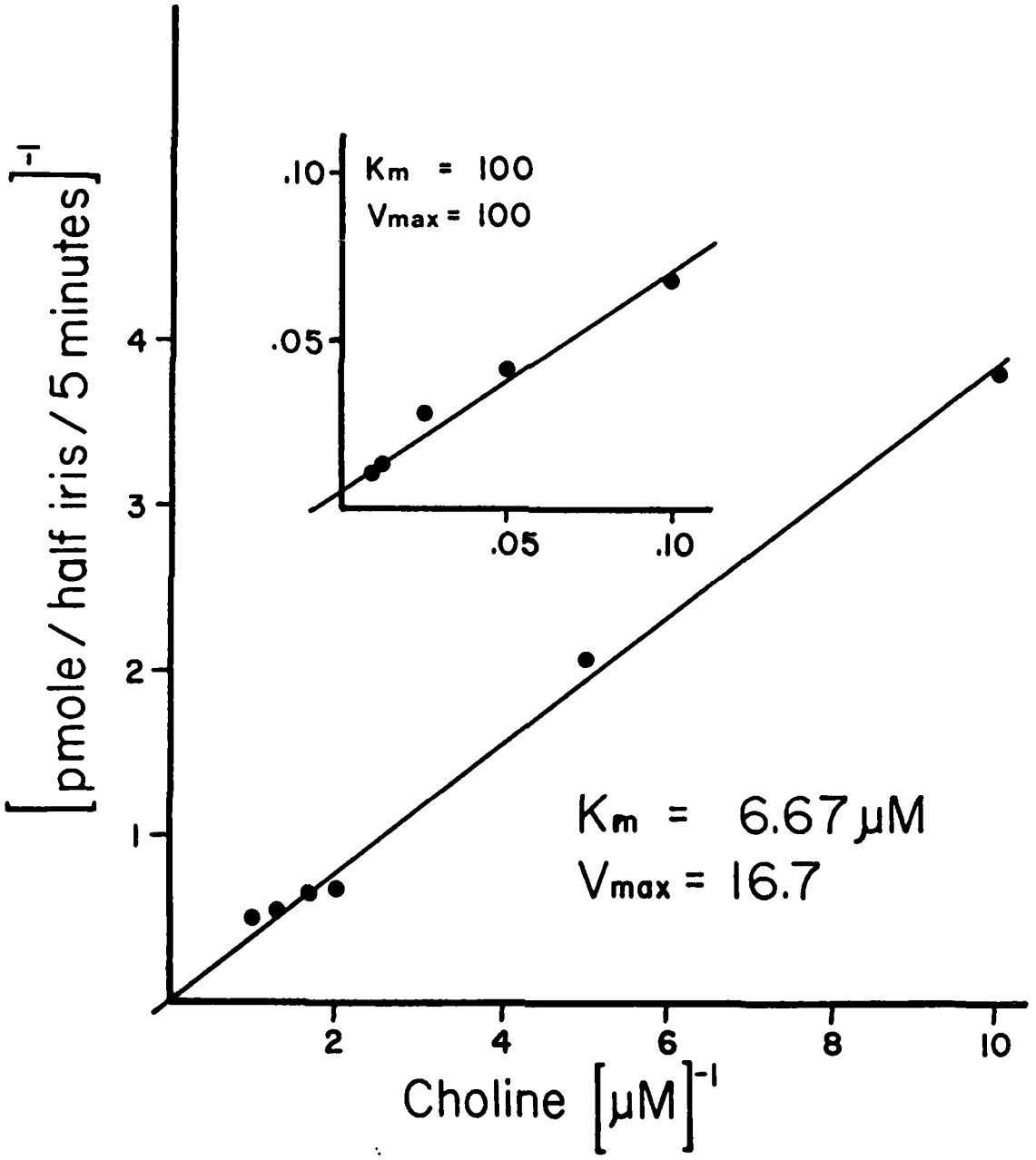


Figure 3 - EFFECT OF VARIOUS DRUGS ON CHOLINE UPTAKE

GIACOBINI, Ezio
472-50-2140

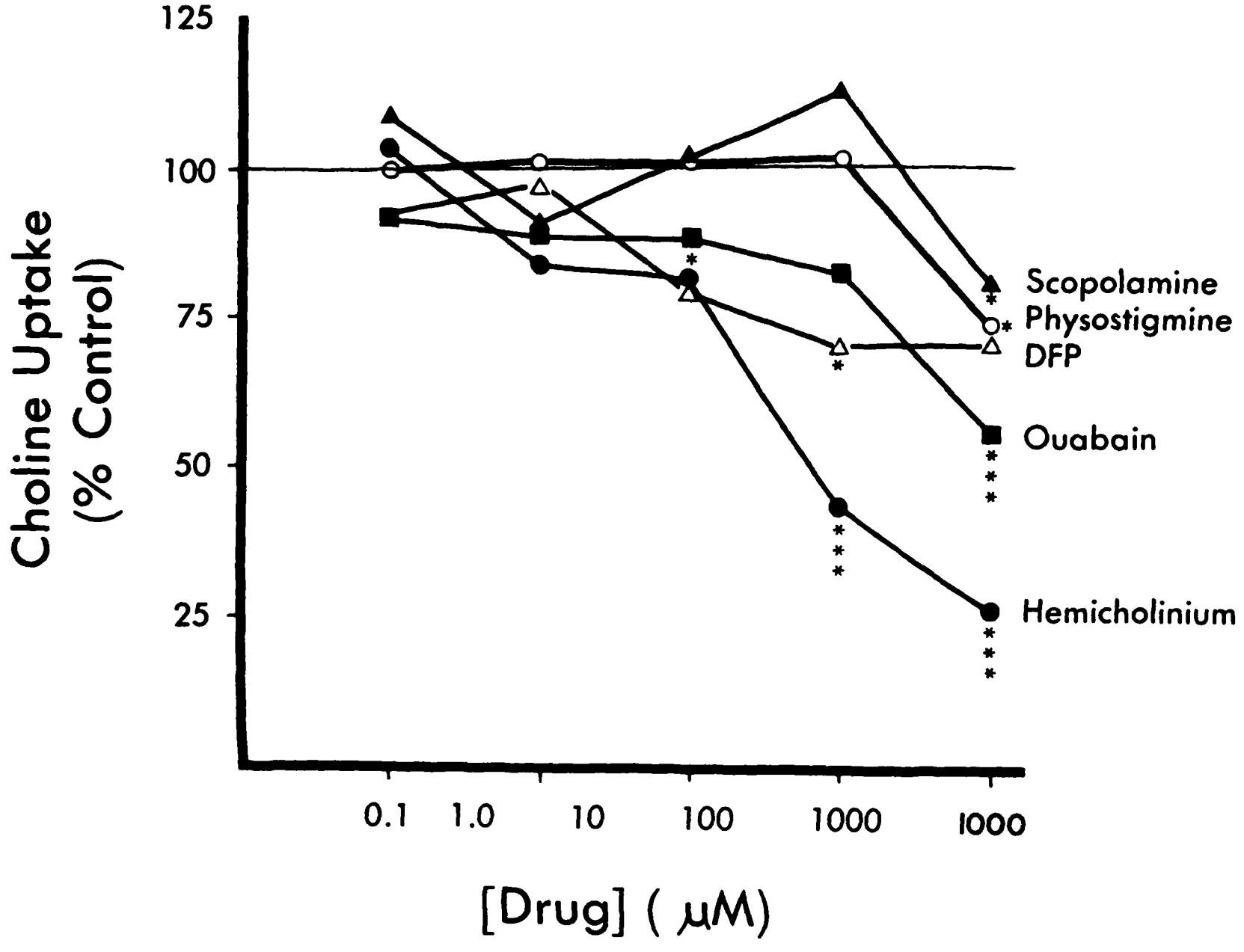


Figure 4 - RELEASE RATIO OF ACETYLCHOLINE AT FREQUENCIES

GIACOBINI, Ezio
472-50-2140

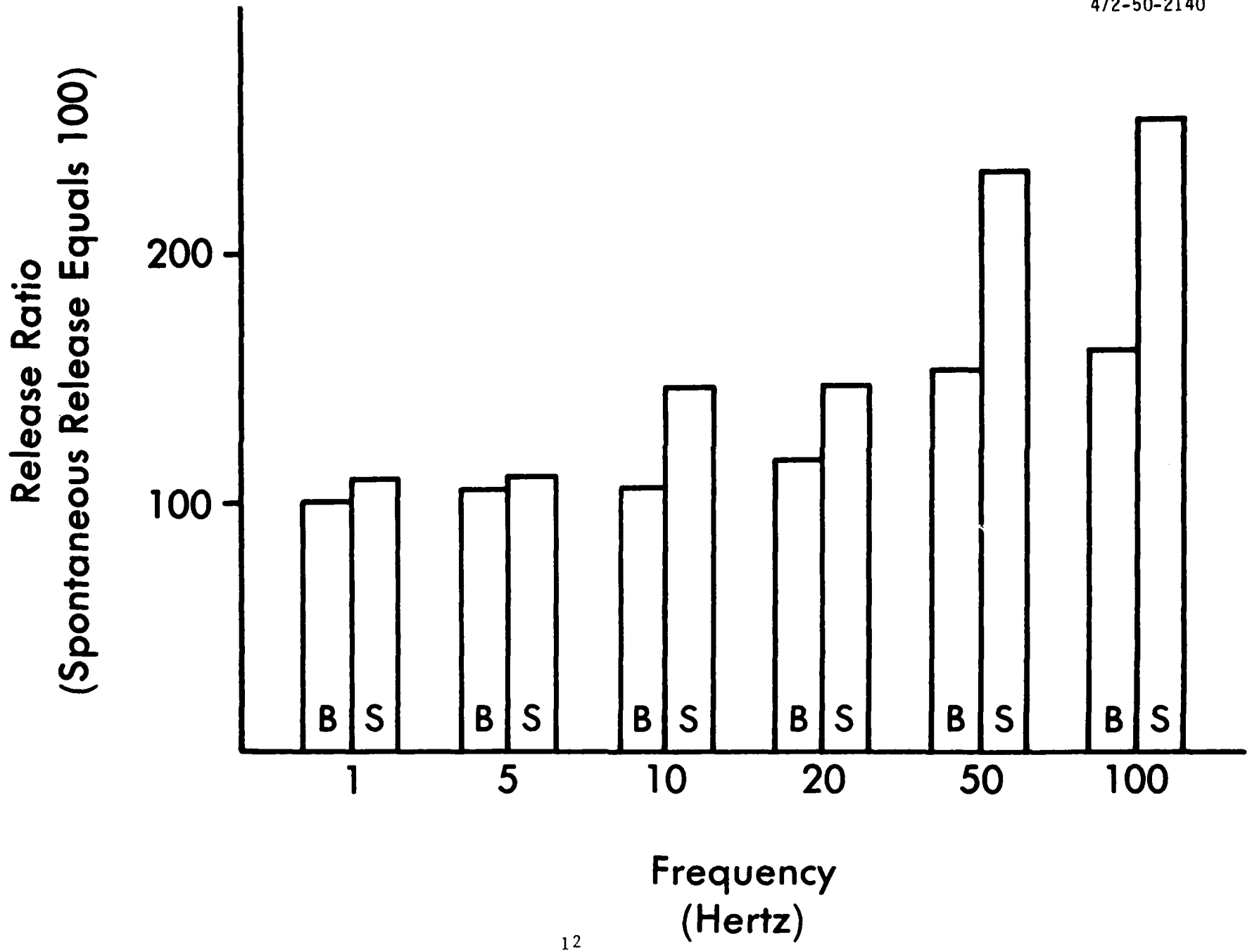


Figure 5

EFFECT OF VARIOUS DRUGS ON THE RELEASE OF ^{3}H-ACh FOLLOWING PRELOADING WITH 1 µM ^{3}H-Ch (20 mA, 5 ms, 100 Hz)

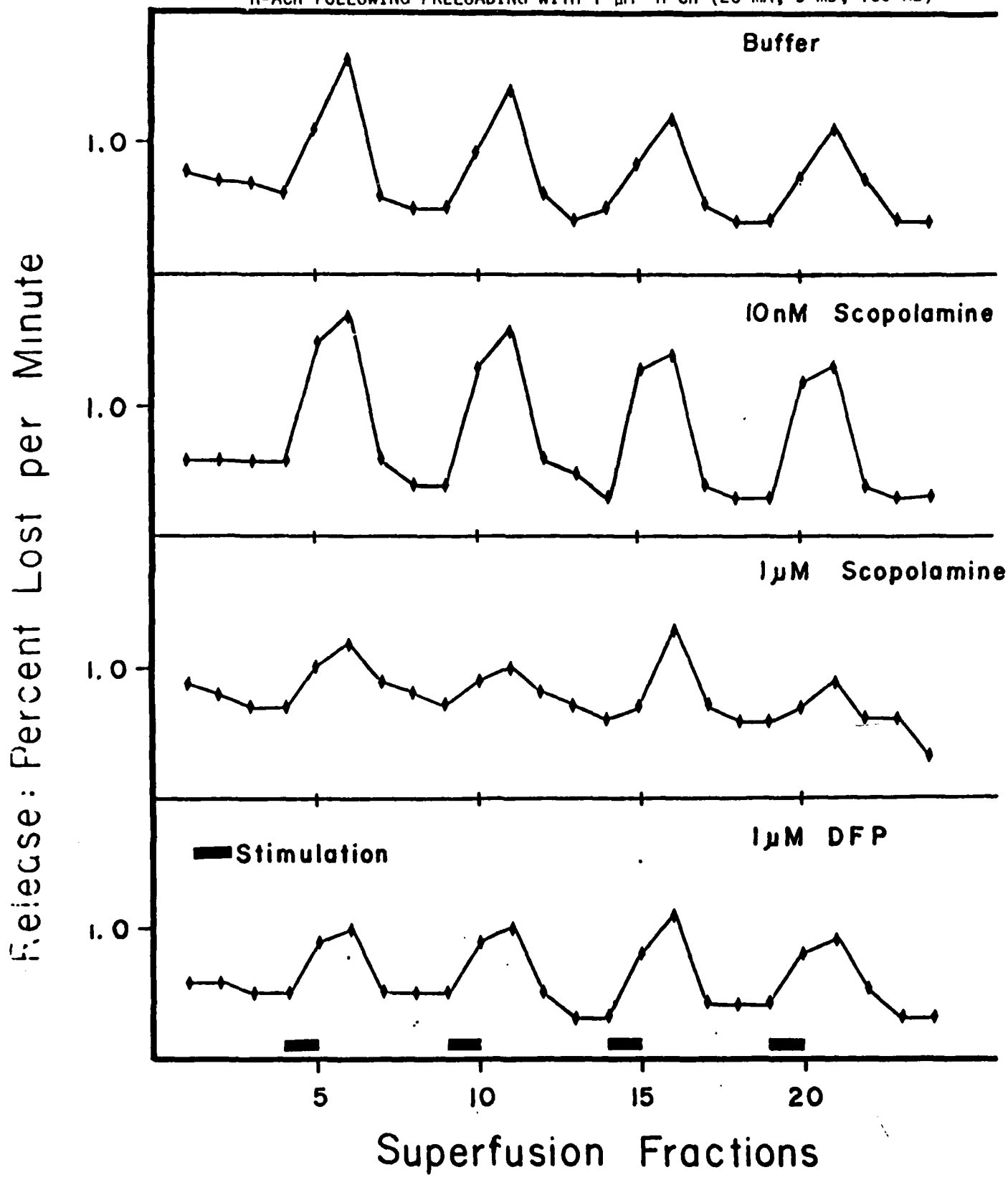


Figure 6
CHANGES IN AChE ACTIVITY, PUPIL DIAMETER, ACh AND Ch LEVELS
IN RATS INSTILLED WITH DFP (5 mM) IN THE CONJUNCTIVAL SAC

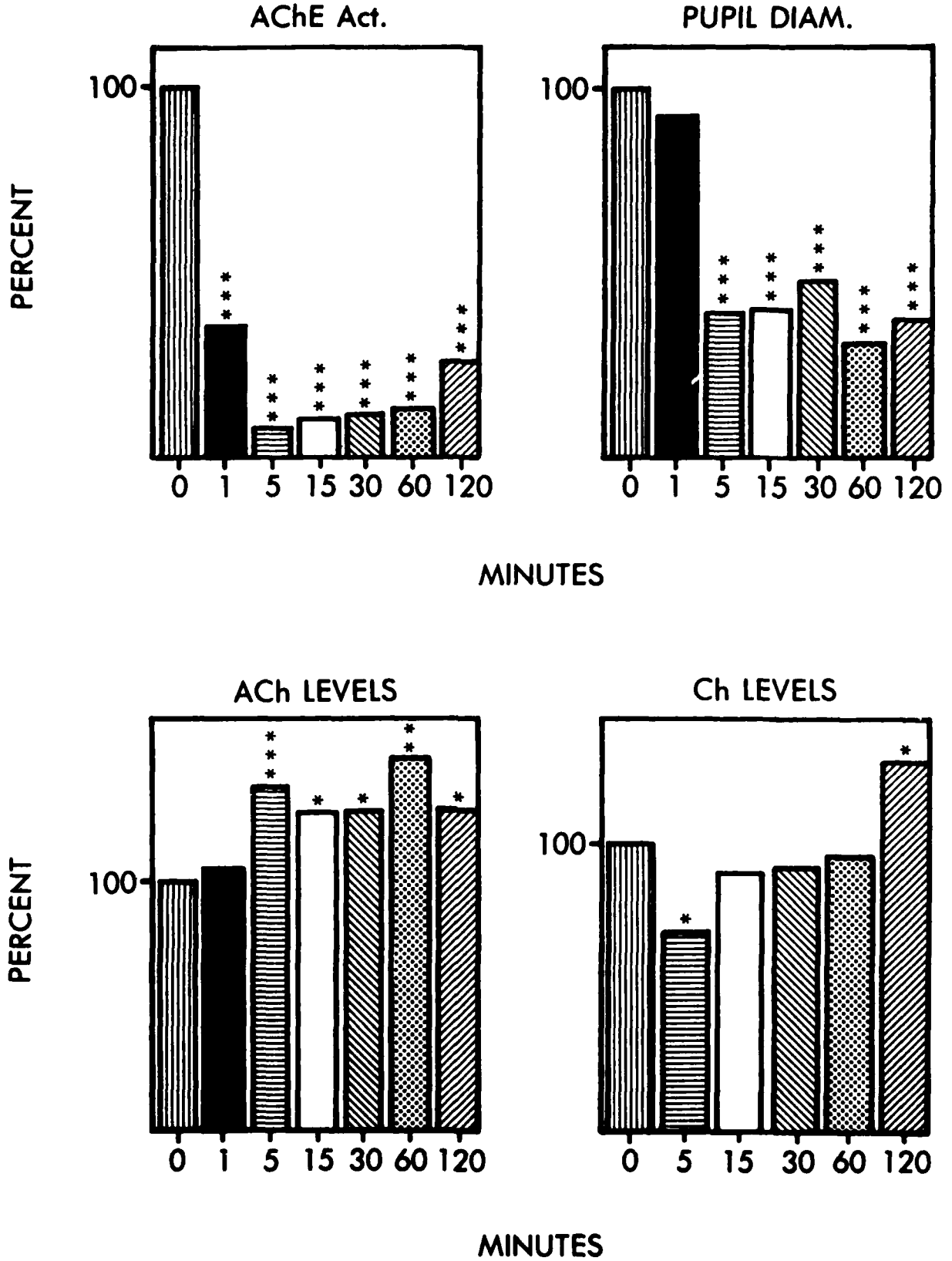
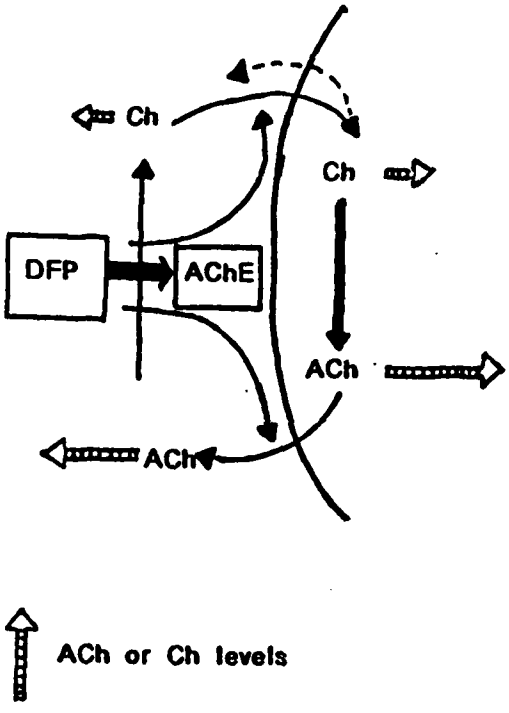
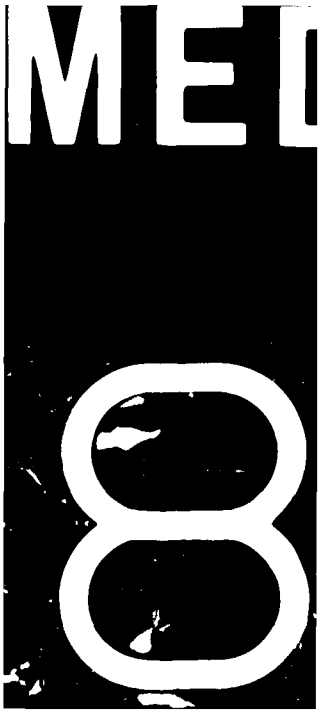


Figure 7

MULTIPLE EFFECTS OF DFP ON CHOLINERGIC SYNAPSES

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