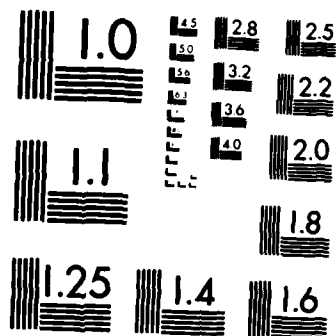


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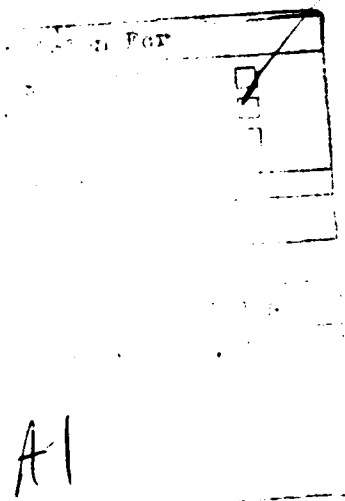
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ACUTE EFFECTS OF ANTICHOLINESTERASE AGENTS
ON PUPILLARY FUNCTION

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PROGRESS REPORT

(Period March 15, 1984 through September 15, 1984)

General description of the work in progress

Three main directions of our research have been pursued. First, we have accumulated new pharmacological evidence for a mechanism of ACh release related to a muscarinic autoreceptor present in the rat iris. Secondly, we have continued our study of drug effect on the release of ACh, adding a new group of drugs, the aminopyridines, which enhance Ca^{++} influx into the neuron. Finally, we have studied the effect of aging on pupillary function and ACh metabolism. These three lines of work have each produced novel and intriguing results which are summarized in the following section. The results described in this report have been communicated at several national and international meetings. The abstracts of the communications are attached (Appendix V).

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A. EVIDENCE FOR THE PRESENCE OF MUSCARINIC AUTORECEPTORS IN THE MAMMALIAN IRIS

The release of acetylcholine (ACh) represents one of the most relevant parameters in the study of cholinergic function. This process seems to be regulated by a specific feedback mechanism mediated by presynaptic receptors (autoreceptors) of the muscarinic type located on cholinergic nerve terminals. The presence of muscarinic presynaptic autoreceptors has been demonstrated in both CNS and PNS (Marchi, Paudice and Raiteri, 1984; Kilbinger, 1984). Our pharmacological analysis of the release of [3 H]ACh has for the first time revealed the presence of a presynaptic muscarinic receptor in the mammalian iris. When stimulated either directly through an ACh agonist or indirectly through a cholinesterase inhibitor such as DFP, this receptor will inhibit the release of [3 H]ACh. Inversely, blocking this presynaptic receptor with scopolamine, a muscarinic antagonist, increases the release of [3 H]ACh in a dose-dependent manner. DFP, at concentrations where 90% or more of the AChE activity is inhibited, decreases the release of [3 H]ACh. This effect is reversed by scopolamine 10^{-6} M, a concentration which itself does not increase release. On the other hand, the uptake of choline (Ch) in the iris is also reduced in a dose-dependent manner by DFP. Therefore, we can say that DFP may influence acetylcholine function in the iris two-fold, by acting presynaptically, first on the release of ACh and secondly on the uptake mechanism of Ch. It is difficult to say whether these effects are direct or are indirectly mediated through extraneuronal ACh accumulation. Our knowledge of the existence of presynaptic cholinergic autoreceptors, which might be affected by ChE inhibitors, may help us to develop new antidotes to preserve pupillary function following exposure to anticholinesterase agents (see next section). The evidence for the effect of DFP on mechanisms of uptake and release at the

presynaptic site have been described in detail in two publications (Mattio et al., 1984a,b) (see Appendices I and II).

The results relevant to the pharmacological characterization of this release mechanism are summarized in the next section of the report.

B. DRUG EFFECTS ON ACETYLCHOLINE RELEASE

Muscarinic drugs - We have demonstrated the presence of a pre-synaptic muscarinic receptor in the rat iris (Mattio et al., 1984b). This receptor controls ACh release. We have further investigated the pharmacology of this receptor and characterized the effect of three muscarinic drugs, two of which have been, or currently are, used in standard ophthalmological procedures.

Atropine and pirenzepine are both muscarinic antagonists and increase ACh release (Table I and Fig. 1). This increase was demonstrated to be Ca^{++} -dependent. Carbachol, a muscarinic agonist, has the ability to increase ACh release by displacing ACh from the cholinergic terminal (Table I). Pirenzepine and atropine demonstrated similar effects on increasing ACh release which was probably due to antagonism of the presynaptic muscarinic receptor. However, carbachol showed a different effect than the muscarinic antagonists with its maximal effect seen at $10^{-9}M$. This drug appeared to have a biphasic effect. Higher concentrations showed a marked increase in ACh release, whereas intermediate concentrations showed little increase and lower concentrations again showed a marked increase.

Calcium enhancers, 2-aminopyridine and 3-aminopyridine, are compounds that enhance calcium influx into the neuron resulting in increased neurotransmitter release (Loffelholz and Weide, 1982; Lemarca and Collier, 1983). The effect of drugs from this class on ACh release in the iris has, until now, not been known.

Both 2-aminopyridine and 3-aminopyridine increased ACh release in the rat iris. These drugs demonstrated a similar profile with 3-aminopyridine being more potent. Both of these drugs effects were Ca^{++} -dependent.

The demonstration of a pre-synaptic muscarinic receptor and the effect of drugs on this receptor in the rat iris is an important finding, because it may help us to elucidate the physiological control mechanisms of ACh release.

C. EFFECTS OF AGING ON PUPILLARY FUNCTION

Changes in cholinergic neurotransmission.

Aging of pupillary and iris mechanisms have been studied very little so far. The chick ciliary-ganglion-iris system has been studied extensively in our laboratory both during development and aging (Giacobini, 1982). The iris preparation of the chick has demonstrated to be particularly suitable as it contains a discrete and homogeneous population of cholinergic terminals which can be tested in vitro as an isolated organ as well as in vivo in the intact non-anesthetized animal. Our studies have shown that structural and biochemical changes related to cholinergic nerve fibers and nerve endings are surprisingly similar in the chick and in humans suggesting common degenerative mechanisms. In our study we have found that signs of aging can be identified in the chick iris as early as 18-24 months of age. These are mainly localized to the nerve endings and to the axoplasm of the nerve fiber proximal to the neuromuscular junction. Early kinetic changes (V_{max}) in the uptake process of Ch have been detected in the same period, leading to a decreased access of the precursor to the ending. The degenerative processes which we have observed lead to a progressively wider synaptic cleft which is filled by Schwann cells. A significant reduction of the neuronal junctional (appositional) membrane is more evident at 5 years (Giacobini et al., 1984). However, already at 3 years a very pronounced decrease occurs in synaptic vesicles, their relative volume

becoming reduced by more than 50%. These results have been obtained by means of a quantitative morphometric analysis performed on electron micrographs of cross sections of 4-month, 3-year and 5-year-old animals. This observation of a decrease in vesicular volume and synaptic membrane length lead us to the hypothesis of a decreased ability of the iris neuromuscular junction for neurotransmitter release. To verify the presence of a junctional deficit we designed a study of release of ACh comparing 4-month-old with 3-year-old animals. The experiment was designed to determine the ability of the 3-year-old iris to undergo strenuous depletion-reloading-release as compared to younger tissues. Our neurochemical results supported the previous morphological findings showing a decreased ability, dependent only on age and not on experimental design, of the 3-year-tissue to release [^3H]ACh when compared to a 4-month tissue. This functional decline is possibly related to modifications of membrane carriers and receptors regulating uptake and release of neurotransmitter molecules and precursors. The reason for a selective breakdown of such critical membrane attributes is not clear.

The results of these investigations were communicated at two meetings, first at the Oglesbay meeting on Dynamics of Cholinergic Function (October, 1983, In Press, 1984, Plenum Press), and at the 5th meeting of the International Society for Developmental Neuroscience (July, 1984) (Proceedings In Press, 1984, Elsevier). These results will also be presented at the 14th meeting of the Society for Neuroscience, Anaheim, California (October, 1984). Copies of these papers are enclosed (Appendices III and IV).

As discussed in our application (Significance, page 4), the problem of a progressive decay of visual function in military pilots has been particularly emphasized. Senile miosis, a progressive age-dependent reduction in pupil size, seems to be a very frequent, if not constant, symptom in humans. This

reduction involves a decrease in the amount of light which reaches the retina, adversely affecting vision. Our study is the first to address this problem in terms of structural, biochemical and functional terms in animal models.

Directions for Future Investigations

We do not foresee any major modification in the direction of our investigation in the third year of our grant.. The lines of research devised in our original proposal will be followed with only minor changes which emphasize particular aspects or results obtained during the first two-year period. A short account of the major emphasis of our future experiments is given below.

a) Characterization of subtypes of muscarinic receptors in the iris and long-term effects of DFP.

Recent reports in the literature have suggested the possibility that presynaptic muscarinic receptors may include subtypes (Kilbinger, 1984). One type when stimulated by ACh or oxotremorine results in release inhibition. We assume that DFP via increased ACh levels, acts at this receptor to inhibit ACh release (Mattio et al., 1984b). The other type when activated by muscarine, methacholine, or pilocarpine results in an increased ACh release. The difference between these two receptor subtypes appears to be in their ability to respond to certain antagonists. For example, scopolamine acts on both receptor subtypes whereas pirenzepine is approximately fifty times more potent as an antagonist of the excitatory receptor that facilitates ACh release. These data have been reported in the guinea pig ileum. Our results in the iris show that pirenzepine may either antagonize the first type of receptors or activate the second subtype resulting in an increased release of ACh.

It is of great interest to determine whether this situation exists in the rat iris and to pharmacologically identify the characteristics of these

receptors. We have some preliminary evidence that after acute exposure to DFP pupillary function is disturbed, even after acetylcholinesterase activity has returned to normal values. It is possible that pupillary function is impaired due to a long-lasting effect of DFP on ACh release. Certain drugs such as pirenzepine may be able to over-ride this phenomenon and potentially be developed as a preventive measure to improve pupillary function after acute exposure to DFP.

Our experiments will be divided into two groups. First, after acute exposure to DFP, pupil function will be monitored by infra-red pupillometry and correlated with the reappearance of acetylcholinesterase (AChE) activity. Secondly, by using a variety of compounds (agonists and antagonists) with a demonstrated ability to differentiate between receptors, their effect on ACh release and pupil function will be determined. Following this characterization, the drugs will be topically applied to the eye at the proper dosage after DFP treatment, and the recovery of pupillary function will be correlated to the effect on ACh release.

These experiments are important for two major reasons. First, the possibility to recover pupillary function after DFP exposure in a shorter time than required by the normal time-course, may represent a valid treatment after intoxication. Secondly, the ability to alter cholinergic neurotransmission presynaptically to the extent that it will over-ride DFP poisoning, is important in understanding the control mechanism of ACh release in the iris.

b) Muscarinic binding sites and kinetics.

Based on our results (see previous sections A and B and Receptor section of our application, p. 15-17) experiments will be focused upon specific aims and expanded to encompass new experiments related to muscarinic receptor

identification. Experiments will be carried out to study the binding properties of the rat iris with available muscarinic ligands.

The following muscarinic ligands will be employed:

- 1) (N-methyl-³H)oxotremorine M acetate
- 2) 1-quinacridinyl[phenyl-4-³H]bensylate
- 3) 1-[N-methyl-³H]scopolamine methylchloride
- 4) [N-methyl-³H]pirenzepine
- 5) [³H(G)]pilocarpine
- 6) (N-methyl³H)-acetylcholine

The determination of kinetic properties of binding will add information useful to further characterize cholinergic mechanisms in iris (see previous Section B).

We have postulated an effect of DFP on ACh release, which could be modulated by muscarinic presynaptic autoreceptors. Based on this observation, the displacement of specific muscarinic ligands by DFP will be determined.

In addition, the kinetics of binding of these ligands will be studied following AChE inhibition by DFP.

c) Action of peptides on acetylcholine release in the iris.

Following the directions indicated in our previous progress report (September 15, 1983, p. 3), we have extended our study on release of ACh to other neurohormones, particularly peptides, which have recently been involved in pupillary function. Particularly the presence, distribution and origin of substance P (SP) immunoreactive axons in the rat iris and cornea has been demonstrated (Miller et al., 1981). These fibers are of trigeminal origin and release SP upon receptors of the pupillary sphincter to cause their contraction (Shimizu et al., 1982). Other peptides such as bradykinin have been found to contract the pupillary sphincter through release of neuronal SP (Bynke et al.,

1984). More recently, sparse VIP- and CCK-positive fiber systems have been identified in the iris, together with a varicose fiber network containing leu-enkephalins (Bjorklund et al., 1984). Using our technique of stimulated release, we will investigate the role played by these various peptides in the physiology of the pupil. These experiments will involve the use of appropriate agonists and antagonists.

LITERATURE CITED

- Bjorklund, H., A. Seiger, T. Hokfelt and L. Olson. Distribution of neuropeptide and tyrosine hydroxylase immunoreactive nerves in iris and choroid membrane of the rat. Proc. 7th intl. Congr. Histochem. Cytochem. (Helsinki, Finland), p. 43, 1984.
- Bynke, G., R. Hakanson, J. Horig and S. Leander. Bradykinin contracts the pupillary sphincter and evokes ocular inflammation through release of neuronal substance P. Eur. J. Pharmacol., 91:469-475, 1983.
- Loffelholz, K. and Weide, W. Aminopyridine and the release of acetylcholine. Trends in Pharmacological Sciences, 3(4):147-149, 1982.
- Marchi, M., P. Paudice and M. Raiteri. Studies on presynaptic muscarinic autoreceptors. Proceedings of the Intl. Mtg. Neurochemistry, 1984.
- Kilbinger, H. Facilitation and inhibition by muscarinic agonists of acetylcholine release from guinea pig myenteric neurones: mediation through different types of neuronal muscarinic receptors. Trends in Pharmacological Sciences (Suppl.), 49-52, 1984.
- Mattio, T.G., J.S. Richardson and E. Giacobini. Acute effects of cholinesterase inhibitors on choline uptake in the rat iris. Neuropharmacology (submitted), 1984a.
- Mattio, T.G., J.S. Richardson and E. Giacobini. Effects of DFP on iridic metabolism and release of acetylcholine and a pupillary function in the rat. Neuropharmacology (In Press), 1984b.

Miller, A., M. Costa, J.B. Furness and I.W. Chubb. Substance P immunoreactive sensory nerves supply the rat iris and cornea. Neurosci. Letters, 23:243-249, 1981.

Shimizu, Y., I. Ishimoto, S. Shiosaka, Y. Kuwayama, M. Fukuda, S. Inagaki, M. Sakanaka and m. Tohyama. A direct contact of substance P-containing nerve fibers with pupillary sphincter muscles of the rat: an immunohistochemical analysis. Neurosci. Letters, 33:25-28, 1982.

Vella Lamarca, M. and Collier, B. Effects of 4-aminopyridine on the cat superior cervical ganglion. J. Pharmacol. Exptl. Therap., 226:249-257, 1983.

APPENDICES: I, II, III, IV, IV

TABLE I

Drug	Molar Concentration								
	10 ⁻³ M	10 ⁻⁴ M	10 ⁻⁵ M	10 ⁻⁶ M	10 ⁻⁷ M	10 ⁻⁸ M	10 ⁻⁹ M	10 ⁻¹⁰ M	10 ⁻¹¹ M
Pirenzepine	192**	204**	187*	139***	144**	114			
Atropine	190***		176**		154***		131**		115
Carbachol	151*		108		112		162*		136
2-aminopyridine	108		115		150***		123*		
3-aminopyridine	118		127		152***		153***		

Percent increase in acetylcholine release by different concentrations of various drugs.

* $p < .05$, ** $p < .01$, *** $p < .001$

PIRENZEPINE EFFECT ON ACh RELEASE

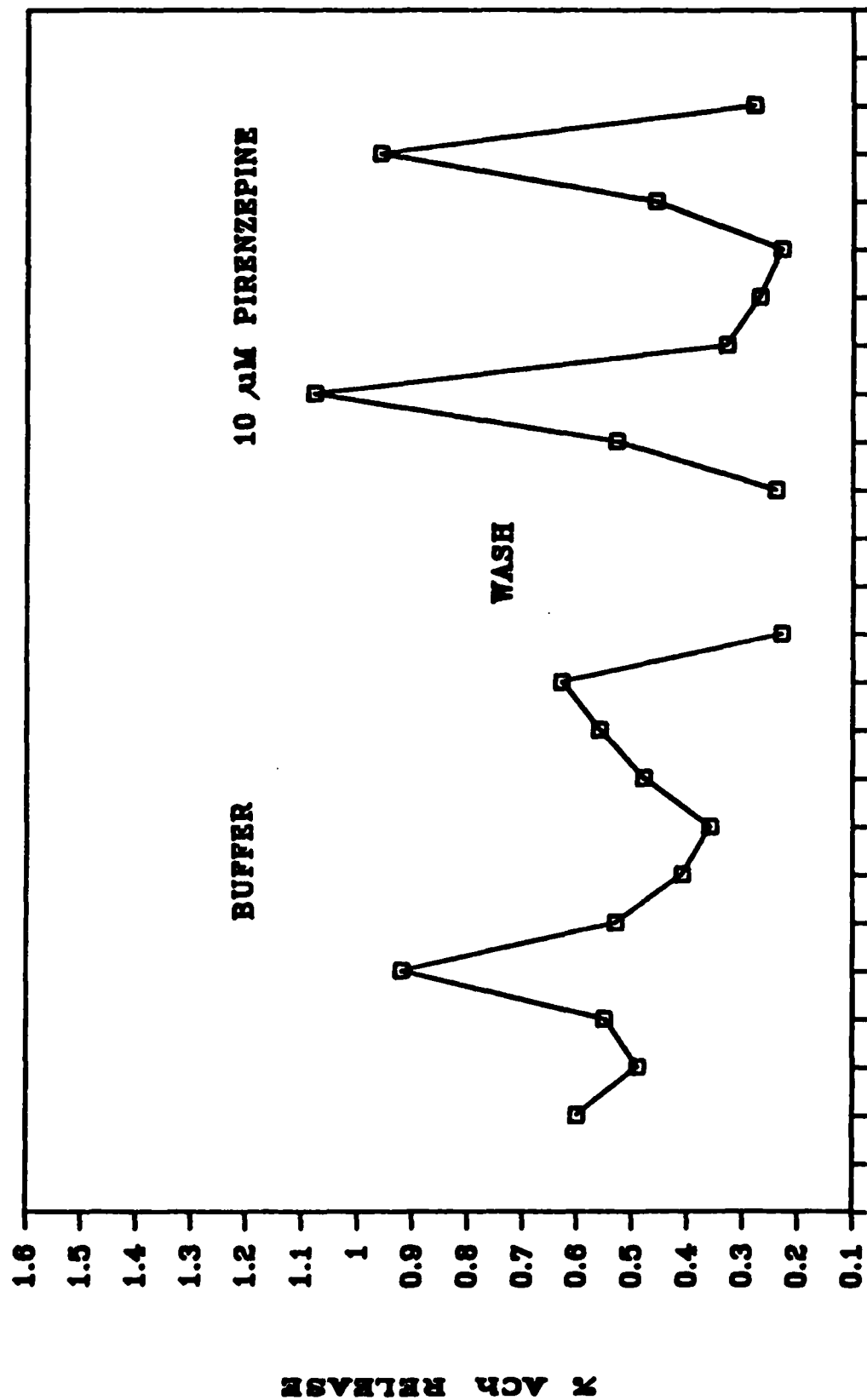


Fig. 1

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