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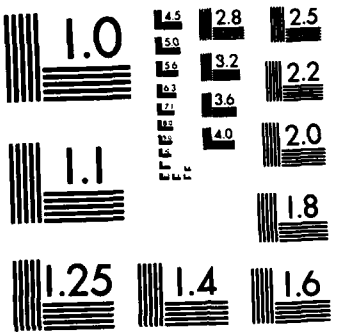

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ANTICHOLINESTERASE EFFECTS ON NUMBER AND FUNCTION OF BRAIN MUSCARINIC RECEPTORS AND CENTRAL CHOLINERGIC ACTIVITY: DRUG INTERVENTION

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

This study endeavors to elucidate the acute mechanisms adapted by the body to reduce cholinergic tone in order to fend off the toxic effects of anticholinesterase poisons. The effect of DDVP on rat brain acetylcholine content was characterized. The drug increased ACh in hemispheric structures(striatum, hippocampus,cortex) but not in cerebellum or midbrain-hindbrain. Pretreatments with atropine or reserpine only partially prevented the DDVP-induced increases. These and other experiments suggest that DDVP acted through a feedback mechanism secondarily to muscarinic receptor stimulation by the protected synaptic ACh.

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The feedback activation mediated by a monoamine leads to intraneuronal storage of ACh. Another fraction, perhaps smaller, accumulates extraneuronally and likely is responsible for the toxicity.

PREFACE

Of pertinence to this study on possible feedback mechanisms that DDVP may activate as an acute adaptive response to its toxic actions, is ongoing work on elucidating feedback loops in the striatum and hippocampus using neuropharmacological and lesion techniques.

Figure 1 shows the arrangements of afferent pathways and neurons intrinsic to the striatum and hippocampus as they are presently tentatively being conceived.

Of importance to this project too is a highly sensitive radio-enzymatic assay for acetylcholine that has been recently devised in this laboratory. This method will permit the investigation of the effect of anticholinesterase poisons in particular brain nuclei which may lead to greater understanding of their neurotoxicological mechanisms. In this report some preliminary data are presented on the levels of acetylcholine in parts of the hippocampal formation and the effects of anticholinesterase poisons.

TABLE OF CONTENTS

1. Statement of work

The ultimate objective of the proposed research is to clarify a basic, still unresolved problem connected with the mechanisms by which the anticholinesterases affect cholinergic nerves which should lead to a more throrough understanding of their most adverse reactions, i.e., the generalized cholinergic stimulation, convulsions and neuromuscular paralysis. This may lead to safer management of the agents through new pharmacological means for preventing or counteracting the toxic effects.

The organophosphorous cholinesterase inhibitor, DDVP (dichlorvos) will be employed. Dose-response effects and time course effects of DDVP on brain regional cholinergic biochemical markers, i.e., acetylcholine and choline contents, acetylcholine turnover, sodium-dependent high affinity uptake of choline, specific muscarinic receptor binding, acetylcholinesterase activity and choline acetyltransferase activity as well as brain monoamine levels in rats will be carried out. Highly sensitive and specific radioenzymatic methods and chromatographic methods will be utilized for the measurements. The curves obtained will be compared with one another to search for correlations.

Rats will be treated chronically with DDVP for the purpose of looking for a change in specific muscarinic receptor binding and then to correlate this with tolerance and cross tolerance to muscarinic agonists on brain regional acetylcholine content.

Experiments will be designed to determine whether putative neurotransmitters play a role in mediating the action of DDVP. To this end various drug classes will be employed to interfere with, or facilitate, the neuropharmacological and psychopharmacological cholinergic effect of DDVP. Among drugs to be used are: reserpine, alphamethylparatyrosine, parachlorophenylalanine, pimozide, atropine, phenoxybenzamine, prazosin and choline. As these powerful drugs affect known neuronal systems, their influence on DDVP will provide clues as to the mediator in a feedback loop.

Lesions of known, central monoaminergic pathways will also be made to remove any suspected neuron before challenge with DDVP.

It is anticipated that the results will reveal : a) whether DDVP induces an intracellular as well as extracellular accumulation of acetylcholine and thus whether a negative feed-back loop is operative; b) whether monoaminergic pathway(s) are activated by DDVP; c) whether the feedback loop can be manipulated pharmacologically; d) whether such manipulation can prevent or reduce the toxicity of cholinesterase inhibition; and e) whether DDVP-induced changes in muscarinic receptor number leads to changes in the sensitivity of muscarinic receptors to stimulation.

2. Status of the Research

a. Source, purity, stability and toxicity of DDVP

Dichlorvos (DDVP) was obtained from Shell Italia, S.p.A., Milan. The technical grade liquid was 97.6% pure as analyzed at Shell Research Limited, Kent and was supplied in individually packaged glass ampoules (100 x 5 g) sealed under nitrogen. The material was stored at 4°C. The material in an opened ampoule was stable for at least six months as shown in a test of pharmacological stability; equal doses of DDVP (20 mg/kg, p.o.,30 min) taken from a freshly opened ampoule or from 0.5., 1,2,4 and 6 months opened ampoules yielded identical increases in rat striatal acetylcholine content.

When dissolved in saline or in corn oil, DDVP (40 mg/kg, p.o., 30 min) yielded identical increases in rat striatal acetylcholine content but mortality was found to be greater in saline (deaths: 3/8 in saline; 2/14 in corn oil) in 30 min.

b. Neuropharmacological characterization of DDVP

A dose-response effect of DDVP (5,10,20,40 and 80 mg/kg p.o.) was performed at 30 min after its administration (Table 1). Significant increases of 20-30% were attained between 5-20 mg/kg. No mortality was produced at these doses although symptoms of cholinergic hyperactivity were induced. A 50% increase was attained at 40 mg/kg and mortality was 2/6.
The dose of 80 mg/kg produced convulsions and death within 5 min in 6/6 rats.

The non-lethal, cholinergic symptoms-producing doses of DDVP, 15-20 mg/kg, were used in subsequent experiments.

A time course at 20 mg/kg, p.o. showed that the peak increase in ACh was attained by 30 min in striatum and the effect persisted for at least 240 min before declining and terminating by 16h after administration. By contrast, the effect of DDVP on hippocampal and cortical ACh contents was much shorter-lasting. The peak increases were attained within 30 min but the ACh level returned to

normal by 60 min. (Figure 2). This unusual phenomenon may indicate that acetylcholinesterase influences cholinergic activity differently in these brain regions.

In a study of the regional distribution of the effect of DDVP, 20 mg/kg, 30 min, it was found that the cholinesterase poison increased ACh content only in the hemispheric regions (striatum, cortex and hippocampus) but not in the cerebellum and midbrain-hindbrain regions. Choline contents, on the other hand, were unaltered in the hemispheric regions but were increased in the cerebellum (+ 64) and midbrain-hindbrain (+ 30%) (Table 2).

Acetylcholine was measured in 3 parts of the hippocampal formation (dorsal hippocampus, ventral hippocampus and the dentate gyrus) using a recently developed more sensitive radioenzymatic assay for its determination (see Preface). The purpose was the compare the regional distribution of the effect of anticholinesterases with oxotremorine), a selective centrally-acting muscarinic receptor agonist. Table 3 shows in fact that both DDVP and physostigmine produced marked increases in ACh content in the ventral hippocampus, differently from oxotremorine which was ineffective. In the other two regions, physostigmine and oxotremorine were equally active.

At the dose of 15 mg/kg p.o., DDVP produced a small (+21%) but significant increase in sodium-dependent high affinity uptake of choline as measured ex vivo on the P_2 pellets from striatum but not from hippocampus (Table 4).

In vitro, DDVP, 10^{-4}M, depressed the electrically-evoked release of acetylcholine (Table 4). In vitro at this concentration DDVP had no effect on the Na^+-dependent high affinity uptake of choline by striatal or hippocampal P_2 pellets.

The drug did not alter the contents of monaomines present in the striatum (noradrenaline, dopamine and serotonin) and hippocampus (noradrenaline and serotonin)(Table 5).

c. Interactions of DDVP with drugs

Pretreatment with atropine sulfate (5 mg/kg, i.p., 60 min) significantly prevented the increase in ACh content induced by DDVP in the hemispheric regions. In cortex (Table 6), DDVP was blocked completely by atropine whereas the block appeared to be about 65% complete in striatum (Table 7) and approximately 50% complete in the hippocampus (Table 8). The marked increase in ACh content of the ventral hippocampus induced by physostigmine was also markedly but not completely inhibited (Table 9). Increasing the dose of atropine to 10 mg/kg still did not produce a greater block of DDVP.

These results indicate that in the cortex the anticholinesterase increased ACh content by a feedback mechanism secondarily to muscarinic receptor stimulation by the protected synaptic ACh. The action of DDVP in striatum and hippocampus is more complex. Part of the increase in these regions too is due to feedback through muscarinic receptor activation. But in addition, another fraction of the increase in ACh content may be due to extrasynaptic accumulation of ACh and/or feedback through nicotinic receptor activation.

Depletion of monoamines by reserpine pretreatment (5 mg/kg, i.p., 16 h) partially prevented the effect of DDVP in striatum (Table 10) and hippocampus (Table 11) suggesting the involvement of a reserpine-sensitive monoamine in the feedback loop.

Reserpine produced death in 1 of 8 rats treated with this normally non-lethal dose. Attempts to identify the monoamine(s) involved are not yet completed. Blockade of noradrenaline synthesis by the inhibition of tyrosine hydroxylase with alphamethyl-paratyrosine (300 mg/kg, i.p., 16h + 200 mg/kg, i.v., 4h) did not prevent the increase in hippocampal or striatal ACh and instead appears to have potentiated DDVP. This drug also caused 2 deaths in 10 rats treated with the normally non-lethal dose of DDVP. Inhibition of serotonin synthesis by pretreatment with parachlorophenylalanine (100 mg/kg, p.o., 72 h,

48h and 24h prior to the experiment) was similarly ineffective (Tables 12 and 13).

In striatum the pretreatment with other drugs designed to block β-adrenergic receptors (propranolol), α-adrenergic receptors (phenoxybenzamine) and dopamine receptors (pimozide) did not mitigate the increase produced by DDVP (Table 13).

d. Tentative conclusions

DDVP at the non-lethal but cholinomimetic dose of 20 · kg, strongly increased in content of acetylcholine in rat b .. hemispheric regions.

Pretreatment with atropine blocked the action to vari · · degrees in the cortex > striatum > hippocampus suggesting that part of the increase in ACh content is provoked by feedback mechanisms shutting of the cholinergic terminals and resulting in an intraneuronal accumulation of ACh. This is inferred because atropine is known as an ACh-releaser and as such would not have interfered with, but rather would have potentiated the action of DDVP.

The scheme in Figure 3 depicts how oxotremorine and DDVP increase intraneuronal ACh. As oxotremorine does not inhibit acetylcholinesterase, it is evident that the increase in ACh that it produces is due to an accumulation in intraneuronal stores. Any synaptic ACh is destroyed by intact acetylcholinesterase. In addition, the feedback inhibition of ACh release provoked by muscarinic receptor stimulation by oxotremorine could result in less then normal synaptic acetylcholine.

Differently, DDVP first inhibits the esterase and causes ACh to accumulate in the synaptic cleft.. The ACh provokes the feedback inhibition of ACh release which also leads to an intraneuronal as well as the synaptic accumulation of ACh.

Drugs designed to interfere with the positive feedback loop

(i.e. reserpine, α-Mpt, α-adrenoceptor blockers) can strongly prevent the increase in ACh induced by oxotremorine (Fig.3) are thus prevent the accumulation of intraneuronal stores of ACh. The data of the present study show that drug treatments only weakly prevented the increase in ACh content induced by DDVP. It is inferred that feedback inhibition was blocked by these drugs and the intraneuronal accumulation of acetylcholine was prevented. However, the extraneuronal intrasynaptic accumulation of acetylcholine (perhaps the fraction responsible ofr the toxicity) was not mitigated by the drug. The net result appears therefore to be a shift in the proportion of intraneuronal to extraneuronal acetylcholine in anticholinesterase poisoned rats (Figure 3). The aim of future work is to find means of strengthening the feedback mechanism and decrease acetylcholine release in anticholinesterase poisoned rats.

3. *Professional personnel*

Silvana Consolo, Doctorate in Biological Sciences
Silvio Garattini, M.D.
Anna Maria Vezzani, Doctorate in Biological Sciences
Herbert Ladinsky, Ph.D.

4. Interactions

The data in this report were presented at the Review of Air Force Sponsored Basic Research in Biomedical Sciences, 26-28 July 1983 at the University of California, Irvine, California.

TABLE 1

DOSE-RESPONSE OF THE INCREASE IN STRIATAL ACETYLCHOLINE CONTENT BY DDVP			
DDVP (mg/kg)	ACETYLCHOLINE (nmoles/g)	MORTALITY	
CONTROLS	70.7 ± 2.2 (6)	–	
5	84.8 ± 1.8* (6)	0/6	Stereotypy
10	87.2 ± 2.9* (6)	0/6	Sweating
20	89.8 ± 1.5* (6)	0/6	Sweating, tremor, diahrrea
40	106.2 ± 10 * (4)	2/6	Sweating, tremor, salivation
80	–	6/6	Convulsions & death (5 min)

The rats were killed 30 min after DDVP, p.o.

TABLE 2

EFFECT OF DDVP ON THE ACETYLCHOLINE AND CHOLINE CONTENTS IN RAT BRAIN REGIONS				
REGION	ACETYLCHOLINE (nmoles/ g)		CHOLINE (nmoles/ g)	
	CONTROLS	TREATED	CONTROLS	TREATED
STRIATUM	65.8 ± 1.5	98.6 ± 2.7 *	28.2 ± 1.1	29.0 ± 1.6
HIPPOCAMPUS	21.0 ± 0.8	30.7 ± 1.4 *	20.8 ± 1.7	21.4 ± 1.1
CORTEX	20.6 ± 1.0	26.6 ± 1.1 *	19.6 ± 0.7	22.4 ± 1.5
BRAINSTEM	24.9 ± 0.6	26.4 ± 0.4	20.0 ± 1.3	26.1 ± 2.7
CEREBELLUM	3.8 ± 0.5	4.4 ± 0.2	18.7 ± 0.5	30.6 ± 2.7 *

THE DATA ARE MEANS ± S.E.M. (n = 6)

* = $p < 0.01$

TABLE 3

COMPARISON OF THE EFFECTS OF DDVP, PHYSOSTIGMINE AND OXOTREMORINE ON REGIONS OF THE HIPPOCAMPAL FORMATION

	Acetylcholine (nmoles/ g)		
	Dorsal hippocampus	Ventral hippocampus	Dentate gyrus
Control	–	27.3 ± 0.6 (6)	–
DDVP	–	35.0 ± 1.7 * (6)	–
Control	21.7 ± 0.7 (10)	26.0 ± 1.5 (10)	27.0 ± 1.3 (7)
Physostigmine	30.7 ± 1.2 * (10)	40.1 ± 2.3 * (10)	38.7 ± 1.8 * (11)
Control	20.9 ± 0.5 (6)	27.9 ± 1.3 (10)	28.6 ± 0.7 (10)
Oxotremorine	27.6 ± 1.4 * (6)	31.5 ± 1.2 (11)	34.9 ± 0.9 * (9)

DDVP, 15 mg/kg, p.o. , 30 min ; physostigmine, 1 mg/kg, i.p., oxotremorine, 0.54 mg/kg, i.p., 20 min.

* = $p < 0.01$ vs control

TABLE 4 - Effect of DDVP ex vivo and in vitro on the sodium-dependent high affinity choline uptake and electrically-evoked release of acetylcholine in hippocampus and/or striatum

Brain region	Na^+-dependent high affinity choline uptake (nmoles/min/g prot)				Electrically evoked ACh release (S_2/S_1)	
	ex vivo[a]		in vitro[b]		in vitro[b] (slices)	
	control	DDVP	control	DDVP	control	DDVP
hippocampus	1.47 ± 0.12(9)	1.31 ± 0.09(9)	1.40(2)	1.43(2)	-	-
striatum	2.83 ± 0.16(14)	3.43 ± 0.23(14)°	2.76(2)	2.82(2)	0.79 ± 0.08(4)	0.58 ± 0.09(4)°

[a] DDVP was dissolved in distilled water and administered at the dose of 15 mg/kg, p.o. The rats were killed after 30 min and the P_2 pellets of striatum and hippocampus were prepared

[b] DDVP, 10^{-4}M final concentration

The data are the means ± S.E.M. (n)

° = $p < 0.05$ vs controls

TABLE 5 - Lack of effect of DDVP treatment on noradrenaline (NE),serotonin (5-HT) and dopamine (DA) contents in rat hippocampus and striatum

Treatment	Hippocampus (ug/gT)		Striatum (ug/gT)		
	5-HT	NE	5-HT	NE	DA
Vehicle	203.0 ± 10	343.8 ± 18.6	217.8 ± 26	122 ± 15.6	11060 ± 784
DDVP	207.9 ± 7.7	395.8 ± 34.9	217.6 ± 13	110 ± 5	10752 ± 529

The data are the means of 5-7 animals

The animals were killed 30 min after DDVP administration, 15 mg/kg, p.o.

TABLE 6

EFFECT OF ATROPINE ON THE DDVP-INDUCED INCREASE IN ACh CONTENT IN RAT CORTEX	
DRUG	ACETYLCHOLINE (nmoles/g) CORTEX
CONTROL	19.1 ± 0.6 (6)
DDVP	26.2 ± 0.7 * (6)
ATROPINE	17.1 ± 0.6 (6)
DDVP + ATROPINE	18.8 ± 1.0 (6)
INTERACTION	$p < 0.02$

ATROPINE SULFATE, 5 mg/kg, i.p. ; DDVP 20 mg/kg, p.o. , 30 min

* = $p < 0.01$ vs controls

TABLE 7

EFFECT OF ATROPINE ON THE INCREASE IN STRIATAL ACh INDUCED BY DDVP					
	Striatal acetylcholine (nmoles/ g)				
Atropine dose	Vehicle	DDVP	Atropine	Atropine + DDVP	Interaction
5 mg/kg	63.5 ± 2.0 (8)	95.3 ± 3.3 * (8)	60.9 ± 3.0 (7)	72.3 ± 3.7 ** (7) 1/8	$p < 0.01$
10 mg/kg	67.1 ± 1.5 (8)	87.7 ± 3.8 * (8)	57.6 ± 1.6 * (7)	62.8 ± 2.1 (7) 1/8	$p < 0.01$

DDVP, 20 mg/kg, p.o., 30 min ; atropine 60 min

* = $p < 0.01$ vs vehicle

** = $p < 0.01$ vs atropine

TABLE 8

EFFECT OF ATROPINE ON THE INCREASE IN HIPPOCAMPAL ACh INDUCED BY DDVP					
		Hippocampal acetylcholine (nmoles/ g)			
Atropine dose	Vehicle	DDVP	Atropine	Atropine + DDVP	Interaction
5 mg/kg	21.8 ± 1.0 (8)	26.3 ± 1.1 * (8)	18.5 ± 0.5 (7)	21.1 ± 0.9 (7) (1/8)	$p < 0.01$
10 mg/kg	20.5 ± 1.5 (8)	27.0 ± 0.7 * (8)	18.7 ± 0.9 (7)	22.8 ± 1.9 ** (7)	$p < 0.01$

DDVP, 20 mg/kg, p.o., 30 min ; atropine sulfate, 60 min

* = $p < 0.01$ vs vehicle

** = $p < 0.01$ vs DDVP

TABLE 9

EFFECT OF ATROPINE ON THE INCREASE IN ACh CONTENT INDUCED BY PHYSOSTIGMINE IN THE VENTRAL HIPPOCAMPUS	
Drug	**V.H. acetylcholine (nmoles/ g)**
Control	22.2 ± 1.6 (6)
Physostigmine	40.0 ± 1.7 * (6)
Atropine sulfate	22.7 ± 0.8 (6)
Atropine + Physo.	27.7 ± 1.8 (6)
Interaction	$p < 0.01$

Atropine sulfate, 5 mg/kg, i.p., 60 min

physostigmine sulfate, 1 mg/kg, i.p. , 20 min

* = $p < 0.01$ vs controls

TABLE 10

EFFECT OF RESERPINE PRETREATMENT ON THE INCREASE IN STRIATAL ACh INDUCED BY DDVP

	Striatal acetylcholine (nmoles/g)				
DDVP dose	Vehicle	DDVP	Reserpine	Reserpine + DDVP	Interaction
20 mg/kg	66.8 ± 1.8 (8)	91.2 ± 1.5 * (8)	60.3 ± 1.4 (8)	78.6 ± 3.2 (7) (1/8)	$p < 0.05$
15 mg/kg	65.8 ± 1.8 (8)	95.0 ± 2.0 * (8)	62.1 ± 1.8 (8)	84.0 ± 4.1 (7) (1/8)	$p < 0.05$

Reserpine, 5 mg/kg, i.p., 16 h ; DDVP, p.o., 30 min

* = $p < 0.01$ vs vehicle

TABLE 11

EFFECT OF RESERPINE PRETREATMENT ON THE INCREASE IN HIPPOCAMPAL ACh INDUCED BY DDVP

	Hippocampal acetylcholine (nmoles/ g)				
DDVP dose	Vehicle	DDVP	Reserpine	Reserpine + DDVP	Interaction
20 mg/kg	21.6 ± 0.7 (8)	31.4 ± 1.3 * (8)	23.4 ± 0.8 (8)	28.1 ± 1.2 (7) **	$p < 0.05$
15 mg/kg	21.9 ± 0.9 (8)	32.3 ± 1.7 * (8)	20.8 ± 0.5 (8)	27.7 ± 0.9 (7) **	$p < 0.05$

** Mortality : 1/8

* $p<0.01$ vs vehicle-treated group

TABLE 12

EFFECT OF TREATMENT WITH VARIOUS DRUGS ON THE DDVP-INDUCED INCREASE IN HIPPOCAMPAL ACETYLCHOLINE CONTENT

Drug in Columns C and D	HIPPOCAMPAL ACETYLCHOLINE (nmoles/g)				
	A	B	C	D	
	Vehicle	DDVP	Drug	Drug + DDVP	Interaction
α MpT	22.1 ± 0.8 (6)	27.5 ± 0.6* (8)	18.3 ± 0.6** (7)	27.8 ± 1.0* (8)	n.s.
PCPA	24.9 ± 1.6 (7)	30.1 ± 1.3 (8)	23.7 ± 0.7 (7)	29.8 ± 1.1* (6)	n.s.

* = $p < 0.01$ vs vehicle

* = $p < 0.01$ vs vehicle

α-methylparatyrosine, 300 mg/kg, i.p., 16 h + 200 mg/kg, i.v., 4h; parachlorophenylalanine, 100 mg/kg, p.o., 72h, 48h and 24h prior to the experiment.

** Mortality, 2/10

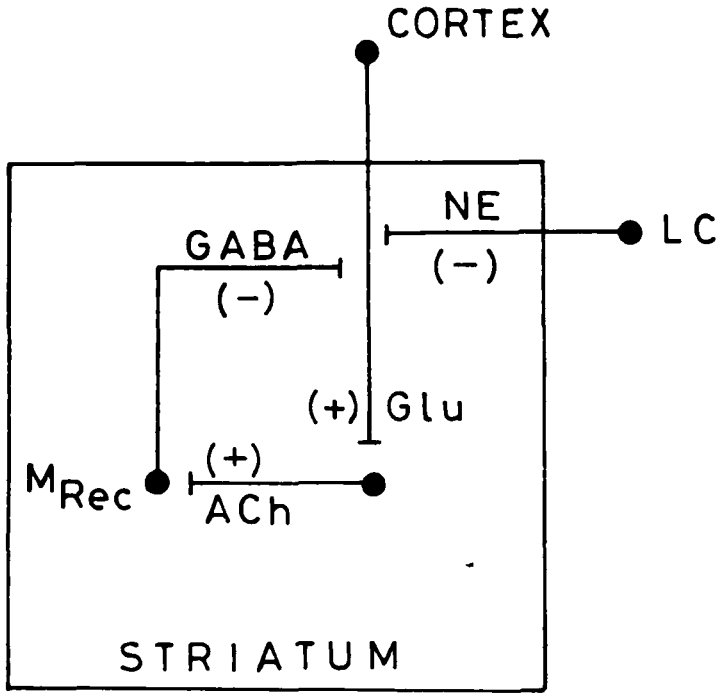
TABLE 13

EFFECT OF TREATMENT WITH VARIOUS DRUGS ON THE DDVP - INDUCED INCREASE IN STRIATAL ACETYLCHOLINE CONTENT

Drug in columns C and D	Striatal acetylcholine (nmoles/ g) A Vehicle	B DDVP	C Drug	D Drug + DDVP	Interaction
∝ - MpT	64.7 ± 2.9 (6)	88.1 ± 1.9 * (7)	63.0 ± 2.5 (7)	97.5 ± 2.6 * (8) 2/ 10	n.s.
Propranolol	64.0 ± 4.0 (6)	90.3 ± 2.8 * (10)	67.5 ± 1.7 (6)	83.2 ± 6.3 * (5)	n.s.
Phenoxybenzamine	67.3 ± 1.5 (5)	85.3 ± 3.9 * (5) 2/8	62.1 ± 2.0 (6)	83.9 ± 2.8 * (7)	n.s.
Pimozide	65.6 ± 1.8 (6)	86.3 ± 2.3 * (6)	42.1 ± 2.5 * (6)	67.4 ± 3.2 (6)	n.s.
PCPA	66.2 ± 2.7 (6)	97.5 ± 2.8 * (8)	66.4 ± 2.2 (6)	92.2 ± 2.9 * (6)	n.s.

Propranolol, 10 mg/kg, i.p. , 60 min ; phenoxybenzamine, 20 mg/kg, i.p. , 180 min ; pimozide, 1 mg/kg, i.p. , 240 min ; PCPA, 100 mg/kg p.o. 72 h , 48 h and 24 h before the experiment ; ∝-MpT, 300 mg/kg, i.p. 16 h + 200 mg/kg, i.v. 4 h before the experiment.

* = $p < 0.01$ vs vehicle group



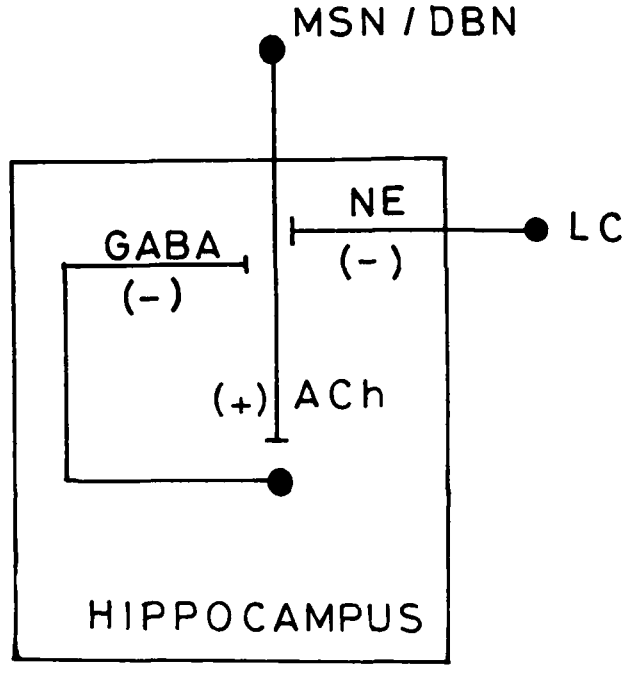


FIGURE 1 - Neuronal organization of cholinergic noradrenergic GABAergic and glutamatergic neurons in the hippocampus and striatum. MSN/DNB, medial septal nucleus/diagonal band nucleus; LC, locus coeruleus; ACh, acetylcholine; NE, noradrenaline; GABA, gamma-aminobutyric acid; Glu, glutamate; Mrec, muscarinic receptor.

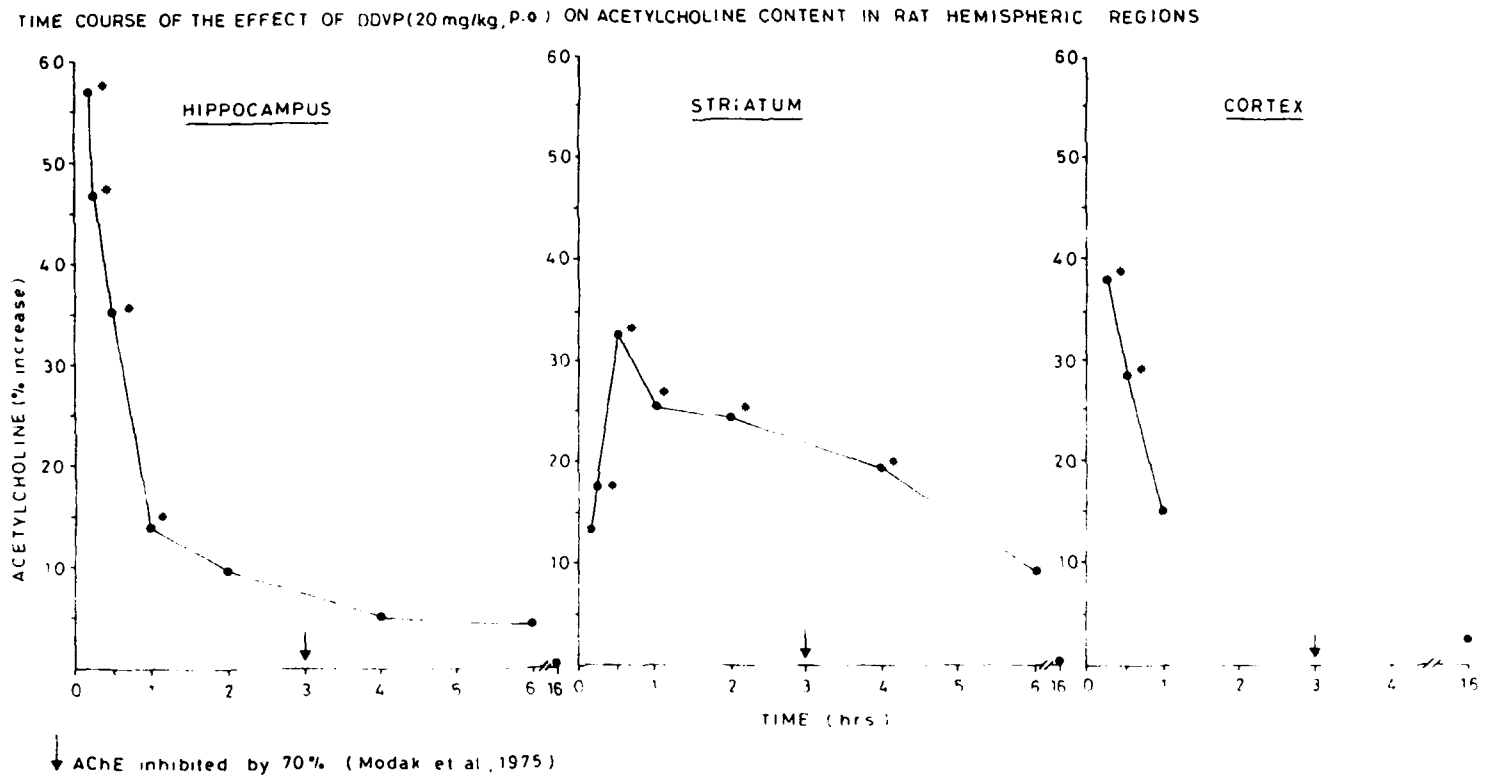


FIGURE 2 - Time course of the effect of DDVP on the content of acetylcholine in the hippocampus, striatum and cortex of rats. DDVP was administered p.o. at the dose of 20 mg/kg. The data show the means of 8-10 rats per time point. The standard errors did not vary by more than 10% from the means.
The asterisks denote significant ($p < 0.01$) difference with respect to the intreated group.

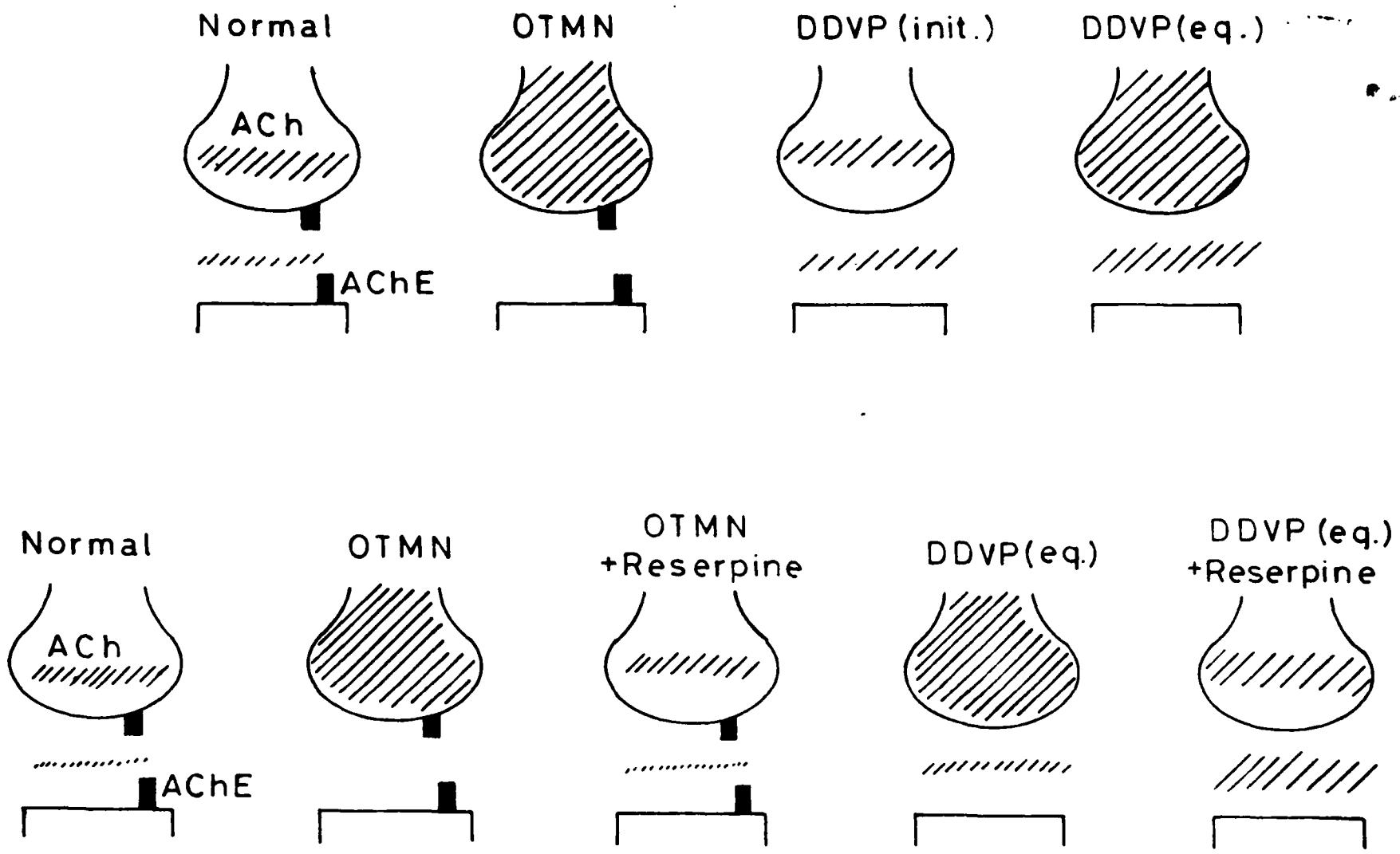


FIGURE 3 - Schematic model of the synapse and control of acetylcholine content and release. Intact acetylcholinesterase present in presynaptic and postsynaptic membranes regulates the intrasynaptic level of acetylcholine in oxotremorine-treated animals. Intraneuronal acetylcholine is regulated by feedback mechanisms. The intrasynaptic accumulation of acetylcholine cannot be regulated in the presence of an acetylcholinesterase inhibitor although feedback control of intraneuronal acetylcholine accumulation may be normal.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