

## Short communications

# Effect of oxotremorine on the acetylcholine content of whole brain and various brain regions in the pigeon

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Oxotremorine (0.125 mg/kg) produces a significant increase in total acetylcholine content in whole pigeon brain. The contribution of different regions to this increase varies. The largest increase occurs in the nucleus basalis (paleostriatum augmentatum), a region which is highly involved in motor control. The mechanism by which oxotremorine increases the acetylcholine content of brain and the causal relationship between the rise in acetylcholine content and tremor are discussed.

Oxotremorine, the active metabolite of tremorine produces tremor of central origin (George, Haslett & Jenden, 1962) and increases the brain acetylcholine content (Holmstedt & Lundgren, 1966). Most studies on tremorogenic agents were done in mammals but it has been shown that birds also respond similarly to these substances (Everett, 1964; Bowman & Osuide, 1967; Igić, Jeličić & Stern, 1968). The importance of cholinergic mechanisms in avian brain has been pointed out by Aprison & Takahashi (1965). This paper describes the effect of oxotremorine on the total acetylcholine content of the whole

brain and different brain regions in the pigeon.

**Methods.**—The experiments were performed on adult pigeons (*Columba domestica*) of both sexes, weighing 250–400 g. Oxotremorine (0.125 mg/kg) was administered intravenously in a volume of 0.1 ml/100 g and the animals were killed by decapitation 15 min after injection. Body temperature was measured by a thermometer in the cloaca, before and 15 min after oxotremorine injection.

Acetylcholine was extracted from the brain by the trichloroacetic acid method as described by MacIntosh & Perry (1950) and assayed on the frog rectus abdominis muscle, sensitized with 0.01 mM physostigmine. In control studies extracts that were boiled in 0.3 M NaOH or 0.3 M HCl and neutralized before assay were used. Recovery of added acetylcholine was 89%. The results are expressed in  $\mu\text{g}$  acetylcholine chloride/g of fresh tissue. The significance of the differences between means was determined by Student's *t*-test. A value of  $P < 0.05$  was taken to be significant.

The brains were divided macroscopically, in ice-cold saline containing 0.1 mM physostigmine, into five regions (see Table 1) and tissue from three animals was used for one sample. The cerebellum was not included in the assay of regions because its acetylcholine content was too low. Determination of acetylcholine in whole brain were carried out on single brains which included the cerebellum.

Drugs used were: acetylcholine chloride (Hoffmann-La Roche), oxotremorine (Le Petite), and physostigmine (eserine) sulphate (Merck).

**Results.**—Oxotremorine injection produced a significant increase in the acetyl-

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TABLE 1. Influence of oxotremorine on the acetylcholine content in whole brain and different regions of the pigeon brain

Region	Acetylcholine content ( $\mu\text{g/g} \pm \text{S.E.}$ )		Per cent change	Significance
	Controls	Oxotremorine injected		
Epibasalis complex of nuclei	$1.31 \pm 0.18$ (5)	$1.47 \pm 0.16$ (5)	+12	N.S.
Nucleus basalis	$1.36 \pm 0.14$ (5)	$2.52 \pm 0.16$ (5)	+85	$P < 0.001$
Optic lobes	$2.68 \pm 0.20$ (5)	$3.11 \pm 0.14$ (5)	+16	N.S.
The rest of brain (except cerebellum)	$1.96 \pm 0.14$ (5)	$2.77 \pm 0.18$ (5)	+41	$P < 0.05$
Whole brain (including cerebellum)	$1.59 \pm 0.11$ (6)	$2.32 \pm 0.13$ (6)	+46	$P < 0.001$

Oxotremorine (0.125 mg/kg) was injected intravenously and animals were sacrificed 15 min later. The number of experiments is given in parenthesis. The figures represent  $\mu\text{g}$  acetylcholine chloride, not corrected for the loss (11%) which occurred during extraction. N.S., not significant.

choline content of whole brain (Table 1). This increase was greatest in the nucleus basalis but was also partly attributable to a rise in the acetylcholine concentration of the thalamus, hypothalamus, medulla oblongata and the rest of mid-brain (Table 1).

Before decapitation, animals injected with oxotremorine showed pronounced tremor: they could not walk or fly and showed autonomic symptoms (defaecation and salivation) and the muscular resistance to forced flexion (rigidity) was remarkably increased. Body temperature dropped from  $42.2 \pm 0.2^\circ \text{C}$  to  $36.0 \pm 0.3^\circ \text{C}$  (mean  $\pm$  S.E.,  $n=6$ ) 15 min after injection of oxotremorine.

It is interesting that oxotremorine did not significantly change the acetylcholine content in the optic lobes which have the highest brain acetylcholine content. These results agree with previous observations in dogs (Hadžović, Potkonjak & Stern, 1966) and cats (Bartolini, Bartolini & Pepeu, 1970).

**Discussion.**—The results show that oxotremorine raises the acetylcholine content in whole pigeon brain *in vivo*. The largest increase occurs in the nucleus basalis, which may be considered homologous with the 'striatum' of mammals and plays an important part in motor control. It should be mentioned that the macroscopic division of hemispheres into nucleus basalis and the epibasalis complex of nuclei cannot be made with complete certainty in pigeons. The nucleus epibasalis centralis may be included in the 'nucleus basalis'.

The mechanism by which oxotremorine increases the acetylcholine content of brain is obscure. The increase is unlikely to be due to inhibition of acetylcholinesterase (Holmstedt, Lundgren, Schubert & Sundwall, 1965) and there are controversial reports on activation of choline acetylase (Ratković, Stern & Bošković, 1965; Holmstedt, *et al.*, 1965). It is possible that the utilization of acetylcholine in the brain is reduced since oxotremorine has pronounced muscarinic effects (Cho, Haslett & Jenden, 1962) and probably competes with acetylcholine for receptors. The hypothermic effect could lead to under-utilization of acetylcholine as well.

We do not know whether a causal relationship exists between tremor and increase in brain acetylcholine. In rats

oxotremorine produces tremor before the acetylcholine content in the whole brain is raised (Cox & Potkonjak, 1969) but it is possible that changes in discrete regions may be present at this time. The exact relationship between the onset and duration of tremor and acetylcholine concentration in extrapyramidal structures thus warrants further investigations.

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