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Reduction of acetylcholine output from the indirectly stimulated rat diaphragm preparation by some carbamates and phenols

During a comparative study of some cholinesterase inhibitors on the extra-cellularly recorded endplate potential of the rat phrenic diaphragm preparation it was observed that 3-isopropylphenylmethylcarbamate (OMS 15) caused a slower rate of rise of the endplate potential than paraoxon, eserine, 2-methyl-2-methylthiopropion aldehydeo-(methylcarbamoyl)oxime (OMS 771) or 2-oxo-1,3-dithiolane *O*-(methylcarbamoyl) oxime (OMS 744) when tested in concentrations which gave a similar degree of cholinesterase inhibition. It was thought likely that this difference could be due to a relative reduction of acetylcholine output from the phrenic nerve terminals in the presence of the phenylcarbamates.

Phrenic-diaphragm preparations from male rats 200–250 g, were set up as described by Bülbring (1946) in a Perspex bath containing 7·0 ml Krebs solution at 37°. In every experiment paraoxon 10^{-4} M was added to the bath 20 min before stimulating to ensure complete inhibition of cholinesterases.

All the compounds tested, and paraoxon, were made up as concentrated solutions in acetone and a small volume added to the diaphragm bath to give the desired concentrations. It had previously been established that the maximum concentration of acetone used in the bath did not itself reduce acetylcholine output.

Preparations were stimulated at 50 Hz for 15 min then the bath fluid was removed by pipette and assayed for acetylcholine within 5 min using the leech dorsal muscle preparation (Murnaghan, 1958). Responses to the test solutions were matched with those elicited by standard solutions of acetylcholine perchlorate in Krebs also containing the same concentrations of drugs as the diaphragm bath fluid samples.

Three collections of acetylcholine were made from each preparation. (i) Paraoxon 1×10^{-4} M alone in the bath, (ii) paraoxon 1×10^{-4} M and the compound under test, (iii) paraoxon 1×10^{-4} M alone.

Acetylcholine output in the presence of paraoxon 1×10^{-4} M alone ranged from 21–84 ng with a mean of $44{\cdot}22 \pm 1{\cdot}73$ ng per 15 min stimulation period.

The percentage reduction of acetylcholine output was calculated by comparing the mean of periods (i) and (iii) with period (ii); see Table 1.

In view of the marked reduction of acetylcholine output with the phenylcarbamate OMS 15, the effects of 3-isopropylphenol and some other phenols were also tested; see Table 1. These results may appear to be at variance with those of Otsuka & Nonomura (1963), but their observations on the effects of phenol on the endplate potential only extended up to 7 min after adding the phenol compared with the 20 min incubation period used in these experiments.

The effect of OMS 15 on acetylcholine output may possibly be due to the formation of 3-isopropylphenol from carbamylated cholinesterase at motor nerve endings.

Table 1. *Depression of acetylcholine output from the rat phrenic diaphragm preparation by some carbamates and phenols.*

Compounds (M) present in diaphragm bath in addition to paraoxon 1×10^{-4} M	Acetylcholine output as the % of that when paraoxon 1×10^{-4} M alone was present		Number of experiments
Eserine 1×10^{-4}	93		16
OMS 15 1×10^{-6}	50		6
OMS 15 1×10^{-4}	45		6
OMS 771 1×10^{-6}	91	100, 100, 80, 85	4
OMS 744 1×10^{-6}	89	85, 100, 83	3
3-Isopropylphenol 1×10^{-6}	48	50, 44, 50	3
3-Isopropylphenol 1×10^{-4}	5	0, 16, 0	3
2-Isopropoxyphenol 1×10^{-6}	60	67, 50, 62	3
Phenol 1×10^{-6}	67	67, 67	2
3-Methyl-4-nitrophenol 1×10^{-6}	87	75, 100, 85	3
p-Nitrophenol 1×10^{-6}	83	85, 80	2
Phenyl-n-butyrate 1×10^{-4}	79	67, 100, 50, 100	4
Phenetole 1×10^{-4}	100	100, 100	2

The mode of action of the phenols which were active in these experiments awaits elucidation. However, Nathan & Sears (1960) showed that phenol affects nerve condition in a similar manner to procaine and Harvey (1939) suggested that procaine depressed acetylcholine output because of a selective action on terminal branches of motor nerves. The effect of OMS 15 on acetylcholine output may partly account for its lower mammalian toxicity than OMS 771, OMS 744 or eserine.

I thank Dr. M. Thain of the Tropical Products Institute for preparing the samples of 3-isopropylphenol and 2-isopropoxyphenol and gratefully acknowledge the guidance of Dr. J. M. Barnes.

Toxicology Unit,
Medical Research Council Laboratories,
Woodmansterne Road,
Carshalton, Surrey, U.K.

P. J. FORSHAW

May 2, 1972

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