Br. J. Pharmac. (1981), **73**, 481–483

THE EFFECTS OF ATROPINE AND OXOTREMORINE ON ACETYLCHOLINE RELEASE IN RAT PHRENIC NERVE-DIAPHRAGM PREPARATIONS

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1 Atropine (10^{-5}M) enhanced the release of [^{3}H]-acetylcholine from rat isolated hemidiaphragms, previously incubated with [^{3}H-methyl]-choline, stimulated via their phrenic nerves.

2 Oxotremorine (10^{-5}M) did not affect the stimulated release of [^{3}H]-acetylcholine but antagonized the facilitatory effects of atropine (10^{-5}M).

3 It is suggested that there are presynaptic inhibitory muscarinic receptors that modulate the release of acetylcholine in the phrenic nerves of the rat.

Introduction

Ganguly & Das (1979) showed that oxotremorine increased and that atropine decreased the release of acetylcholine (ACh) from rat phrenic nerve-diaphragm preparations. They suggested that ACh release might be locally controlled by excitatory presynaptic muscarinic receptors. Recently, Gundersen & Jenden (1980) demonstrated that oxotremorine did not enhance ACh release and indicated that presynaptic muscarinic receptors were unlikely to be present in this tissue. In this paper we describe our results with oxotremorine and with atropine which indicate that there may be presynaptic muscarinic receptors on the terminals of rat phrenic nerves but that these receptors are inhibitory.

Methods

Left hemidiaphragms from adult Wistar rats (200 to 250 g) were dissected with a short length of phrenic nerve attached and with their costal margins intact and were immersed for 4 h in 1 litre bathing medium (Potter, 1970) aerated with 95% O_2 and 5% CO_2 at room temperature. They were then placed in 5 ml aerated fresh bathing medium, containing 10 μCi/ml [^{3}H-methyl]-choline made up to a choline concentration of 3×10^{-5}M with non-radioactive material, at 37°C and were stimulated via their phrenic nerves at supramaximal voltage for 90 min with square-wave pulses of 0.2 ms duration at 0.5 Hz. From this point onwards the bathing medium contained 3×10^{-5}M neostigmine and was aerated with 95% O_2 and 5% CO_2 at 37°C. Each preparation was washed in 10 ml bathing medium for 10 min; this was repeated a further three times. After withdrawal of the final 10 ml of bathing medium, a further 5 ml was added and left in contact with the preparation for 10 min. This fluid was then withdrawn and constituted the resting release sample, R_1. The preparation was then rinsed with 5 ml bathing medium for 1 min. This was removed and a further 5 ml bathing medium was added. Phrenic nerves were stimulated using the above parameters but at 1 Hz for 7.5 min and after a further 2 min the bathing medium was removed and constituted the stimulation release sample, S_1. This procedure was repeated to produce samples R_2, S_2, R_3, S_3 and R_4, S_4.

Samples (R_1, S_1 etc) were acidified to pH 4 with 4 N HCl and were evaporated under reduced pressure to dryness. Residues were dissolved in 250 μl of 40% v/v ethanol, containing 1 mg of both acetylcholine chloride and choline chloride, at pH 4 and were centrifuged; 10 μl aliquots of the supernatant fluid were 'spotted' on a thin-layer plate of silica gel and were subjected to electrophoresis for 3 h (Storey & Wyn Jones, 1977). This allowed quantitative separation of [^{3}H]-ACh and [^{3}H]-choline. Appropriate areas of the thin-layer plate were eluted with 1 ml distilled water, and 10 ml biofluor scintillant (New England Nuclear) was added. The radioactivity in the samples was counted in a liquid scintillation spectrometer.

Oxotremorine was used as the sesquifumarate, atropine as the sulphate and neostigmine as the methyl sulphate.

Results

Atropine (10^{-6} and 10^{-5}M) had little effect on the resting release of [^{3}H]-ACh with the exception of the increase in R_4 after withdrawal of the larger concentrations of the drug (Table 1). The drug did however increase the stimulated [^{3}H]-ACh release. Atropine

0007-1188/81/060481-003 $01.00

Table 1 Release of [^{3}H]-acetylcholine ([^{3}H]-ACh) from rat isolated phrenic nerve-hemidiaphragm

Resting release		*Period [^{3}H]-ACh released (% release in R_1)*		
Treatment	R_1	R_2	R_3	R_4
None	100	81 ± 1.5(11)	67 ± 7.7(7)	62 ± 3.8(7)
Atropine (10^{-6}M)	100	82 ± 16.0(4)	78 ± 13.0(4)	85 ± 17.0(4)
Atropine (10^{-5}M)	100	80 ± 6.0(7)	82 ± 8.0(7)	*90 ± 11(7)
Oxotremorine (10^{-5}M)	100	80 ± 6.6(10)	67 ± 4.5(10)	71 ± 11.2(10)
Atropine (10^{-5}M) + oxotremorine (10^{-5}M)	100	82 ± 4.2(6)	66 ± 4.5(6)	63 ± 4.6(6)
Stimulated release (1 Hz)		*Period [^{3}H]-ACh released (% release in S_1)*		
Treatment	S_1	S_2	S_3	S_4
None	100	77 ± 2.7(11)	65 ± 3.9(6)	60 ± 6.0(6)
Atropine (10^{-6}M)	100	102 ± 16.0(4)	85 ± 15.0(4)	70 ± 17.0(4)
Atropine (10^{-5}M)	100	*103 ± 12.0(7)	**94 ± 7.0(6)	*100 ± 12.0(7)
Oxotremorine (10^{-5}M)	100	85 ± 7.8(10)	79 ± 8.0(10)	68 ± 5.6(10)
Atropine (10^{-5}M) + oxotremorine (10^{-5}M)	100	75 ± 2.8(6)	62 ± 2.8(6)	61 ± 4.4(6)

Samples R_1–R_4 and S_1–S_4 were collected at 20 min intervals. Drugs were present during the collection of R_2 and R_3 and the collection of S_2 and S_3.
Results are the mean±s.e. of n experiments. Numbers in parentheses are numbers of observations.
*$P < 0.05$; **$P < 0.01$; (Student's t test).

(10^{-5}M) increased the stimulated release of [^{3}H]-ACh in periods S_2, S_3 and S_4 (Table 1).

Oxotremorine (10^{-5}M) had no effect either on the resting or stimulated release of [^{3}H]-ACh (Table 1) but it antagonized the facilitatory action of (10^{-5}M) atropine on the stimulated release of [^{3}H]-ACh. A combination of 10^{-5}M atropine and 10^{-5}M oxotremorine did not affect the resting release of [^{3}H]-ACh.

Discussion

The results obtained with oxotremorine confirm the recent observations of Gundersen & Jenden (1980) and conflict with those of Das, Ganguly & Vedasiromoni (1978) and Ganguly & Das (1979); the drug at 10^{-5}M does not affect the release of [^{3}H]-ACh in the indirectly-stimulated isolated hemidiaphragm of the rat.

Our results with atropine also conflict with those of Ganguly & Das (1979) who, in direct contrast to our results, described an inhibitory effect of the drug on acetylcholine release. In trying to explain the discrepancies between our results and theirs we, like Gundersen & Jenden (1980), have had to conclude that the difference probably lies in the greater specificity of our method in separating, identifying and measuring only acetylcholine.

The facilitation of the release of [^{3}H]-ACh by atropine and the antagonism of its effects by oxotremorine suggests that there may be presynaptic inhibitory muscarinic receptors in the rat phrenic nerve-diaphragm preparation. Evidence for the presence of such receptors has been provided, in guinea-pig ileum, by Kilbinger & Wagner (1975), Sawynok & Jhamandas (1977) and Fosbraey & Johnson (1980) and in rat hippocampus, by Nordström & Bartfai (1980).

References

DAS, M., GANGULY, D.K. & VEDASIROMONI, J.R. (1978). Enhancement by oxotremorine of acetylcholine release from the rat phrenic nerve. *Br. J. Pharmac.*, **62,** 195–198.

FOSBRAEY, P. & JOHNSON, E.S. (1980). Modulation by acetylcholine of the electrically-evoked release of [^{3}H]-acetylcholine from the ileum of the guinea-pig. *Br. J. Pharmac.*, **69,** 145–149.

GANGULY, D.K. & DAS, M. (1979). Effects of oxotremorine demonstrate presynaptic muscarinic and dopaminergic receptors on motor nerve terminals. *Nature*, **278,** 645–646.

GUNDERSEN, C.B. & JENDEN, D.J. (1980). Oxotremorine does not enhance acetylcholine release from rat diaphragm preparations. *Br. J. Pharmac.*, **70,** 8–10.

KILBINGER, H. & WAGNER, P. (1975). Inhibition by oxot-

remorine of acetylcholine resting release from guinea-pig ileum longitudinal muscle strips. *Naunyn-Schmiedebergs Arch. Pharmac.*, **287,** 47–60.

NORDSTRÖM, Ö. & BARTFAI, T. (1980). Muscarinic autoreceptor regulates acetylcholine release in rat hippocampus: *in vitro* evidence. *Acta physiol. scand.*, **108,** 347–353.

POTTER, L.T. (1970). Synthesis, storage and release of [^{14}C]-acetylcholine in isolated rat diaphragm muscles. *J. Physiol.*, **206,** 145–166.

SAWYNOK J. & JHAMANDAS, K. (1977). Muscarinic feedback inhibition of acetylcholine release from the myenteric plexus of guinea-pig ileum and its status after chronic exposure to morphine. *Can. J. Physiol. Pharmac.*, **55,** 909–916.

STOREY, R. & WYN JONES, R.G. (1977). Quaternary ammonium compounds in plants in relation to salt resistance. *Phytochemistry*, **16,** 447–453.

(*Received September 29, 1980.*
Revised October 30, 1980.)