Brit. J. Pharmacol. (1966), **26**, 56–67.

THE CENTRAL AND PERIPHERAL ACTIVITY OF ACETYLENIC AMINES RELATED TO OXOTREMORINE

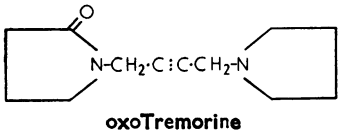
BY

A. BEBBINGTON, R. W. BRIMBLECOMBE AND D. SHAKESHAFT

From the Ministry of Defence, Chemical Defence Experimental Establishment, Porton Down, Wilts.

(*Received April* 5, 1965)

In 1961 Cho, Haslett & Jenden isolated and independently synthesized a metabolite, 1-(4-pyrrolidin-1′-ylbut-2-ynyl)pyrrolid-2-one (oxoTremorine), of Tremorine which was highly active in producing tremors, spasticity, hypokinesia and parasympathomimetic effects. The peripheral pharmacology of this compound was later studied in more detail by Cho, Haslett & Jenden (1962) and it was concluded that the peripheral effects could be explained solely in terms of stimulation of the postganglionic parasympathetic receptors. OxoTremorine inhibited cholinesterase only at high concentrations and it was devoid of nicotinic properties.



oxoTremorine

The high degree of potency of oxoTremorine has led to some speculation as to whether it represents a new class of muscarinic agent (Cho *et al.*, 1962). The work described in this paper is concerned with an investigation of this aspect, with special reference to the function of the acetylenic linkage and the amide carbonyl group.

METHODS

Chemical syntheses

The physical properties of the compounds used in this investigation are listed in Tables 1 and 2.

Compounds No. 1, 2, 3, 7, 8, 9, 20, 21, 23, 24, 26 and 27 were obtained by a Mannich reaction from the appropriate terminal acetylene in dioxan solution according to the method described by Bebbington & Shakeshaft (1965) for oxoTremorine. Keto-groups were protected by prior conversion to ethylene ketals. The carbamates, compounds No. 17 and 18, were prepared in pyridine solution by the reaction of dimethylcarbamoyl chloride with the alcohols obtained by alkaline hydrolysis of the corresponding esters (compounds No. 26 and 27). Hydrolysis of the esters was achieved by heating at 100° C for 15 min with sodium hydroxide solution and then slowly cooling with stirring until complete solution was effected. The solution was heated at 100° C for an additional 15 min and the product was then extracted with chloroform.

The acetylenes required for the Mannich reactions were obtained by the reaction of prop-2-ynyl bromide with the potassium salt of pyrrolid-2-one in toluene solution at 67° C for 30 min (for compounds No. 1 and 2), with the sodium salt of succinimide in dimethyl sulphoxide at 100° C for 30 min (for compound No. 7), with potassium phthalimide in dimethylformamide at 100° C for 2 hr (for compound No. 8), with

sodium ethoxide in ethanol at 30° C for 18 hr (for compound No. 9), with the sodium salt of ethyl acetoacetate in ethanol (for compounds No. 23 and 24), with cyclopentenylmorpholine in methanol at 5 to 10° C and subsequently at 20° C for 4 hr (for compounds No. 20 and 21) and by acetylation of prop-2-ynyl alcohol with acetyl chloride in pyridine (for compounds No. 26 and 27).

The amides and ureas (compounds No. 11 to 16) were most conveniently prepared from the appropriately substituted 4-aminobutynols via the chloride hydrochlorides. The chloride hydrochloride was added to a large excess of 33% ethanolic solution of the methylamine and the solution was heated under reflux for 5 hr. Sodium iodide was added and heating was continued for an additional 5 hr. The mixture was cooled and filtered and the solvent was removed by distillation. The residue was dissolved in water and sodium hydroxide was added with cooling. The product was extracted with chloroform. The amides were obtained from the amines by heating with acetic anhydride at 100° C for 45 min. The ureas were obtained by the addition of dimethylcarbamoyl chloride to a solution of the amine in benzene at 0 to 5° C in the presence of triethylamine followed by heating under reflux for 1 hr.

Methiodides were prepared in either acetone or methanol solution.

Compound No. 4 was obtained by hydrogenation of oxoTremorine in ethanolic solution at atmospheric pressure over 5% palladium on barium sulphate treated with quinoline.

Compound No. 5 was obtained from 1-(4-bromobutyl)pyrrolid-2-one (prepared from pyrrolid-2-one sodium and a thirtyfold excess of 1,4-dibromobutane) by treatment with pyrrolidine.

The synthesis of compound No. 6 involved the preparation of 1-*p*-nitrosophenylpyrrolidine by conventional methods and reduction to yield 1-*p*-aminophenylpyrrolidine. A stirred mixture of ethanol (250 ml.), potassium carbonate (27 g) and the substituted pyrrolidine (31 g) was heated under reflux during the dropwise addition of ethyl γ-iodobutyrate (47 g). After the addition was complete, heating was continued for 6 hr under nitrogen. All subsequent operations were carried out under nitrogen. The reaction mixture was filtered and the filtrate was evaporated. The residue was extracted with ether; the ether extract was filtered and evaporated and the product was recrystallized from methanol. It was further purified by sublimation *in vacuo*.

Pharmacological tests

Toxicity

An indication of the intravenous toxicity of each compound, using male albino mice (20 to 30 g), was obtained by injecting groups of two mice per dose. The highest dose used was 50 mg/kg. The LD50 was then determined using four groups of five mice for each compound with a ratio between doses of 1 : 1.5. LD50s were calculated using Thompson's (1947) method of moving averages and the tables calculated by Weil (1952).

Muscarinic activity

Two preparations were used. The terminal piece of ileum from guinea-pigs (200 to 250 g body weight) was suspended in a 5-ml. organ-bath of Tyrode solution at 37° C. A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the bath fluid. Contractions were recorded on a kymograph using an isotonic frontal-writing lever. Acetylcholine (0.1 and 0.2 μM) was used as a standard. Concentrations of the test substances were adjusted to give contractions of the gut of similar magnitude to those produced by the standard doses of acetylcholine, and four-point assays were carried out. Activity of all test substances was then expressed in terms of acetylcholine activity.

In some experiments log concentration/response curves were obtained by cumulative administration of increasing concentrations of the test substances allowing the contraction of the ileum to develop fully after each administration. This procedure was continued until maximum contraction was obtained.

For the second preparation cats (neutered males, 1.8 to 2.5 kg) were anaesthetized with chloralose and urethane (2.5 ml./kg intraperitoneally of an aqueous solution containing 25 mg/ml. of chloralose and 250 mg/ml. of urethane). Arterial blood pressure was recorded from a cannulated carotid artery using a mercury manometer. Drugs were administered in a volume of 0.1 ml./kg through a cannula in a femoral vein and were washed in with 1 ml. of 0.9% saline. The depressor responses to each compound were

TABLE 1

PREPARATION OF ACETYLENES

$X.CH_2.C{:}C.CH_2.Y$

Values of n_D^{25} are given in parentheses under Boiling Point.

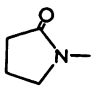
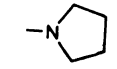
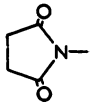
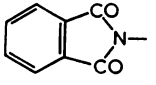
No.	X	Y	Name	Melting point (°C)	Boiling point (°C)	Formula	Calculated (%) C	Calculated (%) H	Found (%) C	Found (%) H	Solvent of recrystallization	Yield (%)
1			1-(4-Pyrrolidin-1′-ylbut-2-ynyl)pyrrolid-2-one (oxoTremorine)		124/ 0·1 mm Hg (1·5156)	$C_{12}H_{18}N_2O$	69·9	8·8	69·9	8·6		72
2		$-N(CH_3)_2$	1-(4-Dimethylaminobut-2-ynyl)pyrrolid-2-one		98/ 0·02 mm Hg (1·4981)	$C_{10}H_{16}N_2O$	66·6	9·0	66·5	9·1		15
3		$-N(C_3H_7)_2$	1-(4-Dipropylaminobut-2-ynyl)pyrrolid-2-one		105/ 0·01 mm Hg (1·4868)	$C_{14}H_{24}N_2O$	71·1	10·2	71·6	10·6		35
7			*N*-(4-Pyrrolidin-1′-ylbut-2-ynyl)succinimide	89–91		$C_{12}H_{16}N_2O_2$	65·4	7·3	65·7	7·6	Cyclohexane	61
8			*N*-(4-Pyrrolidin-1′-ylbut-2-ynyl)phthalimide	112–114		$C_{16}H_{16}N_2O_2$	71·6	6·0	71·6	5·8	Ethyl acetate	11
9	C_2H_5O-		1-(4-Ethoxybut-2-ynyl)-pyrrolidine		97/ 3 mm Hg (1·4698)	$C_{10}H_{17}NO$	71·8	10·3	71·9	10·5		55
10		$-\overset{+}{N}(CH_3)_3I^-$	Trimethyl[4-(2-oxopyrrolidin-1′-ylbut-2-ynyl]-ammonium iodide	154–156		$C_{11}H_{19}IN_2O$	41·0	5·9	41·0	6·0	Ethyl acetate/ methanol	

TABLE 1—*Continued*

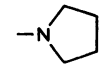
11	$CH_3.CO.N(CH_3)$–		*N*-Methyl-*N*-(4-pyrrolidin-1′-ylbut-2-ynyl)acetamide		106/ 0·05 mm Hg (1·5010)	$C_{11}H_{18}N_2O$	68·0	9·3	67·9	9·7		91
12	$CH_3.CO.N(CH_3)$–	$-N(CH_3)_2$	*N*-(4-Dimethylaminobut-2-ynyl)-*N*-methylacetamide hydrogen oxalate	86–87		$C_{11}H_{18}N_2O_5$	51·2	7·0	50·9	6·8		68
13	$CH_3.CO.N(CH_3)$–	$-\overset{+}{N}(CH_3)_3I^-$	Trimethyl[4-(*N*-methylacetamido)but-2-ynyl]ammonium iodide	140–142		$C_{10}H_{19}IN_2O$	38·7	6·2	38·5	6·7	Ethyl acetate/ methanol	
14	$(CH_3)_2N.CO.N(CH_3)$–		*NNN′*-Trimethyl-*N′*-(4-pyrrolidin-1′-ylbut-2-ynyl)urea hydrogen oxalate	117		$C_{14}H_{23}N_3O_5$	53·7	7·4	53·8	7·4	Acetone/ ethanol	Free base 42
15	$(CH_3)_2N.CO.N(CH_3)$–	$-N(CH_3)_2$	*N*-(4-Dimethylaminobut-2-ynyl)-*NN′N′*-trimethylurea hydrogen oxalate	85–87		$C_{12}H_{21}N_3O_5$	50·2	7·4	50·4	7·6	Acetone	Free base 70
16	$(CH_3)_2N.CO.N(CH_3)$–	$-\overset{+}{N}(CH_3)_3I^-$	Trimethyl[4-(*NNN′*-trimethylureido)but-2-ynyl]ammonium iodide	88–89		$C_{11}H_{22}IN_3O$	39·0	6·5	39·1	7·0	Acetone/ ethyl acetate	
17	$(CH_3)_2N.CO.O$–		4-Pyrrolidin-1′-ylbut-2-ynyl dimethylcarbamate		80–84/ 0·05 mm Hg (1·4885)	$C_{11}H_{18}N_2O_2$	62·8	8·6	62·6	9·0		57
18	$(CH_3)_2N.CO.O$–	$-N(CH_3)_2$	4-Dimethylaminobut-2-ynyl dimethylcarbamate hydrochloride		Free base 68/ 0·5 mm Hg (1·4681)	$C_9H_{17}ClN_2O_2$	49·0	7·8	49·2	8·0	Acetone	Free base 61
19	$(CH_3)_2N.CO.O$–	$-\overset{+}{N}(CH_3)_3I^-$	(4-Dimethylcarbamoyloxybut-2-ynyl)trimethylammonium iodide	240 decomp.		$C_{10}H_{19}IN_2O_2$	36·8	5·9	36·6	6·1	Methanol	
20			1-(4-Pyrrolidin-1′-ylbut-2-ynyl)cyclopentan-2-one hydrochloride	110–115		$C_{13}H_{20}ClNO$	64·6	8·3	64·8	8·7	Ethyl acetate	86

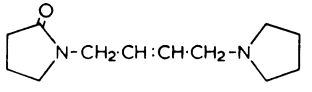
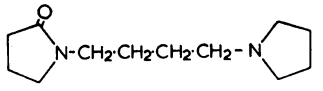
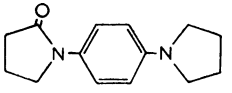
TABLE 1—*Continued*

No.	X	Y	Name	Melting point (°C)	Boiling point (°C)	Formula	Calculated (%) C	Calculated (%) H	Found (%) C	Found (%) H	Solvent of recrystallization	Yield (%)
21		$-N(CH_3)_2$	1-(4-Dimethylaminobut-2-ynyl)cyclopentan-2-one hydrochloride	119–120	Free base 86/ 0·1 mm Hg (1·4827)	$C_{11}H_{18}ClNO$	61·2	8·4	60·9	8·6	Ethyl acetate	77
22		$-\overset{+}{N}(CH_3)_3I^-$	Trimethyl[4-(2-oxocyclopentyl)but-2-ynyl]-ammonium iodide	109		$C_{12}H_{20}INO$	44·9	6·3	45·0	6·4	Acetone	
23	$CH_3.CO.CH_2-$		7-Pyrrolidin-1′-ylhept-5-yn-2-one hydrogen oxalate	100–102		$C_{13}H_{19}NO_5$	58·0	7·1	58·0	7·3	Acetone	Free base 3
24	$CH_3.CO.CH_2$	$N(CH_3)_2$	7-Dimethylaminohept-5-yn-2-one hydrogen oxalate	91–93		$C_{11}H_{17}NO_5$	54·3	7·0	54·6	7·5	Acetone	Free base 15
25	$CH_3.CO.CH_2$	$-\overset{+}{N}(CH_3)_3I^-$	Trimethyl(6-oxohept-2-ynyl)ammonium iodide	137–140		$C_{10}H_{18}INO$	40·7	6·2	40·9	6·0	Ethanol	
26	$CH_3.CO.O-$		4-Pyrrolidin-1′-ylbut-2-ynyl acetate		68/ 0·1 mm Hg	$C_{10}H_{15}NO_2$	66·3	8·3	66·1	8·2		61
27	$CH_3.CO.O-$	$-N(CH_3)_2$	4-Dimethylaminobut-2-ynyl acetate		47–50/ 0·02 mm Hg	$C_8H_{13}NO_2$	61·9	8·4	62·0	8·8		47
28	$CH_3.CO.O-$	$-\overset{+}{N}(CH_3)_3I^-$	(4-Acetoxybut-2-ynyl)trimethylammonium iodide	138		$C_9H_{16}NO_2$	36·4	5·4	36·9	5·6	Ethanol	

TABLE 2

PREPARATION OF MISCELLANEOUS COMPOUNDS

Values of n_D^{25} are given in parentheses under Boiling Point

No.	Structure	Name	Melting point (°C)	Boiling point (°C)	Formula	Calculated (%) C	Calculated (%) H	Found (%) C	Found (%) H	Yield (%)
4	 *cis*	*cis*-1-(4-Pyrrolidin-1′-ylbut-2-enyl)pyrrolid-2-one		95/ 0·01 mm Hg (1·5080)	$C_{12}H_{20}N_2O$	69·2	9·7	69·5	9·7	30
5		1-(4-Pyrrolidin-1-ylbutyl)-pyrrolid-2-one hydrogen oxalate	105–106	Free base 100/ 0·02 mm Hg (1·4932)	$C_{14}H_{24}N_2O_5$	56·0	8·1	55·7	8·4	36
6		1-*p*-Pyrrolidin-1-ylphenyl)-pyrrolid-2-one	190–191		$C_{14}H_{18}N_2O$	73·0	7·9	73·2	7·9	47

compared with those to standard doses of acetylcholine (usually 0.625 and 2.5 mμM/kg) and the relative potency was calculated on the basis of a four-point assay.

In both preparations tests were made to confirm that the effects produced were blocked by atropine sulphate.

Tremor production

In the toxicity tests on mice the lowest dose at which tremors occurred was noted. Visual observation of the animals was found to be quite satisfactory for this purpose but in other experiments, where it was desirable to assess the tremors quantitatively, the following technique was used. The mouse was placed in a cage made from a 150-ml. polystyrene weighing-bottle with ventilation holes drilled in the bottom. A boss with a screw attachment was glued to the side of the bottle and the end of the bottle was closed with a polyethylene cap. This cage was attached to a transducer comprising a Goodman's vibrator firmly mounted with the stem projecting downwards. The signal from the vibrator was fed into a " long-tailed pair " transistorized amplifier, the circuit being essentially that given in the Mullard Manual of Transistor Circuits (1960). This design had the advantage that " in phase " changes cancelled out, whereas " push-pull " signals were amplified. Drift associated with temperature change had little or no effect and the amplifier was extremely stable. Being a D.C. amplifier, it was ideally suited to amplification of the low frequencies (50 cycles/sec and less) associated with mouse movement and tremors.

A diode was placed across the output to rectify the signal which was fed into a low inertia motor and counter (Electro Methods Ltd.). A balancing potentiometer was placed across the emitters of the first pair of transistors. The potentiometer could be adjusted until the output was zero (in balance). In this condition the counter integrated the positive portion of any signal over a predetermined period.

This equipment was used to investigate the effect of either atropine sulphate or atropine methonitrate upon incidence of tremors. The atropine was injected intraperitoneally to mice immediately before injection of the muscarinic agent, and a tremor count was carried out between 9 and 11 min after the injection. It was assumed that tremors of central origin were blocked by the tertiary atropine sulphate but not by the quaternary atropine methonitrate.

RESULTS

The pharmacological results are summarized in Tables 3 and 4.

Table 3 shows the muscarinic activities of oxoTremorine and some of its structural analogues. Among these compounds, apart from oxoTremorine (compound No. 1), only compound No. 2 showed any appreciable activity; compound No. 7 was slightly active but none of the others had agonist activity. Three of them (compounds Nos. 5, 8 and 9) antagonized to some extent the contractions of the guinea-pig ileum caused by acetylcholine.

With the exception of compounds Nos. 1 and 2 (details given in Table 4) tremors were not seen after administration of any of the compounds listed in Table 3.

Table 4 gives results from compounds selected in order to study the effect of changing the character of the carbonyl group. In each class of compound an *NN*-dimethyl tertiary base, a pyrrolidine tertiary base and a trimethylammonium salt were included. All the compounds tested showed muscarinic activity. The ureas were partial antagonists and so the values for muscarinic activity obtained for these compounds are rather lower than those obtained for other members of the series.

Six tertiary bases (compounds Nos. 1, 2, 11, 12, 14 and 15) produced tremors in mice. Tremors were not produced by quaternary trimethylammonium salts nor by tertiary bases with muscarinic activities less than one-hundredth of that of acetylcholine.

TABLE 3

TOXICITY AND MUSCARINIC ACTIVITY OF SOME MISCELLANEOUS STRUCTURAL ANALOGUES OF OXOTREMORINE

See Tables 1 and 2 for formulae of compounds. LD50s are intravenous in mice. Inactive means <0·001

Compound No.	LD50 (mg/kg)	Muscarinic activity (acetylcholine=1)	
		Guinea-pig ileum	Cat blood pressure
1	1·4	1·48	1·72
2	2·3	0·16	0·18
3	>50	Inactive	Inactive
4	>50	Inactive	Inactive
5	>50	Inactive (antagonist)	Inactive
6	12·9	Inactive	Inactive
7	35	0·06	0·03
8	19·5	Inactive (antagonist)	Inactive
9	>50	Inactive (antagonist)	Inactive

TABLE 4

TOXICITY AND MUSCARINIC ACTIVITY OF SOME ACETYLENIC LACTAMS, AMIDES, UREAS, KETONES, CARBAMATES AND ESTERS RELATED TO OXOTREMORINE

See Table 1 for formulae of compounds. LD50s and minimal effective doses (approximate) for tremors are intravenous in mice. * Tremors not seen at doses less than the LD50

Compound No.	LD50 (mg/kg)	Muscarinic activity (acetylcholine=1)		Minimal effective dose for tremors (mg/kg)
		Guinea-pig ileum	Cat blood pressure	
1	1·4	1·48	1·72	0·05
2	2·3	0·16	0·18	0·8
10	0·27	1·74	1·49	—
11	4·3	1·15	0·28	0·12
12	6·3	0·06	0·07	1·5
13	1·2	0·87	0·56	—
14	>50	0·04	0·04	12
15	>50	0·05	0·04	6
16	14·1	0·03	0·01	—
17	35	0·004	0·009	*
18	50	0·009	0·005	*
19	3·0	0·15	0·08	—
20	30	0·003	0·002	*
21	>50	0·005	0·003	*
22	15	0·11	0·05	—
23	35	0·005	0·003	*
24	50	0·01	0·009	*
25	1·7	2·10	0·36	—
26	>50	0·005	0·003	*
27	>50	0·008	0·008	*
28	2·6	0·47	0·39	—

The tremors produced were blocked by atropine sulphate but unaffected by atropine methonitrate, though the latter compound was effective in blocking all peripheral parasympathomimetic effects (salivation, lachrymation, diarrhoea, etc.). Results of a typical experiment are shown in Fig. 1. Here, oxoTremorine (0.5 mg/kg) produced a high tremor count in animals treated with doses of atropine methonitrate of up to 31.6 mg/kg, while tremors were blocked by doses of atropine sulphate of 3.16 mg/kg and above.

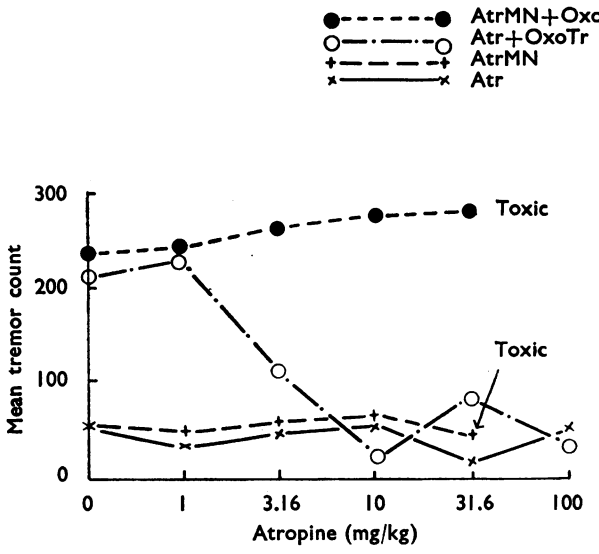


Fig. 1. The effect of intraperitoneal atropine sulphate (Atr) and atropine methonitrate (AtrMN) on oxoTremorine-induced tremors in the mouse. 0.5 mg/kg of oxoTremorine (oxoTr) was injected intraperitoneally immediately before the atropine. Tremor recordings were taken 10 min after the injection.

Table 5 shows the carbonyl absorption maxima and pK_a values for the pyrrolidine tertiary bases of linear compounds (amides, ureas, carbamates, ketones and esters). Also included in this table are values for the ratio of the muscarinic activity (guinea-pig ileum) of each compound relative to that of the corresponding quaternary trimethylammonium salt.

TABLE 5

MUSCARINIC ACTIVITIES AND PHYSICAL CONSTANTS OF PYRROLIDINE TERTIARY BASES

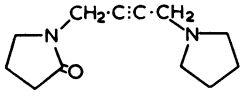
See Table 1 for formulae of compounds

Compound No.	Muscarinic activity relative to corresponding quaternary salt	C=O absorption maximum (cm^{-1})	pK_a
11	1·32	1,647	8·2
14	1·33	1,652	8·1
17	0·03	1,700	8·0
23	0·002	1,715	8·5
26	0·01	1,736	7·9

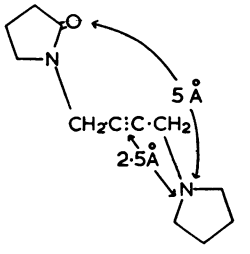
DISCUSSION

The results in Tables 3 and 4 suggest very strongly that there is a close correlation between central tremorogenic activity and peripheral muscarinic activity in the tertiary amines examined. Compounds with high muscarinic activity evoked tremors, while those with low activity did not. In addition, tremorogenic potency tended to parallel muscarinic activity. The tremors were blocked by atropine sulphate (a tertiary base) but not by atropine methonitrate (a quaternary salt). From this evidence it may perhaps be concluded that the

structure of the tremorogenic site located in the central nervous system very closely resembles that of the peripheral postganglionic parasympathetic acetylcholine receptor. The structure of this peripheral receptor has been discussed by Beckett, Harper, Clitherow & Lesser (1961), Beckett, Harper & Clitherow (1963), Waser (1961) and Belleau & Puranen (1963) with particular reference to the activity of muscarine, muscarone and the highly active quaternary salts of 5-dimethylaminomethyl-2-methyl-1,3-dioxolane. Beckett *et al.* (1963) have suggested that muscarinic drugs may interact with the receptor at an anionic site (site 1), and a cationic site (site 2), separated by 3 to 3.5 Å, and also at a third site located at a distance of 5 to 7 Å from the anionic site. Because of the existence of free rotation about the $\equiv C\text{–}CH_2$ bond oxoTremorine can exist in two planar forms, cisoid or transoid.

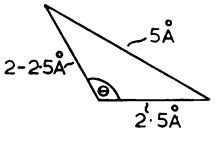


Cisoid form of oxoTremorine



Transoid form of oxoTremorine

The distances between active centres in the molecule in the transoid form correspond with those in muscarone and in the dioxalanes. An essential difference, however, between the structure of oxoTremorine and that of many previously described muscarinic agents is the presence of the acetylenic bond. It is suggested that the triple bond represents a region of high electron density which is capable of binding at the muscarinic site 2, the site at which the furan oxygen atom of muscarone and the ester oxygen atom of acetylcholine interact with the receptor. The amide carbonyl group of oxoTremorine could then adopt the correct spatial configuration for binding at site 3 in a manner similar to the binding of the carbonyl group of muscarone and acetylcholine. That three such groups arranged at the corners of a triangle having the dimensions shown below are essential for high muscarinic activity is well illustrated by the results in Tables 3 and 4.

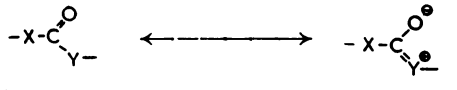


θ = approximately 130°

In compounds having groups which conform to these dimensions maximum activity is associated with compact structures such as the ketone (compound No. 25), lactam (compound No. 10), amide (compound No. 13) and ester (compound No. 28). Jenden & Cho (1963) have shown that increasing the size of the lactam ring is associated with the appearance of antagonist properties and in the present work a similar observation has been made with regard to the phthalimide (compound No. 8). Compounds Nos. 5 and 9 also antagonize the action of acetylcholine on the guinea-pig ileum but to a lesser degree. The urea (compound No. 16) has partial antagonistic properties.

In the oxoTremorine series tertiary amines are able to interact with the receptor. Previously the only two muscarinic agents known to be active in the tertiary base form were pilocarpine and arecoline, and neither of these substances is as active as oxoTremorine. A possible explanation of this anomaly is suggested by the results in Tables 4 and 5, in which compounds designed to examine the contribution of the carbonyl group to the total interaction with the receptor are listed. The small variation in pK_a values shows that in the series of compounds shown in Table 5 the pyrrolidinyl groups can be considered to be equivalent at physiological *p*H. Any gross variation in the muscarinic activities must therefore be due to variations in the degree of interaction of the carbonyl group with the receptor.

From the results in Table 4 it is apparent that there are variations in the muscarinic activities of the quaternary salts which are probably due to steric and stereochemical factors. This variation is, however, not much more than one order of activity except in the case of the urea (compound No. 16) which is a partial antagonist. On the other hand, a comparison of the activities of the tertiary bases reveals some very striking differences. OxoTremorine (compound No. 1) is 200-times more active than the corresponding carboxylate ester (compound No. 26); the carbamates, ketones and ureas have intermediate activities. In the case of the linear pyrrolidine tertiary bases (compounds Nos. 11, 14, 17, 23 and 26) these changes in activity have been compared with the changes in the frequency of the carbonyl absorption in the infrared region (see Table 5). As the frequency of the carbonyl absorption decreases, the activity of the pyrrolidine tertiary base approaches that of the corresponding quaternary trimethylammonium salt. This decrease in the frequency of maximum absorption corresponds to a delocalization of electrons in the carbonyl group, that is, there is an increase in the contribution of the structure II to the total resonance, of the molecule.



Structure I Structure II

The degree of interaction of amido groups (C=O; 1,647 cm^{-1}) should therefore be greater than that of ester groups (C=O; 1,736 cm^{-1}) and this difference is manifested in the increased activity of the amide (compound No. 11) compared with the ester (compound No. 26).

It is of some interest that Barlow, Scott & Stephenson (1963) have suggested that the affinity of acetylcholine for the postganglionic receptor of the guinea-pig ileum depends on both the onium group and the carbonyl group. In the case of the esters (compounds Nos. 26, 27 and 28) it appears that the contribution of the carbonyl group to the affinity of the agonist for the receptor is small and in order to obtain high muscarinic activity it is essential to have an onium group in the molecule. In contrast there is a larger contribution to affinity from the carbonyl group of ureas and amides (compounds Nos. 11 to 16); consequently the tertiary bases are nearly as active as the corresponding quaternary salts.

There is so far little evidence to indicate the part played by the acetylenic linkage in stimulation of the acetylcholine receptor. By analogy with the compounds related to

acetylcholine studied by Barlow *et al.* (1963), it might be concluded that the acetylenic linkage (possibly together with the protonated amino or onium group) is responsible for the efficacy of these compounds. It is intended that this aspect of the drug receptor interaction will be studied in future work.

SUMMARY

1. The central tremorogenic activities in mice of acetylenic amines related to oxo-Tremorine parallel their peripheral muscarinic activities measured on isolated guinea-pig ileum and an anaesthetized cat preparation. It is suggested, therefore, that the structure of the central tremorogenic site very closely resembles that of the peripheral postganglionic acetylcholine (muscarinic) receptor.

2. The muscarinic activities of twenty-eight acetylenic amines and quaternary salts, all containing a carbonyl group, have been examined. It is concluded that for maximum muscarinic activity such compounds should be capable of interacting with three sites on the acetylcholine receptor.

3. The extent of interaction of the carbonyl group at one of these sites determines whether the muscarinic agent will be active in the free base form or only as the quaternary salt. It is suggested that delocalization of electrons in the carbonyl group assists binding to the receptor. Thus linear amides and ureas are approximately equiactive in the tertiary base and quaternary salt forms, whereas related esters are much more active when quaternized.

We wish to thank Mr D. C. Parkes, Mr J. W. Blackburn and Mr P. Munro for some pharmacological testing, and Mr A. C. Thomas for microanalyses.

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