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Synthesis and pharmacological properties of *N*-[4-(1-azetidinyl)-2-butynyl]-2-pyrrolidone, a highly potent oxotremorine-like agent

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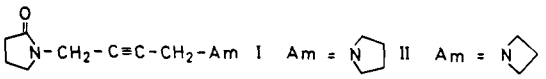
Oxotremorine, *N*-[4-(1-pyrrolidinyl)-2-butynyl]-2-pyrrolidone, I, is a specific muscarinic agent equal in potency to acetylcholine (Cho et al 1962; George et al 1962). Unlike acetylcholine it readily penetrates into the central nervous system after systemic administration. Its extraordinary potency is surprising in view of its structural dissimilarity to other muscarinic agents, the powerful ones possessing a quaternary trimethylammonium group while oxotremorine is a tertiary amine with no methyl groups and an acetylenic bond at the position in the molecule where strong muscarinic agents have an oxygen atom. The structural requirements for muscarinic activity are very specific and oxotremorine apparently possesses an optimal structure as even slight changes lead to loss of the muscarinic activity or to change of the type of action from agonistic to antagonistic (Brimblecombe & Pinder 1972). The only compound of this type which shows appreciable agonistic activity is the dimethylamino analogue of oxotremorine which in different tests had 1/10–1/15 of the activity of oxotremorine (Bebbington et al 1966). As we had observed that in several series of potent oxotremorine antagonists compounds having a dimethylamino or a pyrrolidino group as amino component often showed evidence of agonistic or partial agonistic properties (Dahlbom et al 1966; Karlén et al 1970; Svensson et al 1975), it appeared to us that it would be of interest to use azetidine, which would be close to both dimethylamine and pyrrolidine in its steric requirements, as the amino component in an oxotremorine analogue. This note reports the synthesis and pharmacological properties of this compound (II).



Preparation of N-[4-(1-azetidinyl)-2-butynyl]-2-pyrrolidone (II). Compound II was prepared by the following method. *N*-(4-Diethylamino-2-butynyl)-2-pyrrolidone was synthesized in 65% yield through the Mannich reaction from *N*-(2-propynyl)-2-pyrrolidone, paraformaldehyde and diethylamine using a method described by Lindgren et al (1973), b.p. 123 °C (0·5 mmHg). Oxalate: m.p. 84–86 °C (from ethanol-ether). Anal. ($C_{12}H_{20}N_2O.C_2H_2O_4$) C,H,N. This Mannich base was treated with cyanogen bromide according to a previously described procedure (Resul et al 1979) to give *N*-(4-bromo-2-butynyl)-2-pyrrolidone, b.p. 125 °C (0·1 mmHg), yield 73%. Anal. ($C_8H_{10}BrNO$) C,H,N. Azetidine (1·0 g, 0·017 mol) was added to a stirred solution of the above bromo compound (1·8 g, 0·0085 mol) in dioxane (50 ml). The mixture was kept at room temperature for 1 h and was then filtered. The filtrate was concentrated under vacuum affording the title compound (II) as an oil which was converted to its sesquioxalate. m.p. 103–105 °C (from ethanol-ether). Yield 1·1 g, (40%). Anal. ($C_{11}H_{16}N_2O.1{\cdot}5\ C_2H_2O_4.0{\cdot}5\ H_2O$) C,H,N. IR (KBr): ν_{max} 1680 cm^{-1}; n.m.r. ($CDCl_3$): δ 1·9–2·7 (m, 6H), 3·1–3·7 (m, 6H), 3·70 (s, 2H), 4·15 (s. 2H); m.s.: *m/z* 191 (M^+-1), 164, 137, 109, 108, 107, 106, 98, 94, 80, 79, 70, 56 (base peak).

Pharmacology. Compound II was tested for tremorogenic activity by means of an electronic device to achieve an objective measurement of the tremor intensity (Silverman & Jenden 1970). The method used has been described in detail by Ringdahl et al (1979). In short, the test drug was given by intravenous injection to groups of 6 male NMRI mice, 20–24 g, and the median dose required to evoke a predetermined tremor intensity in 50% of the mice was calculated. This was approximately 111 μg kg^{-1} of the base. The corresponding figure for oxotremorine which was tested on the same occasion was 112 μg kg^{-1}. Compound II is obviously a tremorogenic agent of the same potency as oxotremorine and we decided to investigate its action on the central (c.n.s.) and peripheral (p.n.s.) nervous system more closely.

Oxotremorine (I) or the azetidine analogue (II) was administered subcutaneously to groups of 6 mice (male NMRI, 30 g) at 5 dose levels within a range of 0·05–0·8 mg kg^{-1} of the base. The animals were observed during 1 min every 30 min for 2 h (rats) or 4 h (mice) and the presence of four central nervous system (tremor, head twitch, hypokinesia, rigidity) and three peripheral nervous system (lacrimation, salivation, diarrhoea) symptoms were scored by two experienced observers who were kept blind to the respective treatments.

* Correspondence.

In rats, tremor was scored as absent (0), just present (1) or intense (2). In mice the scoring system by Cho & Jenden (1964) was used, i.e. absent (0), present when handling the animal (1), intermittent (2) or continuous (3). Salivation and lacrimation were scored as absent (0), just present (1) or profuse (2). All other symptoms were either absent (0) or present (1). By addition of these points each animal was given a c.n.s. and a p.n.s. score of 0–5 points, respectively. The sum of scores per group, or group medians, were used for time-response graphs for each dose. The maximal response for each dose level was plotted as a dose-response curve and the dose of the agonist which produced 50% of the maximal effect (ED50) was determined from the graph.

Table 1. Cholinergic effects from the central and peripheral nervous system and body temperature changes after subcutaneous administration of oxotremorine (I) and its azetidine analogue (II). ED50 is given in μmol kg^{-1}.

	Oxotremorine (I)	Azetidine analogue (II)
Mouse		
CNS score, ED50	0·68	0·36
PNS score, ED50	0·63	0·26
Hypothermia, max. response	1·0 (Δ t = −11·7 °C)	0·5 (Δ t = −11·2 °C)
Rat		
CNS score, ED50	0·63	0·68
PNS score, ED50	1·12	0·68
Hypothermia, max. response	1·9 (Δ t = −1·7 °C)	1·0 (Δ t = −2·3 °C)

The colonic temperature was measured with a thermistor inserted 40 mm from the anus and recorded every 30 min (Ellab Electrical Thermometer, Copenhagen). Differences in body temperature (Δ t in °C) between post- and pretreatment values were calculated. Group means were treated as described for the c.n.s. and p.n.s. scores. However, in Table 1 the lowest dose of each compound giving maximal hypothermic response has been given, since the dose-response curves had the shape of an inverted U.

Compound II elicited all symptoms usually observed after administration of oxotremorine, i.e. all central and peripheral signs mentioned above as well as hypothermia. Peak responses occurred within 30 min after administration for both compounds. Compound II appeared to have a slightly longer duration (approximately 30 min) than oxotremorine in both rats and mice. As shown in Table 1 the ED50 values for central and peripheral scores and the hypothermic effect indicate that II with one exception (c.n.s., rat) is approximately twice as active as oxotremorine. Pretreatment of mice with atropine sulphate (10 mg kg^{-1} s.c. 30 min before drug administration) completely abolished all effects of the two compounds (data not shown). Thus, it is concluded that for the first time an oxotremorine analogue has been synthesized which shares both the central and peripheral effects of oxotremorine and is more potent than oxotremorine itself.

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