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α-Methyl analogues of acetylenic amines as striatal muscarinic antagonists

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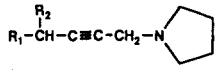
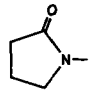
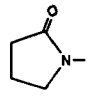
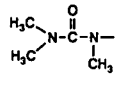
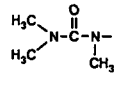
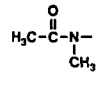
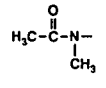
Abstract—The effect of acetylenic amines, with or without α-methyl substitution, on striatal acetylcholine (ACh) concentration in rats was investigated. Oxotremorine, oxotremorine-1, and U-77053 (trimethyl (4-(1-pyrrolidinyl)-2 butynyl)-urea), the unsubstituted amines, increased striatal ACh concentration. On the other hand, the corresponding α-methyl substituted analogues, α-methyl-oxotremorine, BM-5, and α-methyl U-77053, decreased the concentration of ACh in the striatum. The results indicate that substitution of α-methyl in acetylenic amines converts compounds from agonists to antagonists for striatal muscarinic receptors.

Oxotremorine-1 produces central muscarinic agonist effects. These are characterized by induction of tremors in mice (Bebbington et al 1966; Ringdahl & Jenden 1983), increases in brain acetylcholine (ACh) concentrations (Sethy & Francis 1988) and enhancement of phosphatidylinositol hydrolysis (Fisher et al 1984). On the other hand, the α-methyl analogue of oxotremorine-1, BM-5, produces central muscarinic antagonist effects. BM-5 blocks oxotremorine-induced tremors (Ringdahl & Jenden 1983), decreases striatal ACh concentration (Nordstrom et al 1986) and inhibits oxotremorine-induced phosphatidylinositol hydrolysis (Sethy et al 1988). These biological observations indicate that substitution of an α-methyl moiety in the acetylenic amines may convert a compound from a central muscarinic agonist to an antagonist. We have further explored this hypothesis by investigating the effect of two additional pairs of unsubstituted and α-methyl substituted acetylenic amines (Table 1) on striatal ACh concentration.

Table 1. Chemical structures of acetylenic amines.



	R_1	R_2
Oxotremorine		H
α-Methyl Oxotremorine		CH_3
U-77053		H
α-Methyl U-77053		CH_3
Oxotremorine-1		H
α-Methyl Oxotremorine-1		CH_3

Materials and methods

Sprague-Dawley rats, 180–200 g, bred at The Upjohn Company, were kept under diurnal lighting and temperature conditions before use. All animals were killed at approximately the same time of day (0700–0900 h).

Oxotremorine sesquifumarate was obtained from Sigma Chemical Company (St. Louis, MO), and α-methyl oxotremorine oxalate, oxotremorine-1 oxalate, α-methyl oxotremorine-1 hydrochloride (BM-5), U-77053 (trimethyl(4-(1-pyrrolidinyl)-2-butynyl)-urea), and the α-methyl derivative of U-77053 were synthesized at The Upjohn Company. The doses were calculated from the base of the salt of each drug. All drugs were dissolved in 0·9% NaCl (saline) and injected intraperitoneally. Control rats received an equal volume of vehicle (2 mL kg^{-1}). The striatal ACh concentration was determined by the method previously described (Sethy & Francis 1988). Thirty min after treatment,

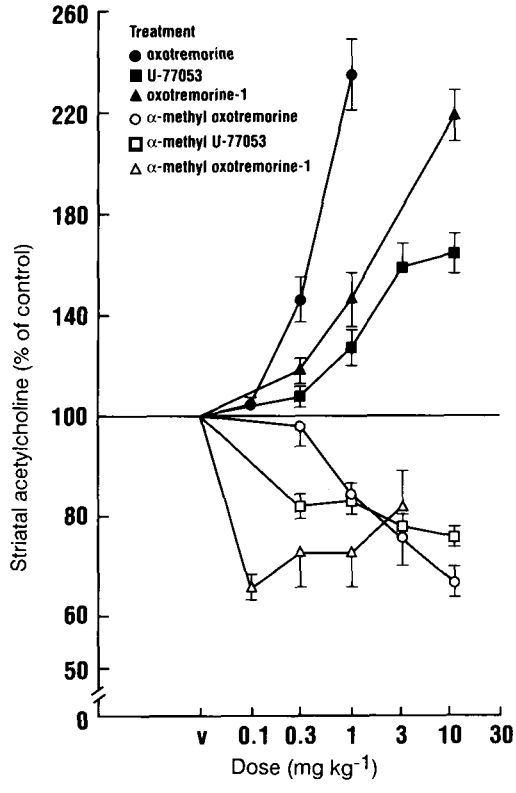


FIG. 1. Effect of cholinergic compounds on striatal acetylcholine concentration. Each observation is the mean ± s.e. of 3–4 experiments.

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animals were killed by decapitation. The brain was quickly removed from the skull and placed in ice-cold 0·32 M sucrose. Bilateral striata were dissected out and homogenized in ice-cold 0·4 M perchloric acid containing ethylhomocholine iodide as internal standard. ACh was extracted by the method of Hanin et al (1972) and estimated by HPLC (Potter et al 1983; Eva et al 1984). The results are expressed as percent of control. Statistical analysis was done using a paired *t*-test.

Results and discussion

Oxotremorine, oxotremorine-1, and U-77053 significantly increased striatal ACh concentration. α-Methyl oxotremorine, BM-5, and α-methyl U-77053 significantly decreased ACh concentration in the striatum (Fig. 1). Muscarinic agonists such as oxotremorine, pilocarpine, and arecoline have been reported to increase ACh concentration in the brain (Haubrich & Reid 1972; Sethy & Francis 1988). Antimuscarinic agents like atropine, benztropine and trihexyphenidyl have been shown to decrease ACh content in the brain (Sethy & Van Woert 1973; Consolo et al 1974; Cheney et al 1976). The results of the present study indicate that substitution of an α-methyl group in the acetylenic amine converts a muscarinic agonist to an antagonist with respect to alterations in striatal ACh concentration.

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Letter to the Editor

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The enantiomeric distribution of propranolol is not influenced by its β-blocking activity

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Propranolol is a lipophilic β-blocker (Hinderling et al 1984). When studying the distribution of propranolol in obese men, Poirier et al (1990) found a decreased volume of distribution as compared with normal subjects. In the rat, propranolol distributes only to a minor extent in adipose tissue (Bianchetti et al 1980). Poirier et al (1990) suggested that the β-blocking activity of propranolol could induce vasoconstriction, and so inhibit the distribution of propranolol to adipose tissues. We have studied this possibility by comparing the tissue distribution of *R*- and *S*-propranolol in rat fat and muscle after intravenous administration of the racemate, with that after administration of the enantiomers separately.

Male Wistar rats (SPF) 12 months old, 553 ± 9·84 g, were purchased from the breeding laboratories of the University of Leuven, Belgium. The rats were fasted for 16 h before drug administration, with free access to water. Silicone catheters were implanted in both jugular veins under ether anaesthesia without administration of anticoagulant (Chindavijak et al 1988). The drugs were administered to conscious animals 2 h after insertion of the catheters and interruption of ether anaesthesia; each rat was used only once. Propranolol racemate (1 mg kg^{-1}), *S*-(−)-propranolol (0·5 mg kg^{-1}) or *R*-(+)-propranolol (0·5 mg kg^{-1}) in 0·9% NaCl (saline) was administered via one of the jugular catheters at volumes of 0·2 mL per 100 g body weight. Sixty min after drug administration, the rats were decapitated and exsanguinated. Blood was collected in heparinized plastic tubes and the haematocrit was measured. For assay of the propranolol enantiomers, 2 blood samples and 2 plasma samples (100 μL) were stored at −20°C in stoppered glass tubes. Suprarenal fat and muscle from the hind limb and the lumbar region were excised, washed in ice-cold phosphate buffer 0·05 M pH 7·4 and homogenized by a Potter Elvehjem homogenizer. Fat was homogenized in three vol of water, muscle in three vol of buffer. The homogenates were stored in plastic tubes at −20°C until analysis.

The propranolol enantiomers were analysed using an indirect HPLC system with fluorescence detection, and (*R,R*)-*O,O*-

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