Metiamide is not an α_2-adrenoceptor antagonist in human platelets

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Metiamide is a well characterized antagonist at histamine H_2-receptors (Black et al 1973). Recently it has been reported that metiamide at concentrations in the range of 10 μM also acts as an α_2-adrenoceptor antagonist when tested on the mouse and rat isolated vas deferens (Griffith et al 1978; Doxey & Everitt 1979) which is a well established in vitro system for examination of α-adrenoceptor drug selectivity (Drew 1976). However studies using the pithed rat failed to detect the in vivo α-adrenoceptor blockade predicted from the results obtained from the isolated tissue preparation (Doxey & Everitt 1979). The aggregatory and secretory responses of human blood platelets induced by adrenaline result from stimulation of an α_2-adrenoceptor on these cells (Grant & Scrutton 1979; Hsu et al 1979). The presence of α_1-adrenoceptors has been detected by functional (Grant & Scrutton 1979) but not by ligand binding (Hoffman et al 1979) studies. These α_1-adrenoceptors have no significant role in the response to adrenaline (Grant & Scrutton 1980). Hence inhibition of the response of human platelets to this catecholamine can be used as a test system for action of a drug as an α_2-adrenoceptor antagonist. We have therefore examined the effect of metiamide on the response of human platelets to adrenaline and to other agonists.

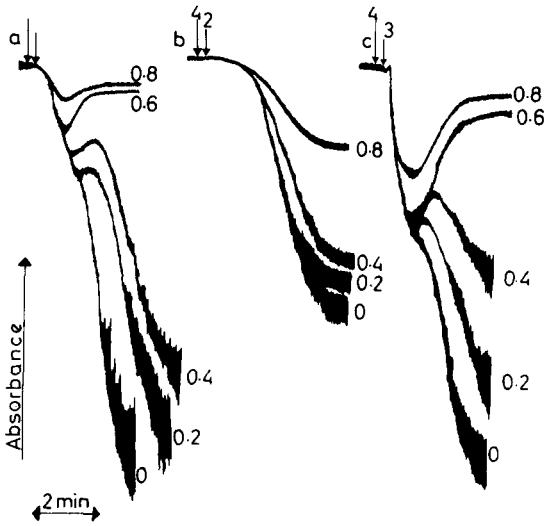

FIG. 1. Effect of metiamide on the response of human platelets to adrenaline (a) collagen (b) and ADP (c). Platelet-rich plasma was prepared and platelet aggregation was monitored as described in the text. The additions were as follows: at 1, 1·2μM adrenaline; at 2, 19μg ml^{-1} collagen; at 3, 5μM ADP; at 4, metiamide at the concentrations (mM) as indicated on the figure.

Human platelet-rich plasma was prepared from drug-free volunteers using acid-citrate dextrose (10 mM citrate) as anticoagulant as described by Pearce et al (1978). The aggregatory response was studied using a Payton Model 300-BD aggregation module and collagen suspensions prepared from bovine achilles tendon collagen as described by Pearce et al (1978). Metiamide was obtained from Smith Kline & French Ltd; and adrenaline bitartrate and ADP from Sigma Chemical Co.

Inhibition by metiamide of the aggregatory response of human platelets to adrenaline is observed only at concentrations of this drug which exceed 100 μM and except at still higher concentrations ($>$ 500 μM) the inhibitory effect is primarily exerted on the secondary phase of the response which is associated with secretion (Fig. 1a). Over this concentration range the effect of metiamide is not selective since addition of this drug also causes inhibition of the response to collagen (Fig. 1b) and to arachidonate (data not shown). It also blocks the second phase of aggregation induced by ADP but has little effect on the primary response (Fig. 1c).

These data indicate that metiamide does not function as an α_2-adrenoceptor antagonist when tested on human blood platelets but rather causes blockade of platelet secretion albeit at high concentration. This latter effect is not unexpected since imidazole acts as an inhibitor of thromboxane synthetase (Moncada et al 1977) and inhibition of thromboxane synthesis by high concentrations of metiamide has been described in isolated microsomal preparations (Allan & Eakins 1978). The α_2-adrenoceptor blockage by metiamide observed in the isolated vas deferens appears therefore to be due to a paradoxical action of the drug in this test system.

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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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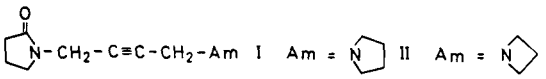
Oxotremorine, *N*-[4-(1-pyrrolidinyl)-2-butynyl]-2-pyrrolidone, I, is a specific muscarinic agent equal in potency to acetylcholine (Cho et al 1962; George et al 1962). Unlike acetylcholine it readily penetrates into the central nervous system after systemic administration. Its extraordinary potency 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.

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