is increased (10^{-5} M) to give between 75–90% of the maximum tone, the sensitivity of the trachea to isoprenaline is significantly reduced and the ability of dimaprit to induce relaxation is virtually abolished.

Duncan et al (1980) induced maximal contraction of the tracheal spiral with acetylcholine, and were unable to relax it with dimaprit. They concluded that there were no H_2 relaxant receptors in the guinea-pig airway smooth muscle. Drazen et al (1979) induced contractions of the tracheal spiral which were 60–80% of the maximum obtainable with histamine, using either 2-(2-pyridyl)-ethylamine (2-PEA), a selective H_1 agonist, or carbachol. The tissue relaxed in response to dimaprit, suggesting a presence of H_2 receptors. These differing results can be explained in terms of the degree of tone induced in the tissue, i.e. functional antagonism. The greater the tone induced, the less likely will dimaprit cause relaxation of the tissue. The results from this work also indicate that the receptor reserve of H_2-receptors is significantly less than that of β-adrenoceptors.

In conclusion, in order to demonstrate that a compound has broncho-relaxant properties it is preferable to use a preparation which exhibits spontaneous tone rather than induced tone, since this may significantly alter the conclusions reached.

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α-Adrenoceptor activity of flutonidine (ST 600) in rat anococcygeus muscle and rabbit jejunum

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Imidazoline compounds are known to stimulate both α-adrenoceptors and histaminergic receptors (Sanders et al 1975; Kobinger & Pichler 1975; Schmitt 1977; Kobinger 1978). Mujic & van Rossum (1965) reported some imidazolines, e.g. naphazoline and tetrahydrozoline, caused relaxations in rabbit intestinal smooth muscles. They further reported that the antihypertensive effect of naphazoline and tetrahydrozoline in cats was mediated through direct stimulation of central α-adrenoceptors. Clonidine, another imidazoline compound is a centrally acting antihypertensive drug the actions of which are mediated through central α-adrenoceptors (Schmitt & Schmitt 1969; Schmitt et al 1973; Finch 1974).

Flutonidine, ST 600 [2-(5 fluoro-*o*-toluidine)-2 imidazoline HCl], a potent antihypertensive drug (Kho et al 1975), acts as a stimulant of central presynaptic and postsynaptic α-adrenoceptors, for its antihypertensive activity (Kho et al 1975; Marmo et al 1976, 1978). The cardiac effects of imidazolines like clonidine and tolazoline in guinea-pig hearts are reported to be mediated through histamine H_2 receptors (Csongrady & Kobinger 1974; Yellin et al 1975a, 1975b; Verma & McNeill 1977a, 1977b). In an earlier investigation, we reported the positive inotropic effects of ST 600 in guinea-pig isolated hearts were mediated via stimulation of histamine H_2 receptors (Veeranjaneyulu & Verma 1979). We have now examined ST 600 for its adrenoceptor activity in the rat anococcygeus muscle and rabbit jejunum. The anococcygeus muscle has a dense adrenergic innervation but apparently no cholinergic innervation (Gillespie 1972) and is a sensitive preparation for the study of the pre and post synaptic actions of α-adrenoceptor agonists and antagonists (Leighton et al 1979).

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Methods

Male rats (300–400 g) were killed by a sharp blow to the head and bled. The two anococcygeus muscles were prepared as described by Gillespie (1972). Each preparation was mounted in a 30 ml organ bath containing modified Krebs solution (composition mM: NaCl 116; KCl 5·4; $CaCl_2$ 2·5; NaH_2PO_4 1·2; $MgCl_2$ 1·2; $NaHCO_3$ 22·00 and glucose 11·00). The bathing solution was bubbled with 95% O_2 + 5% CO_2 and maintained at 37 ± 1 °C, at a pH 7·3. Cumulative dose responses were recorded on smoked drum using an isotonic frontal writing lever with 10 fold magnification. The muscles were maintained under a resting tension of 1 g. The preparation was stabilized for 30 min before the addition of any drug. Paired preparations were mounted at the same time, one preparation serving as control. The preparations were repeatedly washed at intervals of 10 min. Contact times for agonists and antagonists were 45 s and 15 min respectively. In another set of experiments rats were pretreated with reserpine (5 mg kg^{-1}

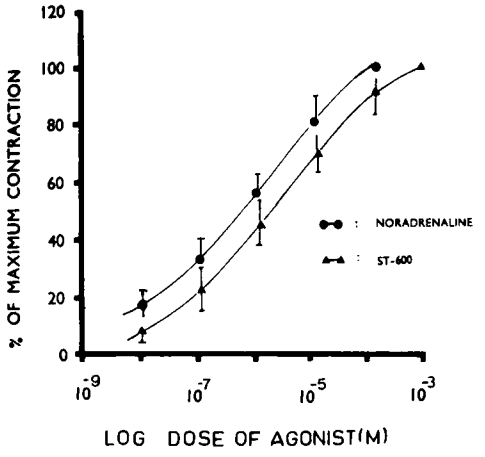

FIG. 1. The dose-dependent contractile effects of ST 600 and noradrenaline (1×10^{-8} to 1×10^{-3} M) in rat anococcygeus muscle. Both the agonists produced parallel dose response curves. Each point represents the mean ± s.e.m. of 6 experiments. Abscissa: log dose of agonist (M).

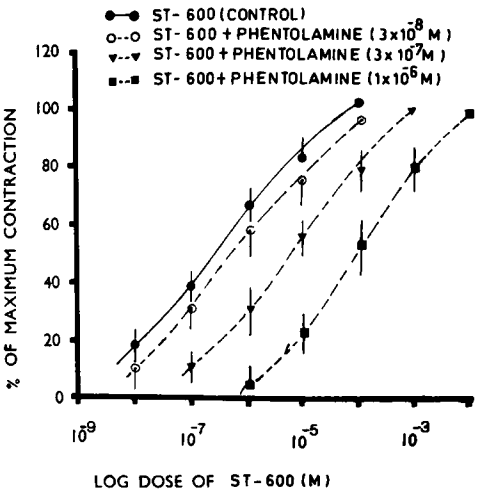

FIG. 2. The effect of ST 600 and its interaction with graded doses of phentolamine (3×10^{-8} to 1×10^{-6} M) in rat anococcygeus muscle. Each point represents the mean ± s.e.m. of 5 experiments. Significant antagonism of the control response occurred at each concentration ($P < 0.05$). Abscissa: log dose of agonist (M).

i.p., 24 h before death) and experiments were done as described before.

Rabbits of either sex were killed by a blow to the head and bled. The abdomen was opened and the jejunum was taken and 2–3 cm lengths were mounted in 30 ml isolated organ baths containing Tyrode as physiological salt solution (composition: mM: NaCl 133·2; KCl 4·7; $CaCl_2$ 1·9; $MgCl_2$ 0·78; NaH_2PO_4 1·2; $NaHCO_3$ 18·6 and glucose 11·1). The bathing solution was bubbled with 95% O_2 + 5% CO_2, maintained at 37 ± 1 °C at pH 7·3. The preparation was stabilized for 45 min before addition of drug and repeated washes were given at an interval of 10 min. Responses were recorded as for rats but with a resting tension of 0·5 g. Contact times were also as for rats. Results were expressed as percentages of the maximal response. Dose effect curves were plotted as percent responses *vs* log agonist concentrations (M). The antagonist pA_2 value was calculated from a Schild plot that was obtained plotting log (ST 600 dose ratio-1) vs log of phentolamine concentration, as detailed by Arunlakshana & Schild (1959).

Drugs used. ST 600, atropine sulphate (C. H. Boehringer Sohn, Ingelheim, W. Germany), adrenaline, noradrenaline (Unichem Labs Ltd, Bombay, India), phentolamine (Ciba Labs, India), propranolol (Imperial Chemicals Ltd, London), cyproheptidine (Merck Sharp & Dohme, India) mepyramine (May and Baker, India) metiamide, (Smith, Klina and French, England) and reserpine (Sigma Labs, St Louis, M. P., U.S.A.). Drugs were added to the bath from the stock solution to yield the final concentration.

Statistical analysis. All results are expressed as means ± standard errors. The two tail Student's *t*-test was used to compare results obtained before/after phentolamine, significance was set at a *P* value of <0·05.

Results

Anococcygeus muscle. ST 600 and noradrenaline caused dose-dependent contractions over the dose range 1×10^{-8} to 1×10^{-3} M. Both the agonists produced parallel dose response curves (Fig. 1). Phentolamine (3×10^{-7} M) produced a parallel rightward shift of the ST 600-induced contractile effects. When different concentrations of phentolamine (3×10^{-8} to 1×10^{-6} M) were used, dose-dependent antagonism of ST 600 responses occurred without any significant change in maxima (Fig. 2). Further, the pA_2 value for phentolamine was obtained as 8·33 with the difference between pA_2 and pA_{10} as 1·33. Neither propranolol (1×10^{-6} M) nor atropine (1×10^{-6} M) had any significant effects on ST 600 elicited responses.

Reserpine pretreatment (5 mg kg^{-1} i.p., 24 h) did not alter the dose-response curve of ST 600 significantly. We also observed that cyproheptidine (1×10^{-6} M), mepyramine (5×10^{-7} M) and metiamide (3×10^{-7} M) had no significant effect to ST 600 induced responses.

Rabbit jejunum. ST 600 produced dose-dependent relaxations in a dose range 1×10^{-8} to 1×10^{-4} M. Phentolamine (3×10^{-7} M) caused parallel rightward shifts of ST 600 induced responses (Fig. 3). Propranolol (1×10^{-6} M) did not alter the ST 600 induced responses to any significance extent.

Discussion

We have found that ST 600 acts as an α-agonist in both rat anococcygeus muscle (Fig. 2) and rabbit jejunum (Fig. 3),

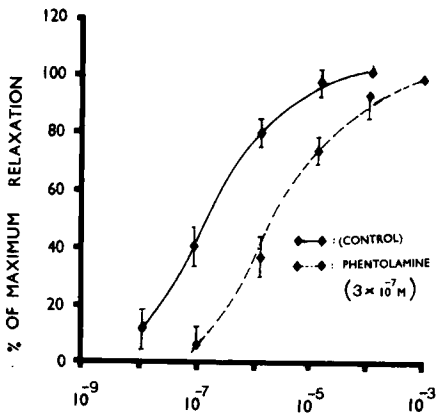

FIG. 3. The dose-dependent relaxations of ST 600 (1×10^{-8} to 1×10^{-3} M) and its interaction with phentolamine (3×10^{-7} M) in rabbit jejunum. Each point represents the mean ± s.e.m. of 5 experiments. Significant antagonism of the control response occurred in presence of phentolamine ($P < 0.05$).

producing dose-dependent contractions and relaxations respectively. The two agonists ST 600 and noradrenaline showed parallel dose response curves in the anococcygeus (Fig. 1). It is suggested that both these agonists are acting on the same receptors, namely α-adrenoceptors. Evidently, phentolamine, an α-antagonist produced a dose-dependent antagonism of the ST 600 responses. The shift of the dose-response curve of ST 600 was parallel and there was no depression of the maxima (Fig. 2) thereby suggesting competitive antagonism, which was confirmed by the difference between pA_2 and pA_{10} (1·33). The responses to ST 600 were unaffected by atropine and propranolol suggesting the absence of cholinergic and β-adrenoceptor mediated effects.

It was observed that the 5-HT antagonist, cyproheptidine, mepyramine and metiamide had no significant effects on ST 600 elicited contractions in the anococcygeus. This rules out the involvement of 5-HT-ergic and histaminergic receptors in the effect of ST 600 on the anococcygeus. The excitatory effects of ST 600 on the anococcygeus appear to be direct since they were not affected by reserpine pretreatment. ST 600 induced dose-dependent relaxations in the jejunum were antagonized by phentolamine (Fig. 3). There was a parallel rightward shift of ST 600 dose response curve in the presence of an α-antagonist, which suggests the presence of an α-adrenoceptor mediated effect. Since propranolol did not alter the responses to ST 600 in the jejunum, this further suggests the absence of a β-adrenoceptor-mediated effect. Our results are in agreement with the previous findings that some imidazoline compounds caused relaxations in rabbit intestinal smooth muscle (Mujic & van Rossum 1965), and contractions in rat anococcygeus muscle (Leighton et al 1979) and rat and rabbit aortic strips (Ruffolo et al 1979; Sanders et al 1975) through the stimulation of α-adrenoceptors.

In conclusion our data suggest that the excitatory effects (in rat anococcygeus) and inhibitory effects (in rabbit jejunum) of ST 600 are mediated through the stimulation of α-adrenoceptors.

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