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Antagonism of 5-hydroxytryptamine-induced bronchospasm in guinea-pigs by 8β-carbobenzyloxy-aminomethyl-1-methyl-10α-ergoline

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The drug 8β-carbobenzyloxyaminomethyl-1-methyl-10α-ergoline (MCE) has been shown to have a potent and prolonged antagonism to 5-hydroxytryptamine-induced bronchospasm in guinea-pigs. 4–5 hr after 2 μg/kg of MCE, given intraveneously, the effects of the 5-HT were still markedly inhibited (dose ratio more than 1:300). A subcutaneous dose of 150 μg/kg of the drug partially counteracted the effects of 5-HT for 4–7 days. Comparison between MCE and 1-methyl-(+)-lysergic acid butanolamide tartrate (methysergide, UML 491) revealed that the antagonism to 5-HT by MCE developed more slowly but lasted longer than that elicited by methysergide. The results show that MCE is a specific antagonist of 5-HT-induced bronchospasm in guinea-pigs as it does not antagonise the bronchospasm-inducing effects of acetylcholine, histamine and eledoisin.

IN a previous paper Beretta, Ferrini & Glässer (1965) reported the strong antagonism of 5-hydroxytryptamine (5-HT) by a new 6-methylergoline derivative synthetised in our laboratories (Bernardi, Camerino, Patelli & Redaelli, 1964), namely 8β-carbobenzyloxyaminomethyl-1-methyl-10α-ergoline (MCE).

The new compound showed a remarkable antagonism to 5-HT *in vitro* and reduced local 5-HT-induced oedema in the rat paw for a long time. This prolonged action is a characteristic of the compound and to provide further confirmation of this property, another test on a different animal species was used. As preliminary experiments had shown that MCE exerted a potent antagonism towards guinea-pig bronchospasm evoked by 5-HT, it was decided to investigate fully its inhibitory effect in this test.

We compared the anti-5-HT action of MCE with that of 1-methyl-lysergic acid butanolamide tartrate (methysergide, UML 491), one of the most potent of 5-HT antagonists (Fanchamps, Doepfner, Weidmann & Cerletti, 1960).

Experimental

METHOD

The method is based on that of Konzett & Rössler (1940) with a water manometer as recording system. Guinea-pigs of 500 to 700 g were anaesthetised with urethane, 1 g/kg i.p. and 1·5 g/kg s.c.

The trachea was cannulated and the lungs inflated by a Starling pump at 36 strokes/min. To suppress the spontaneous respiratory movements, guinea-pigs were injected intravenously with a single dose of 20 mg of gallamine triethiodide (Sincurarina Farmitalia). Optimal stroke volume (varying from 5 to 15 ml) was chosen for each animal.

At the end of each experiment the trachea was clamped and the maximal excursion of the water manometer was measured in mm and recorded as

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"total bronchospasm" (i.e. the maximal resistance to inflation of the air). The effects of doses of bronchoconstrictor agents were expressed as percentage of total bronchospasm. Solutions of drugs in saline were injected at 4 to 8 min intervals through a cannula in the external jugular vein, the volume of each injection (0·5 ml washed in 0·5 ml of saline) being constant. After the resistance to inflation had been increased by a drug, it was returned to the original level by clipping the tube leading to the recording apparatus briefly, thus forcing one full stroke volume of the pump into the lungs.

Two types of experiments were made. In the first, the animals were prepared as described above and drugs (both agonists and antagonists) were administered intravenously. The action of MCE against 5-HT and other bronchoconstrictor drugs was compared with that of other antagonists. 5-Hydroxytryptamine creatinine sulphate, histamine hydrochloride, synthetic eledoisin, synthetic bradykinin and acetylcholine bromide were used as agonists. MCE, methysergide, mepyramine, atropine sulphate, phenoxybenzamine, dihydroergotamine and morphine were used as antagonists.

The second set of experiments was made on groups of guinea-pigs (5 animals in each) treated subcutaneously with different doses (1·5–15 and 150 μg/kg) of MCE or with 150 or 1500 μg/kg of methysergide.

At intervals of 6, 24 and 72 hr after pretreatment, the animals of each group were surgically prepared as described above and dosed intravenously with increasing doses of 5-HT. Three increasing doses of histamine were also administered intravenously to control the sensitivity of the animal preparation.

Injection of these agonists always followed the same regimen: 5-HT (2·25, 4·50 and 9 μg/kg), histamine (2·5, 5 and 10 μg/kg) and then 5-HT again (22·5, 45, 90 and 450 μg/kg).

Thus for each experimental animal a dose-response curve was obtained. For each interval and for each dose of the antagonists a new group of guinea-pigs was used to test the antagonistic effect.

Results

The threshold dose of 5-HT producing bronchospasm in guinea-pigs varied from 2·25 to 4·50 μg/kg i.v. of 5-HT base. Moreover, doses of up to 9 μg/kg i.v. gave a good dose-response relationship. Nearly 90% of total bronchospasm was obtained with 22·5 μg/kg i.v. of 5-HT. No tachyphylaxis was observed. Evaluation of 5-HT effects as a percentage of total bronchospasm seems to be a useful modification of the original method of Konzett & Rössler (1940).

MCE, at a dose of 1 μg/kg i.v., showed little or no antagonism to 5-HT, but 2 μg/kg i.v. had a pronounced inhibitory effect. Inhibition was slow to develop, increased gradually and lasted for a long time (see Fig. 1); the effects of 5-HT induced bronchospasm being still markedly inhibited 4–5 hr after administration of the MCE (dose ratio more than 1 : 300).

Methysergide was also seen to be a potent antagonist of the bronchospasm produced in guinea-pigs by 5-HT. Its action developed more quickly but declined more rapidly than that of MCE. Methysergide, 1 μg/kg i.v.,

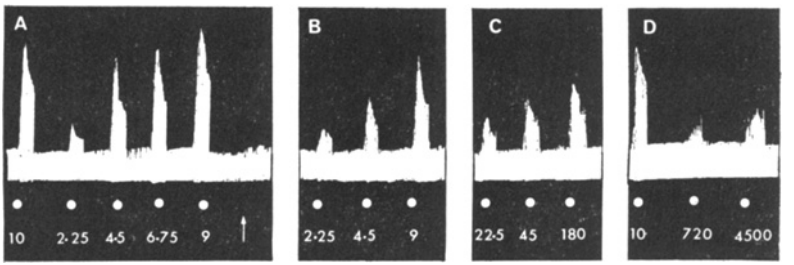

FIG. 1. Bronchospasm induced by 5-hydroxytryptamine 2·25–4500 μg) and by histamine (10 μg) in an anaesthetised guinea-pig) (A) before, (B) 20 min, (C) 1½ hr. and (D) 4 hr after MCE (2 μg) injected at the arrow. All drugs were injected intravenously.

showed a strong and relatively short-lasting inhibitory effect; 2 μg/kg i.v. elicited a stronger longer-lasting inhibition. However 4 hr after the 2 μg/kg dose, the initial effect produced by 5-HT was obtained with doses of 5-HT only 30–40 times higher than those used initially (dose ratio 1 : 30; 1 : 40).

The anti-5-HT action of MCE is specific. At high doses (250–500 times higher than those active against 5-HT) it did not affect the bronchospasm provoked by histamine hydrochloride (10–15 μg/kg i.v.), acetylcholine bromide (5–10 μg/kg i.v.) or synthetic eledoisin (0·2–0·3 μg/kg i.v.). At a dose of 1 mg/kg i.v., MCE exerted a weak and short-lasting anti-bradykinin action (about 5 min) and sometimes itself produced a bronchospasm.

Morphine, phenoxybenzamine and dihydroergotamine, which are known to be powerful antagonists of 5-HT in other tests, were inactive or only slightly active against 5-HT bronchospasm in the guinea-pig, even at high doses (0·5–1·0 mg/kg i.v.). Atropine showed an inhibitory

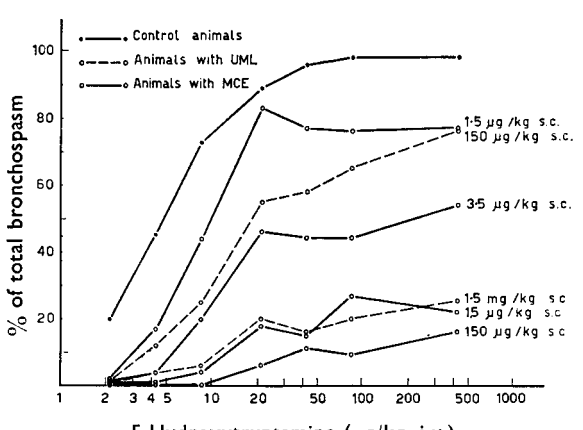

FIG. 2. Bronchospasm-inducing effects of increasing doses of 5-hydroxytryptamine i.v. 6 hr after pretreatment (s.c.) with saline alone (control animals) or with different doses of MCE or of methysergide (UML). Each curve represents the mean of five animals.

effect towards 5-HT at doses of 0·5–1·0 mg/kg i.v., however, the antagonism was not specific as the effect of acetylcholine on guinea-pig bronchospasm was also inhibited by atropine at far smaller doses (1·5 μg/kg i.v.).

The antihistamine drug mepyramine, which specifically antagonised the bronchospasm-inducing effects of histamine when given in small amounts (2–10 μg/kg i.v.), did not modify the response to 5-HT, but at higher doses (0·5–1·0 mg/kg i.v.) it did diminish the 5-HT effects.

The results from the second set of experiments are in Figs 2–4, from which it can be seen that the curves for the control animals have a linear dose response relation for the increasing doses of the agonist (5-HT) up to 9 μg/kg i.v. Higher doses of 5-HT elicited maximal or nearly maximal effects.

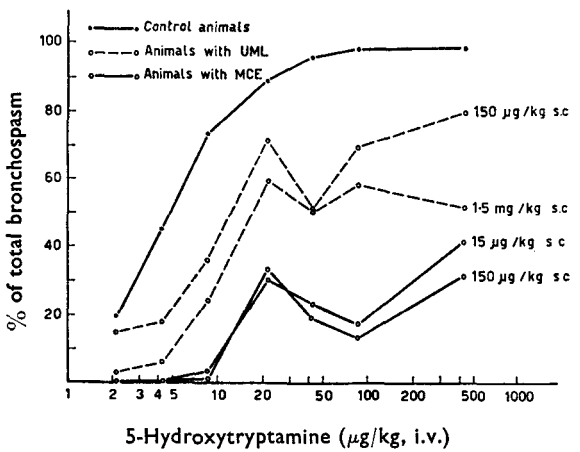

FIG. 3. As Fig. 2 except that the time interval between antagonists and 5-hydroxytryptamine was 24 hr.

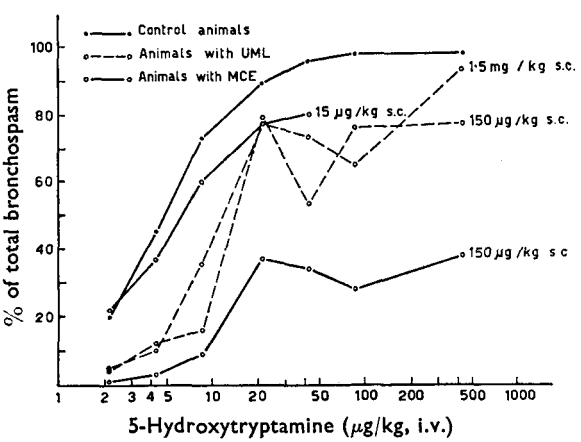

FIG. 4. As Fig. 2 except that the time interval between antagonists and 5-hydroxytryptamine was 3 days.

The curves for the animals subcutaneously pretreated with antagonists showed that both MCE and methysergide were potent anti-5-HT agents. By increasing the doses, 5-HT inhibition increased and persisted longer. From Fig 2–4 it can also be seen that the shapes of the curves for the treated animals differ from those of the control animals, and do not show a simple shift on the log-dose axis (see Fig. 5). Comparison between

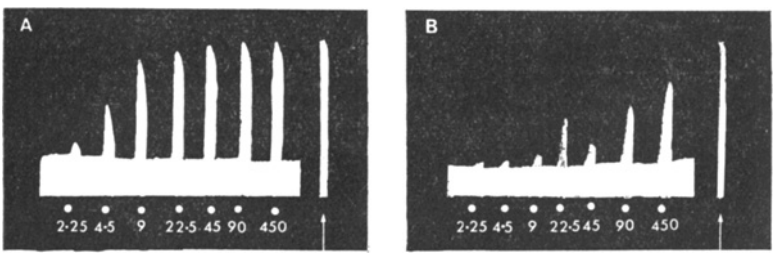

FIG. 5. Bronchospasm-inducing effects of increasing doses of 5-hydroxytryptamine (μg/kg, i.v.) in two anaesthetised guinea-pigs s.c. pretreated 4 days before (A) with saline alone and (B) with 150 μg/kg of MCE. At arrow, total bronchospasm for definition see text).

MCE and methysergide 6 hr after dosing shows that MCE is about 100 times more potent than methysergide, the threshold inhibitory dose of MCE being 1·5 μg/kg s.c. After 24 hr, the inhibitory action of methysergide diminished markedly, while that of MCE at doses even 100 times smaller was still very noticeable.

Three days after treatment (Fig. 4) the actions of methysergide 150 and 1500 μg/kg s.c. and of MCE, 15 μg/kg s.c. were practically undetectable. At this time 150 μg/kg s.c. of MCE was still very active and the activity was still present 4–7 days after dosing (see Fig. 5).

Discussion

MCE has a strong and specific antagonistic action towards 5-HT-induced bronchospasm in guinea-pigs. Its specificity of action was proved when it was tested against other bronchoconstrictor drugs. No inhibition was found against histamine, acetylcholine, and eledoisin even at doses 250–500 times higher than those active against 5-HT. The anti-bradykinin action detectable with 1 mg/kg i.v. of MCE is too slight and short-lasting to be considered important. The action of MCE is characterised by a very slow and gradual onset and by an extraordinary long duration. These results are in agreement with those obtained in other tests both *in vitro* and *in vivo* (Beretta, Ferrini, & Glässer, 1965).

We suggest that these particular properties of the compound may be due to a tenacious fixation of MCE, or of an active metabolite, to the 5-HT receptors.

Phenoxybenzamine or morphine at high doses (1 mg/kg i.v.) failed to antagonise 5-HT bronchospasm. At this dose phenoxybenzamine was found to be very active in blocking the pressor effects of adrenaline in

guinea-pigs (unpublished data). Atropine and mepyramine partly antagonised the action of 5-HT but the effect was not specific as they diminished the bronchoconstrictor action of acetylcholine and histamine respectively at far smaller doses, and their 5-HT antagonism was at least 250–500 times less than that of MCE.

The well known anti-5-HT drug, methysergide was very active in the Konzett-Rössler test, but, compared with MCE, its action developed more rapidly and did not last as long. The shape of the curves for inhibition of 5-HT bronchospasm following s.c. injection of inhibitors, is unusual, e.g. Fig. 3. This might be explained by assuming that when inhibitors are present, doses of 5-HT greater than 20 μg/kg exert both stimulant and tachyphylactic actions, although these are not seen with 5-HT alone.

The last suggestion seems to be supported by the anti-5-HT action exerted by 5-HT itself on guinea-pig bronchospasm as previously described by Courvoisier & Lean (1959). Our results show that the use of 5-HT bronchospasm, as modified by us, is suitable for studying the anti-5-HT drugs with long-lasting action.

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