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DEMONSTRATION OF 5-HYDROXYTRYPTAMINE RECEPTORS THROUGH INHIBITION BY METHERGOLINE IN CAT PIAL ARTERIES *in vitro*

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1 In an attempt to characterize further the nature of the 5-hydroxytryptamine (5-HT)-induced contraction of intracranial vessels, cat's middle cerebral artery was exposed to this amine and the specific 5-HT receptor antagonist, methergoline, under standardized conditions *in vitro*. Methergoline, in increasing concentrations, produced a parallel shift of the log dose-response curve for 5-HT.

2 The Arunlakshana-Schild plot gave a straight line with a slope of -0.85. The figure corresponding to the pA_2 value was 8.80.

3 The findings offer further support for the assumption that the 5-HT-induced intracranial vasoconstriction is mediated by specific 5-HT receptors.

Introduction

It has previously been suggested that 5-hydroxytryptamine (5-HT)-induced contraction of extra- and intracranial vessels is mediated by an effect on postsynaptic 5-HT receptors (Edvinsson, Hardebo & Owman, 1978). In view of the potency ratio for several tryptaminergic agonists and the type of inhibition obtained with various 5-HT antagonists, it was concluded that the character of the 5-HT receptor is different in the two vascular segments (Hardebo, Edvinsson, Owman & Svendgaard, 1978; Edvinsson *et al.*, 1978).

Methergoline (Ferrini & Glässer, 1965) has been reported to be a highly potent, long-lasting antagonist on central 5-HT receptors (Clineschmidt & Lotti, 1974; Fuxe, Agnati & Everitt, 1975). Only at high concentrations is an adrenolytic, cholinolytic and antihistaminic action obtained (Beretta, Ferrini & Glässer, 1965). The present study was performed to analyze the effects of this central 5-HT receptor antagonist on intracranial vessels in an attempt to clarify the nature of the 5-HT-induced vasoconstriction.

Methods

Experiments were performed on blood vessels from 3 adult cats weighing 3 to 5 kg. The animals were exsanguinated and decapitated under Nembutal anaesthesia (30 mg/kg i.p.). The brain was removed, and the middle cerebral arteries (mean diameter 400 μm) were dissected out and placed in an aerated Krebs-Ringer buffer solution at 37.5°C. Pieces, 5 mm long, were mounted in an organ bath (see below) and the remainder of the vessels were stored in a refrigerator at 4°C for up to 24 h.

The vascular preparations were mounted between two L-shaped metal prongs in the same organ bath for registration of isometric circular contractility with Endevco Model 8107-2 force-displacement transducers (Edvinsson, Nielsen & Owman, 1974). The signals were amplified and recorded on a Grass Model 7 B polygraph. Mounting was completed within 20 min after the animal was killed. The arteries were subjected to an initial load of 400 dynes, and were allowed to accommodate for 2 h before testing. The composition of the Krebs-Ringer buffer solution, maintained at 37.5°C ± 0.5°C (range), has been described by Edvinsson *et al.* (1974). The buffer was continuously aerated with 95% O_2 and 5% CO_2, giving a mean pH of 7.39.

A previous study showed that 5-HT causes both dilator and constrictor vascular responses. In the present study on vasoconstrictor effects of 5-HT,

the dilator response was blocked by propranolol 3×10^{-7} M (cf. Edvinsson *et al.*, 1978). After equilibration of the vessels in the organ bath, test doses of the agonist (5-HT) were given cumulatively, and up to three full dose-response curves were run as control before the antagonist (methergoline) was applied to the vessels 20 min before administration of the agonist and present in the bath during the test.

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate (Sigma), propranolol chloride (Scanmeda), and methergoline (methyl-8 β carbobenzyloxy-aminomethyl-10 α-ergoline, Farmitalia). 5-HT and propranolol were dissolved in 0.9% w/v NaCl solution (saline) and methergoline in ascorbic acid at pH 4 immediately before the experiments. All concentrations are given as the salt and expressed as the final molar concentration in the bath.

Results

Administration of 5-HT to the organ bath caused a contraction which fairly rapidly (within 60–90 s) reached a plateau and which was maintained long enough to allow cumulative application of increasing doses. When successive cumulative dose-response curves were obtained at 30 min intervals, no overt changes were noted in ED_{50} (concentration at which half maximum contraction occurred) or E_{max} (maximum contractile effect). Increasing doses of methergoline were applied to test the ability of this antagonist to inhibit the contractile response to 5-HT. Methergoline alone did not contract the artery in the concentrations tested (3×10^{-9} to 3×10^{-6} M). The dose-response curves of 5-HT were shifted in parallel to the right by concentrations of methergoline of 3×10^{-9} M and above (Figure 1a). The response was almost fully reversed by repeated washing for 60 min (cf. Beretta *et al.*, 1965). Since the 5-HT-induced contraction was inhibited by methergoline this interaction was further analyzed and the data plotted according to Arunlakshana & Schild (1959) (Figure 1b). Regression analysis showed a high degree of linearity (0.98). The slope was slightly (−0.85) but significantly (Student's *t*-test, $P < 0.01$) different from unity. The value at the intercept of the straight line with the abscissa was 8.80 (Figure 1b).

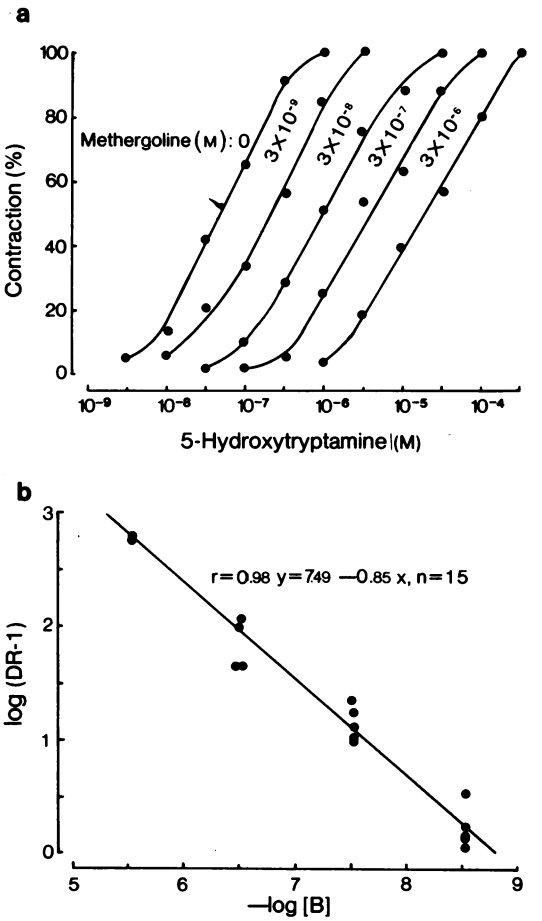

Figure 1 (a) Representative experiment on cat's middle cerebral artery showing contractile effects of 5-hydroxytryptamine (5-HT) before and after addition of increasing concentrations of methergoline. Since the antagonism only involved a parallel shift to the right in the log dose-response curves (without reduction in E_{max}) the value for E_{max} in each curve has been set as 100%. The actual maximum contraction induced by 5-HT was 287 ± 26 dynes (mean ± s.e. mean; number of tests = 5). The bath contained propranolol 3×10^{-7} M in order to inhibit any dilator response. (b) Arunlakshana-Schild plot based on 5 experiments with 5-HT and methergoline (one of the experiments illustrated in a). The regression coefficient and the equation for the line are given. The value at the intercept of the straight line with the abscissa is 8.80. DR = dose-ratio, [B] = concentration of antagonist.

Discussion

One important approach in the pharmacological characterization of receptors is the demonstration of competitive antagonism. This has recently been shown for the extracranial vasculature of cat and man, where methysergide, which is known to be an antagonist of smooth muscle 5-HT receptors (see

Gyermek, 1965), competitively inhibits the 5-HT induced vascular contraction (Edvinsson *et al.*, 1978). On the other hand, in intracranial vessels, a non-competitive inhibition by increasing doses of methysergide was obtained, comprising a weak increase in ED_{50} values and a reduction in E_{max} values (Edvinsson & Owman, 1976; Toda, Hayashi, Fu & Nagasaka, 1976; Edvinsson *et al.*, 1978). However, the presence of 5-HT receptors in intracranial vessels, though apparently different in character from those in extracranial vessels, was suggested by the fact that these were even more sensitive to 5-HT than the extracranial arteries. Furthermore, the potency ratio between different 5-HT agonists was identical in the two vascular preparations, as was the concentration of phenoxybenzamine (considerably above the dose for specific α-adrenoceptor antagonistic effect) needed to block completely the 5-HT-induced contraction.

Methergoline, in contrast to previously tested 5-HT antagonists (Hardebo *et al.*, 1978; Edvinsson *et al.*, 1978), was able to produce a parallel shift of the log dose-response curve of 5-HT towards higher concentrations. The Arunlakshana-Schild plot, showing the relationship between the dose-ratio − 1 and the antagonist concentration in logarithmic form, gave a linear relationship with a slope, slightly different from unity. The slope of the regression line (−0.85) indicates that the blockade at the receptor site is not truly competitive and reversible. In the central nervous system methergoline probably acts by blockade of postsynaptic 5-HT receptors (Fuxe *et al.*, 1975). The present finding offers further support for the presence of specific 5-HT receptors in the wall of intracranial vessels. These 5-HT receptors may be more closely related to the 5-HT receptors in the central nervous system than to those in the extracranial vascular bed, where methysergide produces a parallel shift of the dose-response curve of 5-HT (Edvinsson *et al.*, 1978).

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