TRYPTAMINE RECEPTORS IN RAT PULMONARY ARTERY

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Tryptamine and 5-hydroxytryptamine (5-HT) are equipotent in contracting spiral strips of rat isolated pulmonary artery, in doses between 5 and 20 μg. These 5-HT receptors are blocked by low concentrations (c. 10^{-9} M) of both methysergide and morphine. In rat isolated lung perfused *via* the pulmonary circulation, 5-HT caused increases in perfusion pressure which were also antagonized by methysergide and by morphine. These vascular 5-HT receptors cannot therefore be classified as M or D receptors and, furthermore, are different from those 5-HT receptors mediating release of spasmogens from rat isolated lungs.

We have been investigating the effects of 5-hydroxytryptamine (5-HT) and tryptamine on rat isolated lungs perfused *via* the pulmonary circulation. One of these effects is a rise in perfusion pressure which under our conditions of constant flow represents an increase in vascular resistance and could be due to constriction of the pulmonary artery. We, therefore, decided to study the responses of the isolated pulmonary artery to tryptamines in order to define more closely the 5-HT receptors in this tissue and to compare them with those mediating release of spasmogens from the lung. A preliminary report of some of this work has been made to the British Pharmacological Society (Bakhle & Smith, 1974).

Methods

Isolated pulmonary artery. Wistar rats (200-300 g) of either sex were killed by stunning and exsanguination. The thorax was opened, the pulmonary artery removed from the right ventricle to the first bifurcation of the vessel and cut in a spiral. The arterial strip was tied to an auxotonic lever attached to a Harvard smooth muscle transducer under a resting load of 1 gram. It was superfused at a rate of 8 ml/min with Krebs bicarbonate solution at 37° C, gassed with 95% O_2 and 5% CO_2. Contractions of the tissue were recorded on a Watanabe pen recorder. Agonists were injected into the superfusing Krebs solution and dose-response relationships obtained with at least three concentrations of agonists. The tissues were rested for 15 min and the dose-response relationship again measured. Such cycles were carried out three to four times and the tissues used for antagonism studies only if similar responses were seen on consecutive cycles. Antagonists were then infused (0.07 ml/min into the Krebs solution) and after 15 min, responses to agonists measured in the presence of the antagonist. Regressions for the dose-response relationship were calculated by the least squares method and deviations from linearity of these regressions tested by analysis of variance (Batson, 1956).

Perfused lungs. Isolated lungs were perfused with oxygenated Krebs bicarbonate solution at 37° C, *via* a cannula in the pulmonary artery (Bakhle, Reynard & Vane, 1969). Perfusion pressure was monitored from a side arm in the arterial cannula with a Statham transducer and recorded on a Servoscribe pen recorder. Agonist and antagonist studies were carried out as described above.

Results Both 5-HT and tryptamine caused dose-dependent contractions of the isolated pulmonary artery and Fig. 1a shows the average results from 18 experiments. The dose-response relationships for 5-HT and tryptamine show them to be equipotent on this tissue, both amines giving a linear relationship over a range of 5-20 μg. In three experiments, other amines were compared with 5-HT and tryptamine; adrenaline always induced contractions and was approximately 800 times more potent than tryptamine, whereas histamine contracted only one of the three tissues but on this tissue was equipotent with tryptamine.

The effect of morphine (5×10^{-10} M) and methysergide (5×10^{-10} M) on the responses to 5-HT are shown in Fig. 1b and c respectively. Both drugs antagonized the effect of 5-HT. However, whereas the log dose-response relationship in the absence of antagonists did not deviate significantly from linearity ($P < 0.05$), the relationship in the presence of either antagonist appeared to be non-linear and less steep than the control curve. The change of slope in the shifted dose-response relationship implies that methysergide and morphine were not acting as simple competitive antagonists (Arunlakshana & Schild, 1959). Our results do not therefore allow us to calculate a

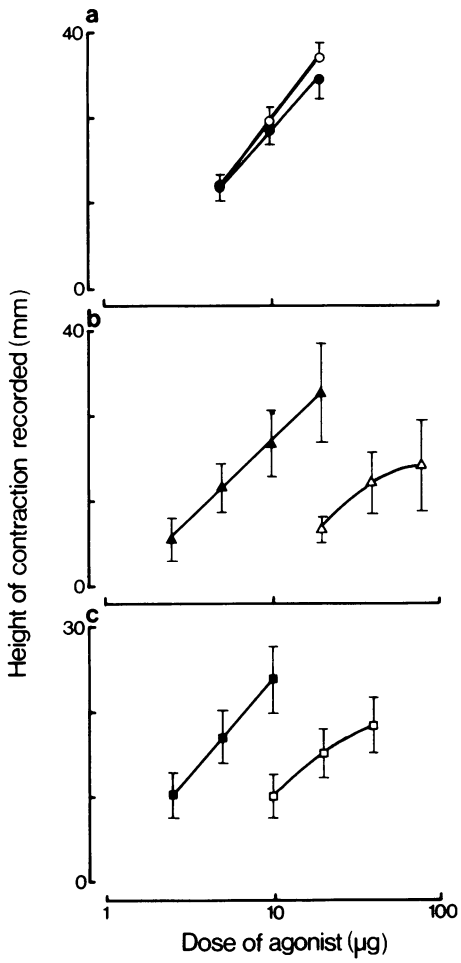

Fig. 1 Dose-response relationships of tryptamines on rat isolated pulmonary artery. The mean (± s.e. mean) of the responses is shown. (a) Comparison of responses to tryptamine (○) and 5-hydroxytryptamine (●). (b) Antagonism by morphine (5×10^{-10} M; six experiments). Responses to 5-HT alone (▲) and in presence of morphine (△). (c) Antagonism by methysergide (5×10^{-10} M; six experiments). Responses to 5-HT alone (■) and in presence of methysergide (□).

pA_2 value for the two antagonists but, as an indication of their relative potency, the dose of 5-HT required to produce a standard contraction (15 mm) was increased by a factor of 5 in the presence of methysergide (5×10^{-10} M) and by a factor of 6.6 in the presence of morphine (5×10^{-10} M). Morphine at this concentration did not antagonize contractions due to adrenaline (2-10 ng) or histamine (5-20 μg).

In rat isolated perfused lungs, injections of 5-HT (5-20 μg) produced dose-related increases in perfusion pressure. Methysergide (5×10^{-10} M) and morphine (5×10^{-10} M) infused through the pulmonary circulation abolished the responses to the lower doses of 5-HT, and reduced those to the higher dose.

Discussion 5-HT causes vasoconstriction in the pulmonary bed of rats *in vivo* (Herget & Paleček, 1972) and in perfused isolated lungs (Greeff & Moog, 1963: Alabaster & Bakhle, 1970), and this response is blocked by methysergide (Hauge, Lunde & Waaler, 1966; Daicoff, Chavez, Anton & Swenson, 1968; Alabaster, 1971). 5-HT also constricts the isolated perfused pulmonary arteries of rabbit and guinea-pig (Starr & West, 1966) and here is antagonized by bromolysergic acid diethylamide (brom-LSD). Our experiments have shown that spiral strips of rat isolated pulmonary artery are contracted by both 5-HT and tryptamine and that these amines are equipotent on this tissue. Generally, 5-HT is more potent than tryptamine and Handschumacher & Vane (1967) have explained this in terms of the faster entry of tryptamine into cells and its subsequent metabolism by monoamine oxidase. The equipotency of these two compounds on the rat pulmonary artery may therefore be due either to an equal rate of entry into cells or to the lack of a suitable monoamine oxidase in this tissue.

Antagonists of 5-HT have been used to classify 5-HT receptors as D-receptors, blocked by dibenzyline and methysergide, or M-receptors, blocked by morphine (Gaddum & Picarelli, 1957; Day & Vane, 1963). However, the 5-HT receptors in the rat pulmonary artery do not fall into either category, being blocked by both methysergide and morphine. Other anomalous 5-HT receptors in vascular tissue occur in the rabbit isolated aorta and these are not inhibited by either morphine or LSD (Wurzel, 1966). The 5-HT receptors in the external carotid bed of the dog and the central ear artery of the rabbit have also been described as of neither M nor D type, but this conclusion is derived from experiments with a newer 5-HT antagonist, mianserin, a derivative of cyproheptadine (Saxena, Houwelingen & Bonta, 1971). We believe our results may be the first description of a vascular 5-HT receptor sensitive to both methysergide and morphine and may represent a characteristic peculiar to the pulmonary vasculature.

In rat isolated perfused lungs, an infusion of 5-HT or tryptamine through the pulmonary artery, in addition to causing a rise in perfusion pressure, also induces the release of a mixture of spasmogens from the lung (Alabaster & Bakhle, 1970). This 5-HT-induced release of spasmogens

was abolished by methysergide (Alabaster, 1971: Bakhle & Smith, 1974) but was not affected by morphine (Bakhle & Smith, unpublished observations). In rat lungs, therefore, there is evidence of two populations of 5-HT receptors with different characteristics; those sensitive to both methysergide and morphine, located in the pulmonary artery involved in the 5-HT-induced increases of pulmonary resistance and those sensitive to methysergide but resistant to morphine, of unknown location, involved in the 5-HT-induced release of spasmogen from rat lung.

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