The nature of the tryptamine receptor mediating spasmogen release from rat isolated lungs

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5-Hydroxytryptamine (5-HT) and tryptamine infused through rat isolated lungs induce the release of spasmogens, including a component with prostaglandin-like activity, from the lung and this release is antagonized by methysergide (Alabaster & Bakhle, 1970; Alabaster, 1971). We have investigated further the tryptamine receptor which mediates spasmogen release by comparing it with those tryptamine receptors in the rat stomach strip (RSS) and in spiral strips of the rat pulmonary artery (RPA). We chose the RSS as an example of classical D tryptamine receptors (Gaddum & Picarelli, 1957; Vane, 1957) and the RPA as an example of pulmonary tryptamine receptors.

5-HT was about 200 times more potent than tryptamine in causing contractions of the RSS but was equipotent to tryptamine on the RPA and in inducing release of prostaglandin-like material from the lung. However, the effects of the tryptamines on the RSS and RPA were related to their concentration whereas release of prostaglandin-like material did not increase with increased concentrations of amine once a threshold (approx. 2 μg/ml) had been exceeded.

We then studied the effects of two 5-HT antagonists, methysergide and BC-105† ('Pizotyline' (Sandoz); Berde, 1972) on these three systems. On the RSS, methysergide was a more potent antagonist than BC-105, concentrations of 3.5×10^{-10} M and 1.3×10^{-8} M respectively giving a dose ratio of 2 for 5-HT. On the RPA, however, BC-105 is more potent than methysergide. Spasmogen release induced by 2 μg/ml of either tryptamine was abolished by methysergide (5×10^{-9} M) or by BC-105 (10^{-6} M). At this concentration of BC-105, the threshold for release was increased to 5 μg/ml, whereas in the presence of methysergide (5×10^{-9} M), the threshold for release was raised to over 10 μg/ml.

In summary: the 'all-or-none' character of spasmogen release is in contrast to the dose related responses of the RSS and RPA; the relative agonist potency of 5-HT and tryptamine for release approximates more closely to the relative potency on the RPA than to that on the RSS; the relative antagonist potency of methysergide and BC-105 against release is closer to that on the RSS than that on the RPA. We conclude that the tryptamine receptor which mediates spasmogen release in the rat lung is different from those in the RSS and the RPA.

† *(4-(9,10-dihydro-4H-benzo (4,5)cyclohepta (1,2-b)thien-4-ylidene)-1-methylpiperidene).*

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The binding affinity of an alkylating muscarinic agonist: acetylcholine mustard

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Acetylcholine mustard (AChM) is a potent agonist at the muscarinic receptor (Hirst & Jackson, 1972; Hudgins & Stubbins, 1972) and at higher concentrations binds irreversibly, presumably in a similar way to benzilylcholine mustard (BCM). However, in contrast to BCM, where the reversible drug-receptor complex (AR) once formed is transformed into the irreversible complex (AR^1)

$$A + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AR \xrightarrow{k_2} AR'$$

faster than it dissociates, i.e. $k_2 \gg k_{-1}$ (Gill & Rang, 1966), with AChM evidence suggests $k_{-1} \gg k_2$. Thus for AChM the free receptor fraction should decline exponentially with time, the apparent rate constant, k_{app}, being related to the

concentration of AChM, [A], by the expression (Kitz & Wilson, 1962):

$$1/k_{app} = 1/k_2 + \frac{1}{k_2 K_a} \cdot \frac{1}{[A]}$$

where $K_a = k_1/k_{-1}$ the apparent binding affinity of AChM.

Experiments were carried out using strips of the longitudinal muscle from guinea-pig small intestine. The equipotent molar ratio for AChM compared to ACh was 4. To determine the free receptor fraction before and after treatment with AChM we have measured the extent of the binding of [^{3}H]-propylbenzilycholine mustard ([^{3}H]-PrBCM), an irreversible muscarinic antagonist (Burgen, Hiley & Young, 1973). The strips were exposed to various concentrations (10^{-6} M to 5×10^{-5} M) of the aziridinium ion form of AChM in Krebs-Henseleit solution at 30°C for a given time in the presence of eserine (10^{-6} M), washed for 30-40 min and then exposed to 2.4 nM [^{3}H]-PrBCM for 10 minutes. The extent of inhibition of [^{3}H]-PrBCM uptake was not significantly altered on varying the washing period after AChM between 1 and 120 minutes. Approximately 30% of the uptake of [^{3}H]-PrBCM could not be blocked by AChM. This has been taken to represent the non-specific portion of the [^{3}H]-PrBCM binding. A plot of $1/k_{app}$ against $1/[A]$ yielded a reasonable straight line, from which values of 1×10^5 M^{-1} for K_a and 2×10^5 s^{-1} for k_2 were derived. The value for K_a is comparable with the value of 1.1×10^5 M^{-1} for ACh deduced from its action as a competitive inhibitor of [^{3}H]-PrBCM uptake (Burgen, Hiley & Young, 1973).

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Factors contributing to the binding of [^{3}H]-5-hydroxytryptamine to butanol extracts of rat brain

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Fiszer & De Robertis (1969) reported the binding of 5-hydroxytryptamine (5-HT) to butanol extracts of mammalian brain and in view of the potential importance of this finding to the isolation and characterization of drug receptors we have investigated some of the parameters which control this binding.

Water washed rat mid-brain was suspended in 50% w/v sucrose and extracted into butanol at room temperature. After washing the extract with deionized water the butanol extracts were dehydrated *in vacuo* at 35°C. Aliquots (6 ml) were incubated with [^{3}H]-5-HT (5×10^{-7} M) at room temperature for 10 min and loaded onto a column of LH_{20} Sephadex equilibrated in chloroform. Radioactivity was eluted with a discontinuous gradient of chloroform: methanol of increasing polarity (Fiszer & De Robertis, 1969). Fractions (5 ml) were collected and sampled for radioactivity, protein and phosphorus. A typical elution pattern for [^{3}H]-5-HT is shown in Figure 1. The

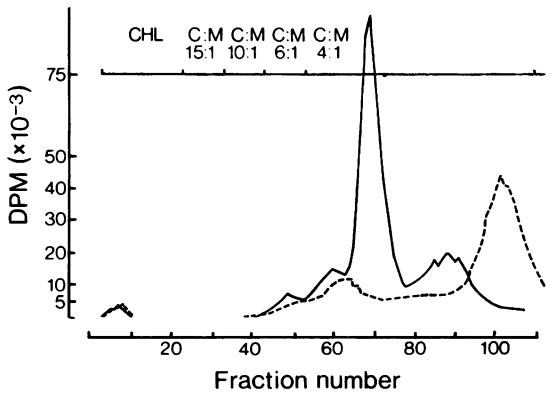

Fig. 1 Hydrophobic chromatography on LH_{20} Sephadex of a butanol extract of rat brain labelled with [^{3}H]-5-HT. Dotted line, butanol; solid line, butanol extract of brain. [^{3}H]-5-HT concentration 5×10^{-7} M. Fractions 5 ml. Discontinuous gradient of chloroform : methanol (C : M).