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Short communication

Potent agonist activity of DOB at 5-HT_2 receptors in guinea pig trachea

William A. Heller and Jay M. Baraban *

Departments of Neuroscience and Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, U.S.A.

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DOB, 4-bromo-2,5-dimethyloxyamphetamine, a potent hallucinogen, displays high affinity for the 5-HT_2 receptor in binding assays. Since 5-HT contractile responses in guinea pig trachea are mediated by 5-HT_2 receptors, we assessed the activity of DOB and related analogs in this preparation. DOB acts as a partial 5-HT agonist with an $EC_{50} = 25$ nM and is blocked by the potent selective 5-HT_2 receptor antagonists ketanserin, pirenperone and LY 53857, as is 5-HT. These findings indicate that DOB may be a useful agent in characterizing 5-HT_2 receptor responses.

Trachea; 5-HT_2 receptors; Hallucinogens; DOB

1. Introduction

Although the 5-HT_2 receptor has been extensively characterized in radioligand binding studies (Peroutka and Snyder, 1979), relatively little is known about the physiological activity of many putative agonist compounds with affinity for this 5-HT receptor subtype. Several lines of evidence point to the 5-HT_2 receptor as the site of action of hallucinogens, which appear to be agonists at this site (Glennon et al., 1985). Thus, it is particularly important to evaluate the activity of hallucinogenic drugs in physiological assays of 5-HT_2 receptor activity. A recent study employing selective 5-HT_2 receptor antagonists indicates that the contractile response to 5-HT in the guinea pig trachea is mediated by 5-HT_2 receptors (Cohen et al., 1985). Accordingly, we have assessed the physiological activity of a series of hallucinogens in this preparation.

* To whom all correspondence should be addressed.

2. Materials and methods

Tracheae from male guinea pigs (400-600 g) were dissected free of surrounding tissue and cut into rings. Three rings were tried loosely into a chain and suspended in a 25 ml organ bath at 37 °C and continuously bubbled with 95% O_2 and 5% CO_2. One end of the chain was fixed and the other tied to to a Grass force displacement transducer at 2.5 g resting tension.

Aspirin (500 μg/l) which enhances 5-HT contractions (Orehek et al., 1975), and prazosin (Pfizer) (2 μM), which blocks stimulation of α_1-receptors, were added to buffer containing (mM): $MgSO_4$ 1.2, $CaCl_2$ 0.8, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 10, NaCl 118 and KCl 4.7. Each preparation was allowed to equilibrate at resting tension for at least 30 min before addition of any drugs. The following drugs were tested: 8-hydroxydipropylaminotetralin (8-OH-DPAT), 1-(2-methoxyphenyl) piperazine (2-MPP), 1-(3-chlorophenyl)-piperazine (mCPP), 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) and ketanserin (RBI, Wayland, MA), bromo-LSD (BOL), 4-ethyl-2,5-dimethoxyamphetamine (DOET), 4-methyl-2,5-di-

methoxyamphetamine (DOM) (S.H. Snyder); DOB and d-LSD (NIDA), pirenperone (Janssen) and LY 53857 (Lilly).

3. Results

3.1. Contractile action of phenethylamine hallucinogens DOB, DOET and DOM

The potent phenethylamine hallucinogen, DOB, produces a slow sustained contraction with an EC_{50} of 25 nM, similar to its affinity at 5-HT_2 receptors in receptor binding studies (Glennon et al., 1985). DOET and DOM hallucinogenic analogues of DOB, also displayed contractile activity (fig. 1). These agents appear to act as partial 5-HT agonists since they produce about half the maximal contraction elicited by 5-HT. Furthermore, application of 5-HT during a maximal DOB contraction produces little further increase in tension.

DOB displayes high potency and selective affinity for 5-HT_2 receptors in binding studies (Glennon et al., 1985). However, to insure that the contractile response elicited by DOB is mediated by 5-HT_2 receptors, we examined the effects of several 5-HT_2 receptor antagonists (Leysen et al., 1982; Cohen et al., 1985). All three agents tested, pirenperone, ketanserin and Ly 53857 blocked contractions produced by both 5-HT and DOB with high potency. These antagonists at a concentration of 10 nM, completely blocked both the 5-HT and DOB responses but did not affect the contractile response to carbachol (0.8 μM) which is mediated by muscarinic receptors, demonstrating a selective action of these drugs for 5-HT.

The 5-HT_1 agonists 8-OH DPAT, 2-MPP (0.5-8.0 μM) and mCPP did not cause contractions arguing against the involvement of either 5-HT_{1A} or 5-HT_{1B} receptor subtypes in this response (Sills et al., 1984; Middlemiss and Fozard, 1983). At high concentrations (> 8 μM), 8-OH DPAT inhibited 5-HT responses consistent with its weak activity at 5-HT_2 receptors in binding studies (Middlemiss and Fozard, 1983).

3.2. Indoleamine hallucinogens block 5-HT response

In contrast to DOB, d-LSD and 5-MeODMT, hallucinogens of the indoleamine class, did not

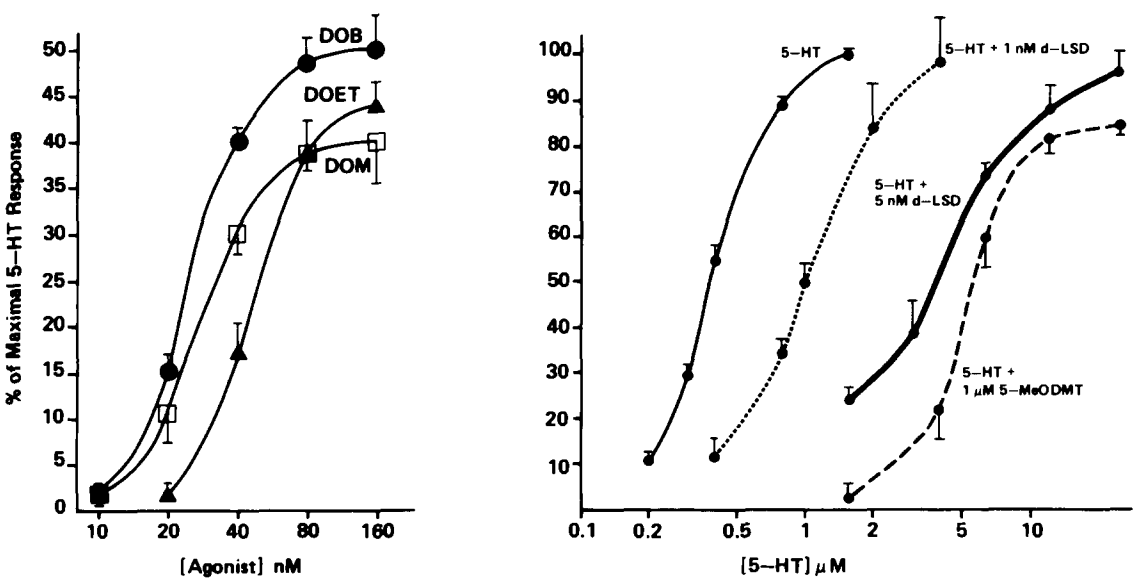

Fig. 1. Contractile effects of hallucinogens in guinea pig trachea. Left panel: phenethylamine hallucinogens produce contraction. Maximal response produced by these agents is between 40-50% of the 2.1 g maximal contraction induced by 5-HT. Right panel: d-LSD and 5-MeODMT block 5-HT-induced contraction and shift the 5-HT contraction response curve to the right. Each point represents the mean ± S.D. for at least three preparations.

cause contractions. Instead, they selectively antagonized 5-HT-induced contractions (fig. 1), without affecting carbachol responses. At 1 nM, 2-bromo LSD, which is not hallucinogenic, also blocks 5-HT's action in a selective fashion.

4. Discussion

In agreement with 5-HT_2 receptor binding studies, DOB and related phenethylamine hallucinogens exhibit potent agonist activity at 5-HT_2 receptors. Since these agents appear to possess higher affinity for the 5-HT_2 receptor than 5-HT_1 receptor subtypes (Glennon et al., 1985) they represent useful tools for characterizing physiological responses mediated by 5-HT_2 receptors. Of note, however, these drugs exert only partial agonist activity not readily detectable in receptor binding studies.

LSD and 5-MeODMT displayed antagonist activity at 5-HT_2 receptors in this preparation. The concentrations of these drugs producing blockade of 5-HT_2 receptors corresponds to their reported affinities for 5-HT_2 receptor binding sites (Glennon et al., 1985). Their effect in the trachea does not appear to be due to agonist activity at 5-HT_1 receptors since several other 5-HT_1 agonists did not block 5-HT responses. In contrast to the discrepancy between the effects of phenethylamine and indoleamine hallucinogens observed in the guinea pig trachea, both classes exhibit agonist activity in human and sheep umbilical artery (Dyer and Gant, 1973), and in brain (Rasmussen et al., 1986). A similar shift from agonist to very weak partial agonist or antagonist activity has been well characterized for several muscarinic agonists at phosphoinositide turnover in different brain regions (Fisher, 1986) Direct comparisons of the physiological activities of phenethylamine and indoleamine hallucinogens at 5-HT_2 receptors in other tissues may be helpful in characterizing their differential effects on 5-HT_2 receptor-mediated responses.

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