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Short communication

Agonism at 5-HT_{2B} receptors is not a class effect of the ergolines

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Abstract

Restrictive cardiac valvulopathies observed in Parkinson patients treated with the ergoline dopamine agonist pergolide have recently been associated with the agonist efficacy of the drug at 5-hydroxytryptamine$_{2B}$ (5-HT_{2B}) receptors. To evaluate whether agonism at 5-HT_{2B} receptors is a phenomenon of the class of the ergolines, we studied 5-HT_{2B} receptor-mediated relaxation in porcine pulmonary arteries to five ergolines which are used as antiparkinsonian drugs. Pergolide and cabergoline were potent full agonists in this tissue (pEC_{50} 8.42 and 8.72). Bromocriptine acted as a partial agonist (pEC_{50} 6.86). Lisuride and terguride, however, failed to relax the arteries but potently antagonized 5-HT-induced relaxation (pK_B 10.32 and 8.49). Thus, agonism at 5-HT_{2B} receptors seems not to be a class effect of the ergolines.

Keywords: Pergolide; Cabergoline; Bromocriptine; Lisuride; Terguride; Valvular heart disease; 5-HT_{2B} receptor agonism

1. Introduction

Pergolide, an ergoline dopamine receptor agonist used to treat Parkinson's disease, has been associated in rare cases with retroperitoneal, pleural and pericardial fibrosis, and, quite recently, also with a high frequency of increased pulmonary arterial pressure and drug-induced restrictive valvular heart disease as demonstrated by cardiac echography (Van Camp et al., 2004; Baseman et al., 2004). Very recently, a few cases have also been reported which have described the occurrence of multivalvular insufficiency of the heart in patients treated with two other ergolines, bromocriptine and cabergoline (Serratrice et al., 2002; Horvath et al., 2004).

Histopathological features are characterized by fibromyoblast proliferation within an avascular myxoid matrix without disruption of the valve structure itself and subsequent thickening of the leaflets and the cords (Redfield et al., 1992) which increase tissue rigidity and can result in either valvular stenosis or insufficiency (Van Camp et al., 2004). Although the pathomechanism through which these ergolines induce valvular remodelling has not yet been fully elucidated, a 5-hydroxytryptamine$_{2B}$ (5-HT_{2B}) receptor mechanism has been proposed to be responsible for this effect (Fitzgerald et al., 2000; Setola et al., 2003). Evidence for an involvement of 5-HT_{2B} receptors is as follows: (i) 5-HT_{2B} receptors are expressed in human heart valves, (ii) 5-HT_{2B} receptor activation has a mitogenic effect on fibromyoblasts, (iii) overexpression of 5-HT_{2B} receptors leads to cardiac hypertrophy in mice, (iv) all ergolines known to cause fibrotic valvulopathy (or at least their metabolites) are agonists at 5-HT_{2B} receptors, (v) large pulses of 5-HT released by carcinoid tumours are also known to cause similar fibrotic heart valvulopathies which can be linked to activation of 5-HT_{2B} receptors, and (vi) chemically unrelated compounds such as the appetite suppressants fenfluramine and aminorex and the designer drug 3,4-methylenedioxymethamphetamine (MDMA) (or at least their metabolites), which have been associated with cardiac valvulopathy and severe pulmonary hypertension, are also 5-HT_{2B} receptor agonists (Fitzgerald et al., 2000; Rothman et al., 2000; Simula et al., 2002; Nebigil et al., 2003; Setola et al., 2003). The majority of the in vitro studies mentioned have been performed using cells express-

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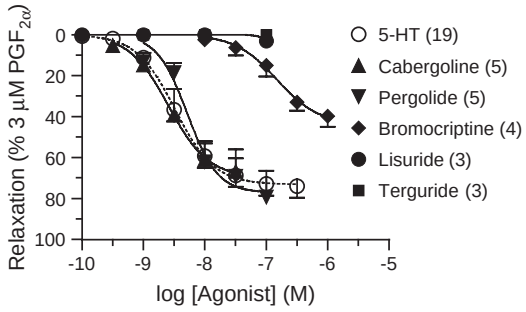

Fig. 1. Relaxant responses to 5-HT and a series of ergolines in porcine pulmonary arteries. E/[A] curves (5-HT: cumulative; ergolines: non-cumulative) are shown. Points are mean values (percentage of the contraction to 3 µM $PGF_{2\alpha}$) ± S.E.M. (vertical bars) for the number of animals indicated in parentheses.

ing recombinant 5-HT_{2B} receptors. It should be emphasized, however, that the functional consequences of receptor–G-protein activation may vary from cell to cell depending on both the level of receptor expression and the component of effector molecules within a given cell. Therefore, studies on cells transfected with recombinant receptors not always mirror the physiological situation (Sanders-Bush and Canton, 1995).

For this reason, we have investigated whether pergolide and other ergolines used to treat Parkinson's disease, viz. cabergoline, bromocriptine, lisuride, and terguride possess agonist efficacy at intact native 5-HT_{2B} receptors. The following experiments were performed using porcine pulmonary arteries, a tissue endowed with endothelial 5-HT_{2B} receptors which have been shown to be quite similar to the human 5-HT_{2B} receptors (Glusa and Pertz, 2000).

2. Materials and methods

2.1. Experimental protocol

Experiments on prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 3 µM)-precontracted isolated rings of porcine pulmonary arteries with intact endothelium were performed as previously described (Glusa and Pertz, 2000). In agonist experiments with ergolines a non-cumulative concentration–response curve (E/[A] curve) to the respective ergoline was established by adding only one concentration of agonist to each tissue. This method was employed, since it is known that many tissues respond only to the first concentration of these drugs and the cumulative concentration–response technique cannot be applied (Müller-Schweinitzer, 1990). Experiments were performed in the absence and presence of the 5-$HT_{2B/2C}$ receptor antagonist SB 206553 (1 µM) added 30 min before the construction of the agonist E/[A] curve. In experiments where the antagonist properties of the ergolines were studied, a cumulative E/[A] curve to 5-HT was constructed on each tissue 60 min after the addition of the respective ergoline. Relaxant responses were expressed as a percentage of the $PGF_{2\alpha}$-induced contraction in each tissue.

2.2. Data analysis and presentation

Data are presented as mean ± S.E.M. for n animals. E/[A] curves were fitted to the Hill equation using an iterative, least-squares method (GraphPad Prism 4.0, GraphPad Software, San Diego, CA, USA) to provide estimates of the maximum response E_{max} (relaxant response relative to the contraction with 3 µM $PGF_{2\alpha}$) and the half-maximum effective concentration pEC_{50} (the negative logarithm of the molar concentration of the agonist producing 50% of the maximum response). To estimate individual differences both in E_{max} and pEC_{50}, control experiments with 5-HT were routinely performed.

In antagonist studies, apparent pK_B values were calculated as described previously (Glusa and Pertz, 2000), according to the equation: $pK_B = -\log[B] + \log(r-1)$, where [B] is the molar concentration of antagonist and r the ratio of agonist EC_{50} measured in the presence and absence of antagonist. Results were compared using Student's t-test. P values < 0.05 were considered to be significant.

2.3. Drugs

The following drugs were obtained as gifts: dinoprost tromethamine ($PGF_{2\alpha}$; Upjohn, Kalamasoo, MI, USA);

Table 1
Vasorelaxant effects of 5-HT, pergolide, cabergoline and bromocriptine, and inhibition of the 5-HT-induced relaxation by bromocriptine, lisuride and terguride in porcine pulmonary arteries

Compound	Agonist profile			Antagonist profile		
	n	pEC_{50}	E_{max} (%)[a]	n	Apparent pK_B	E_{max} (%)[a]
5-HT	19	8.59 ± 0.08	82 ± 3	–	–	–
Pergolide	5	8.42 ± 0.11	74 ± 8	–	–	–
Cabergoline	5	8.72 ± 0.14	69 ± 7	–	–	–
Bromocriptine	4	6.86 ± 0.12	43 ± 6[b]	4	9.39 ± 0.21[c]	50 ± 9[c]
Lisuride	3	–	0	4	10.32 ± 0.10[d]	74 ± 4[d]
Terguride	3	–	0	5	8.49 ± 0.11[c]	75 ± 9[c]

[a] Expressed as percentage of the $PGF_{2\alpha}$ (3 µM)-induced contraction.
[b] Significant difference vs. 5-HT (P<0.05).
[c] Antagonist concentration: 10 nM.
[d] Antagonist concentration: 1 nM.

ketanserin tartrate (Janssen, Beerse, Belgium), bromocriptine, cabergoline, lisuride, pergolide, and terguride, (all from Schering AG, Berlin, Germany). The following compounds were purchased: 5-hydroxytryptamine creatinine sulfate (5-HT; Acros Organics, Geel, Belgium), bradykinin triacetate (Sigma, St. Louis, MO, USA), SB 206553 (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole) hydrochloride (Tocris, Ellisville, MO, USA). The vehicle for stock solutions of drugs (10 mM) was distilled water, ethanol (50% V/V) or equimolar HCl. Stock solutions were diluted in distilled water.

3. Results

In porcine pulmonary arteries precontracted with $PGF_{2\alpha}$ (3 μM), 5-HT produced a concentration-dependent relaxation ($pEC_{50}=8.59\pm0.08$; $E_{max}=82\pm3\%$; $n=19$). Full relaxant responses similar to 5-HT were observed following non-cumulative addition of pergolide and cabergoline, respectively. The potency of both ergolines to elicit relaxant responses in porcine pulmonary arteries was comparable to that of 5-HT itself. Bromocriptine behaved as a partial agonist (Fig. 1; Table 1). No agonist efficacy, however, was observed for lisuride and terguride up to 0.1 μM ($n=3$ each; Fig. 1).

To investigate, whether relaxation induced by pergolide, cabergoline and bromocriptine was mediated by $5\text{-}HT_{2B}$ receptors, we performed experiments in the presence of the $5\text{-}HT_{2B/2C}$ receptor antagonist SB 206553. The E/[A] curves to pergolide, cabergoline, and bromocriptine were significantly shifted to the right in the presence of SB 206553 (1 μM) (Fig. 2). pK_B estimates for SB 206553 against pergolide, cabergoline, and bromocriptine (7.30 ± 0.19, 6.69 ± 0.16, and 6.79 ± 0.26, $n=3$, respectively) were in the same concentration range as those against 5-HT (7.23 ± 0.05, $n=14$) and argue for an involvement of the $5\text{-}HT_{2B}$ receptor subtype in the vasorelaxant response to the agonists (Glusa and Pertz, 2000).

In another set of experiments the antagonist effects of lisuride and terguride have been studied. Both ergolines tested produced a rightward shift of the E/[A] curve to 5-HT with a moderate but significant depression of the maximal response compared to the respective 5-HT control curves in the absence of antagonist. In these experiments lisuride was the most potent antagonist. Interestingly, bromocriptine also potently antagonized the 5-HT-induced relaxation when tested at a low concentration (10 nM) (Table 1).

4. Discussion

$5\text{-}HT_{2B}$ receptor agonist activity of pergolide and cabergoline has been implicated in fibrotic valvular heart disease. The present study provides evidence that several antiparkinsonian drugs which all belong to the class of ergolines show different properties in acting as full agonists, partial agonists or silent antagonists at native $5\text{-}HT_{2B}$ receptors of pig.

The ability of pergolide to act as a potent full agonist at native porcine $5\text{-}HT_{2B}$ receptors shown by us is in line with observations at human recombinant $5\text{-}HT_{2B}$ receptors (Newman-Tancredi et al., 2002). Agonist potency and relative efficacy are in the same order of magnitude. The same is true for cabergoline which also behaved as a potent agonist both at native (our data) and recombinant $5\text{-}HT_{2B}$ receptors (Newman-Tancredi et al., 2002). A notable difference, however, has been observed with bromocriptine. This drug acted as a partial agonist at native $5\text{-}HT_{2B}$ receptors of pig pulmonary arteries but blocked human recombinant $5\text{-}HT_{2B}$ receptors without exhibiting apparent intrinsic activity (Newman-Tancredi et al., 2002). Thus, functional responses across tissues need to be interpreted with caution and results from recombinant receptors need to be complemented by studies involving functional results from native receptors. Nevertheless, the observation that bromocriptine acted as a partial agonist in the present functional study using native $5\text{-}HT_{2B}$ receptors could explain why bromocriptine has also been described as a cause of valvular heart disease (Serratrice et al., 2002; Horvath et al., 2004).

In contrast to the ergot derivatives pergolide, cabergoline and bromocriptine, the 8α-aminoergolines lisuride and terguride lacked agonist activity at $5\text{-}HT_{2B}$ receptors in

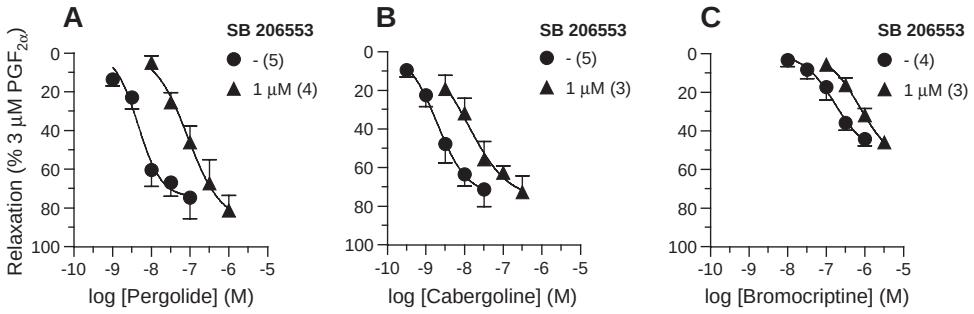

Fig. 2. Relaxant responses to pergolide, cabergoline and bromocriptine in the absence and presence of antagonist in porcine pulmonary arteries. Non-cumulative E/[A] curves are shown. Points are mean values (percentage of the contraction to 3 μM $PGF_{2\alpha}$) ± S.E.M. (vertical bars) for the number of animals indicated in parentheses.

porcine pulmonary arteries even at high concentrations. Both drugs behaved as potent 5-HT_{2B} receptor antagonists. Our data are consistent with those previously published using human recombinant 5-HT_{2B} receptors (Jerman et al., 2001; Newman-Tancredi et al., 2002). It should be noted that lisuride was initially chosen for drug development due to its potent antagonist effect against 5-HT-induced contractions of the isolated rat stomach (Podvalova and Dlabač, 1970). In this tissue contractile responses to 5-HT now are known to be mediated by 5-HT_{2B} receptors (Baxter et al., 1994). Lisuride has been shown to prevent migraine attacks at very low doses (Herrmann et al., 1977) and, in anecdotal reports, also to antagonize carcinoid-induced symptomatology (Podvalova and Dlabač, 1970); both conditions nowadays are believed to be associated with 5-HT_{2B} receptor overactivation. In agreement with these observations, no cardiac valvulopathy has ever been reported for lisuride (Ch. Hofmann and R. Dorow, Schering AG, personal communication).

In conclusion, the present study shows that ergot derivatives such as pergolide, cabergoline and bromocriptine are potent agonists at native 5-HT_{2B} receptors in porcine pulmonary arteries. Agonism at 5-HT_{2B} receptors can be assumed to be a cause or at least a prerequisite for inducing cardiac valvulopathy in patients. In contrast, 8α-aminoergolines such as lisuride and terguride lacked agonism but behaved as potent silent 5-HT_{2B} receptor antagonists. Therefore, we presume that cardiac valvulopathy via 5-HT_{2B} receptor activation is not related to the general chemical class of the ergolines. The individual pharmacological profile of each compound (and if applicable of its metabolites) needs to be considered for further clinical and regulatory evaluation.

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