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Pergolide, Terguride and N,N'-spacer-linked Oligomers of Both Interact with 5-HT_{2A} Receptors of Rat Tail Artery

V. KŘEN, E. EICH[1], H. H. PERTZ[1]

Institute of Microbiology, Laboratory of Biotransformation, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [1]Institut für Pharmazie, Freie Universität Berlin, Germany

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Summary

Pergolide, terguride and N,N'-spacer-linked oligomers of both have been tested for their ability to interact with 5-hydroxytryptamine($HT)_{2A}$ receptors of rat tail artery. Pergolide was a potent partial agonist (pEC_{50} 7.5, E_{max} 55 %) and antagonized 5-HT-induced contractions (pK_P 7.2). Pergolide dimer **3** with a *p*-xylene spacer between the indole nitrogens (N-1) displayed somewhat lower agonist potency than pergolide (pEC_{50} 7.0, E_{max} 55 %, pK_P 6.6). The contractile responses to pergolide and dimer **3** were antagonized by the 5-HT_{2A} receptor antagonist ketanserin (pA_2 9.4, 9.1). In contrast to pergolide dimer **3**, pergolide dimers **5** and **9** with an alkyl and an aralkyl spacer between the piperidine nitrogens (N-6) lacked agonism and displayed low affinity at 5-HT_{2A} receptors ($pA_2 < 5.5$). Terguride behaved as an insurmountable antagonist of 5-HT (pA_2 8.4). Oligomers of terguride showed 5 to 50-fold lower affinity. It is concluded that pergolide and terguride show a high affinity for 5-HT_{2A} receptors, but dimerization (oligomerization) of both drugs fails to increase affinity.

Key words

Pergolide • Terguride • Oligomers of pergolide and terguride • 5-HT_{2A} receptors • Contraction • Rat tail artery

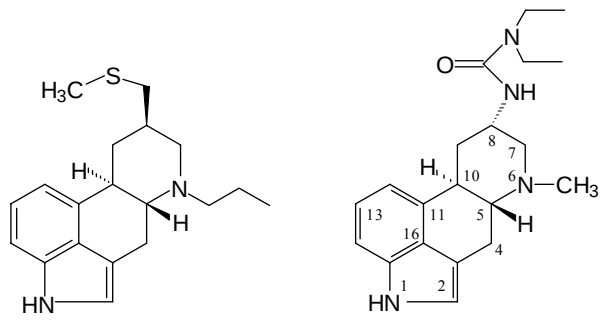
Introduction

Due to their agonist activity at dopamine receptors pergolide (compound **1**) and terguride (compound **2**) (Fig. 1) are currently available for the treatment of Parkinson's disease (Rinne 1987). While pergolide is a potent agonist of the D_1 and D_2 class of dopamine receptors (Fuller and Clemens 1991), terguride (dihydrolisuride) shows partial agonist activity at the D_2 class of dopamine receptors (Stephens et al. 1990). Just as the well established drug bromocriptine, pergolide and terguride are ergoline derivatives of which the spectrum of adverse effects is similar due to the powerful interaction with nearly all subtypes of 5-hydroxytryptamine (5-HT) receptors and α-adrenoceptors (for review see Pertz and Eich 1999).

The aim of the present study was to characterize the interaction of pergolide and terguride and their dimers with 5-HT_{2A} receptors in the isolated rat tail artery. We used the so-called bivalent ligand approach that represents a strategy for medicinal chemists to design potent and selective receptor ligands by combining two or more pharmacophores in a single molecule (Portoghese 1989, 1992, Halazy et al. 1996) (Figs 2 and 3). Previous studies on 5-$HT_{1B/1D}$ receptors have demonstrated that dimerization of 5-HT receptor ligands resulted in higher affinities for this subtype (Perez et al. 1998). We now report the preparation of oligomers of pergolide and

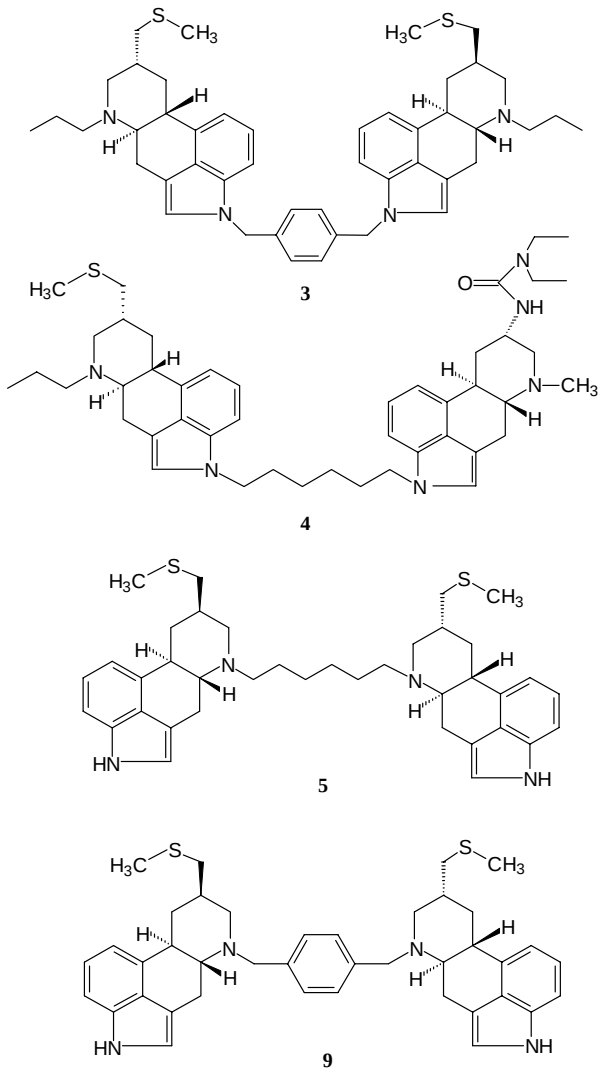
Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

terguride, respectively, in order to find out whether these compounds show activity at vascular 5-HT_{2A} receptors of the rat.



1 (pergolide) **2** (terguride)

Fig. 1. Chemical structures of pergolide and terguride



3 **4** **5** **9**

Fig. 2. Chemical structures of ergot alkaloid dimers

Methods

Materials

Chemicals used for synthesis were reagent-grade mostly from Sigma-Aldrich; solvents were dried and distilled prior to use. Drugs were obtained from the following sources: cocaine-HCl (Merck, Darmstadt, Germany), 5-hydroxytryptamine creatinine sulphate (Janssen, Beerse, Belgium), ketanserin tartrate (Janssen), prazosin-HCl (RBI, Natick, MA, USA).

Chemistry

NMR spectra were measured on a Varian INOVA-400 spectrometer (399.90 and 100.56 MHz for ^{1}H and ^{13}C, respectively) in $CDCl_3$ or d_6-DMSO at 30 ºC. Chemical shifts were referenced to the residual solvent signal (δ_H 7.265, δ_C 77.0; δ_H 2.50, δ_C 39.6. 2D NMR) experiments (gCOSY, TOCSY, HMQC, HMBC) were run using the default parameters in the available Varian software. The pulse sequence for 1D-TOCSY was obtained from the Varian User Library.

Positive ion MALDI mass spectra were measured on a Bruker BIFLEX reflectron time-of-flight mass spectrometer (Bruker-Franzen, Bremen, Germany) equipped with a multiprobe sample inlet, a griddles delayed extraction ion source and a nitrogen laser (337 nm). A saturated solution of α-cyano-4-hydroxycinnamic acid in aqueous 50 % acetonitrile/0.1 % TFA was used as a MALDI matrix. Spectra were calibrated externally using the monoisotopic $[M+H]^+$ ion of α-cyano-4-hydroxycinnamic acid and a peptide standard (angiotensin II, Aldrich).

*Preparation of compounds **3, 4, 5, 6a-e, 7** and **8***

Compounds **3, 4, 5, 6a-e, 7** and **8** were prepared as described previously (Křen et al. 2001, 2002).

*1,4 -Di[8β'- (methylthiomethyl) ergoline - 6'- yl - methyl] benzene (compound **9**)*

The title compound was prepared as described for compound **5** (Křen et al. 2001) in 67 % yield as a yellowish amorphous powder. MS MALDI $[M + H]^+$ (found 647.5, required 647.32 for $C_{40}H_{47}N_4S_2$). ^{1}H NMR (DMSO) δ: 1.01 (ddd, *J* = 12.4, 12.2, 12.2, 2H), 1.77 (dd, *J* = 11.2, 11.2, 2H), 1.92 (m, 2H), 2.00 (s, 6H), 2.34 (m, 4H), 2.44 (dd, *J* = 13.1, 6.4, 2H), 2.62 (ddd, *J* = 14.9, 10.9, 1.4, 2H), 2.71 (m, 2H), 2.89 (m, 2H), 3.03 (m, 2H), 3.38 (d, J = 13.8, 2H), 3.51 (dd, *J* = 14.9, 4.0, 2H), 4.22 (d, *J* = 13.8, 2H), 6.77 (ddd, *J* = 7.2, 1.2, 0.6, 2H), 6.99 (dd, *J* = 2.1, 1.4, 2H), 7.02 (dd, *J* = 8.2, 7.2, 2H), 7.12

(ddd, *J* = 8.2, 0.8, 0.6, 2H), 7.30 (s, 4H), 10.60 (d, *J* = 2.1, 2H). ^{13}C NMR (DMSO) δ: 15.46 q, 27.15 t, 33.96 t, 34.63 d, 38.20 t, 40.60 d, 56.54 t, 58.43 t, 65.12 d, 108.69 d, 110.32 s, 111.97 d, 118.50 d, 122.08 d, 126.09 s, 128.54 d (2 C), 132.92 s, 133.21 s, 137.89 s.

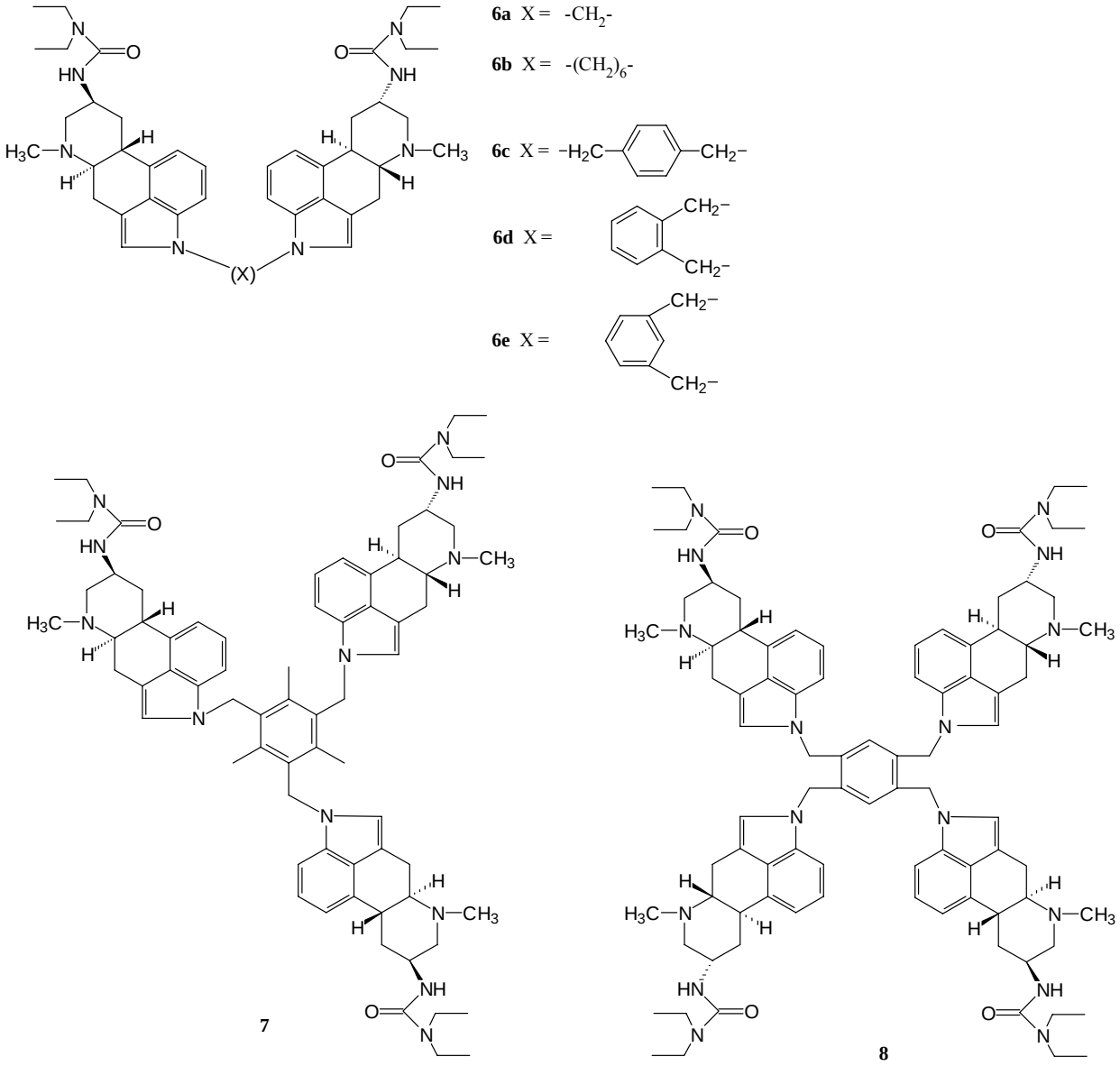


Fig. 3. Chemical structures of terguride dimers and oligomers

*1,6-Di[8β'-(methylthiomethyl)ergoline-1'-yl-]hexane (compound **10**)*

Finely powdered KOH (370 mg, 6.6 mmol) was mixed with DMSO (2 ml) and stirred for 10 min. Depropylpergolide (compound **1a**) (135 mg, 0.5 mmol) was added and the stirring was continued for further 30 min. The mixture turns green indicating deprotonation of N-1. After cooling to 10 ºC, 1,6-dibromohexane (54 mg, 0.22 mmol, dissolved in 2 ml of DMSO) was slowly added in portions. The reaction was completed within 3 h as indicated by TLC: silica gel F_{254} plates (Merck), $CHCl_3$-MeOH-NH_3 94.5 : 5.5 : 0.1 (v/v). The spots were visualised by UV light and charred with 10 % H_2SO_4 in ethanol where N-1-unsubstituted ergolines gave blue spots and substituted compounds (dimers) gave grey spots. The product had quite similar R_f like compound **1a**. The reaction mixture was poured into water (40 ml), the precipitate was filtered off, washed with water till neutral pH and dried affording 130 mg of the crude product. Flash chromatography (silica gel, $CHCl_3$-MeOH-NH_3

97 : 3 : 0.1 (v/v)) afforded pure compound **10** as amorphous yellow foam (52 mg, 33 %). MS MALDI [M + H]$^+$ (found 627.2, required 627.35 for $C_{38}H_{51}N_4S_2$). ^{1}H NMR ($CDCl_3$, 30 ºC) δ: 1.28 (ddd, *J* = 13.3, 12.1, 11.9, 2H), 1.33 (m, 4H), 1.79 (m, 4H), 2.08 (m, 2H), 2.16 (s, 6H), 2.47 (dd, *J* = 11.9, 11.7, 2H), 2.48 (dd, *J* = 13.2, 7.3, 2H), 2.54 (dd, *J* = 13.2, 6.6, 2H), 2.77 - 2.90 (m, 8H), 3.08 (m, 2H), 3.43 (ddd, *J* = 11.9, 4.0, 1.7, 2H), 4.01 (m, 4H), 6.91 (ddd, *J* = 7.0, 1.1, 0.7, 2H), 6.99 (s, 2H), 7.08 (ddd, *J* = 8.2, 0.7, 0.7, 2H), 7.16 (dd, *J* = 8.2, 7.0, 2H). ^{13}C NMR ($CDCl_3$, 30 ºC) δ: 16.17 q, 26.51 t, 29.45 t, 30.33 t, 34.22 t, 36.63 d, 39.00 t, 41.60 d, 46.18 t, 51.98 t, 60.16 d, 107.03 d, 110.51 s, 112.43 d, 121.25 d, 122.45 d, 126.88 s, 133.20 s, 133.88 s.

*Cyclic dimer of pergolide (compound **11**)*

Compound **10** (42 mg, 0.067 mmol) was dissolved in dry DMF (30 ml) at room temperature, three equivalents of finely powdered freshly calcinated K_2CO_3 (36 mg, 0.2 mmol) were added and under stirring *p*-bis(bromomethyl)benzene dissolved in DMF (2 ml) was added and the stirring was continued overnight. Then the temperature was raised to 55-60 ºC to complete the reaction (1 h), the mixture was evaporated in vacuum at the same temperature to the final volume 1-2 ml. The product was precipitated by pouring into water (40 ml), filtered off and washed till a neutral reaction was attained. The dry precipitate (40 mg) was purified by flash chromatography (silica gel, $CHCl_3$ with 0.7 % of methanolic ammonia) to afford compound **11** (20 mg, 41 %). MS MALDI [M + H]$^+$ (found 729.7, required 729.40 for $C_{46}H_{57}N_4S_2$). ^{1}H NMR ($CDCl_3$) δ: 1.08 (dd, *J* = 13.0, 12.7, 12.1, 2H), 1.18 (m, 4H), 1.61 (m, 4H), 1.78 (m, 4H), 2.08 (m, 2H), 2.11 (dd, *J* = 10.9, 10.9, 2H), 2.16 (s, 6H), 2.40 (dd, *J* = 13.1, 7.5, 2H), 2.50 (dd, *J* = 13.1, 6.2, 2H), 2.78 (m, 2H), 2.84 (m, 2H), 3.10 (m, 2H), 3.21 (m, 2H), 3.59 (m, 2H), 3.94 (ddd, *J* = 14.4, 5.2, 5.2, 2H), 4.06 (m, 2H), 4.64 (m, 2H), 6.68 (s, 2H), 6.90 (ddd, *J* = 6.9, 1.0, 1.0, 2H), 7.12 (d, *J* = 8.2, 2H), 7.17 (dd, *J* = 8.2, 6.9, 2H), 7.19 (br s, 4 H). ^{13}C NMR ($CDCl_3$, HMQC + HMBC readouts) δ: 16.2 q, 26.6 t, 27.4 t, 30.7 t, 34.1 t, 36.5 d, 39.1 t, 41.7 d, 45.9 t, 57.0 t, 59.0 t, 63.7 d, 107.4 d, 110.9 s, 112.9 d, 121.5 d, 122.6 d, 126.7 s, 129.5 d (2 C), 133.8 s, 134.1 s, 136.8 s.

Physiology

Male Wistar rats (250-300 g) were killed by asphyxiation. The ventral caudal artery was quickly dissected and cleared of adhering connective tissue. A stainless steel wire (diameter 0.3 mm) was inserted into the artery to rub off the endothelium. Up to 12 cylindrical segments (3-4 mm long) were horizontally suspended between two L-shaped stainless steel hooks (diameter 0.15 mm) and mounted in a 20-ml organ bath filled with modified Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and D-glucose 10. The solution was continuously gassed with 95 % O_2/5 % CO_2 and warmed to constant temperature of 37 ºC. Preparations were connected to an isometric force transducer (W. Fleck, Mainz, Germany) attached to a TSE 4711 transducer coupler and a Siemens C 1016 compensograph for the continuous recording of changes in tension. Resting tension was adjusted to 0.5 g at the beginning of each experiment. During an equilibrium period of 120 min, preparations were stimulated once (after 60 min) with 5-HT (1 μM). In experiments with (partial) agonists, three cumulative concentration-response curves (CRCs) were determined on each arterial segment: the first CRC to 5-HT, the second (60 min after the first) to the test partial agonist and the third (10 min after the second) without washing to 5-HT in the presence of the test partial agonist (0.3-3 μM). In additional experiments, two CRCs were determined at an interval of 60 min as above: the first CRC to 5-HT and the second CRC to the test partial agonist in the presence of ketanserin. Ketanserin (3 nM) was incubated for 30 min. The shift to the right observed in the presence of ketanserin was calculated considering the shift observed for the corresponding control preparation in the absence of ketanserin. In experiments with antagonists two CRCs for 5-HT were determined on each arterial segment at an interval of 90 min. Antagonists were incubated 60 min before the second curve. Prazosin (0.1 μM) and cocaine (6 μM) were present throughout the experiments to block α_1-adrenoceptors and neuronal uptake of 5-HT.

Data presentation and statistical evaluation

Data are presented as mean ± S.E.M. for n separate experiments. Agonist concentration-response curves were fitted using the computer program GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA). Agonist potencies were expressed as pEC_{50} values (negative logarithm of the molar concentration of agonist producing 50 % of the maximum response). Maximal contractile responses were expressed as E_{max} values, i.e. percentage of the maximal contractile response to a reference compound (5-HT). The potencies of the antagonists were expressed as apparent pA_2 values. pA_2 was calculated from the equation:

$pA_2 = -\log c(B) + \log (r - 1)$

where c(B) is the molar concentration of antagonist and r is the ratio of the agonist EC_{50} measured in the presence and absence of the antagonist (Furchgott 1972). Partial agonists were additionally characterized by calculating the equilibrium dissociation constant K_P according to the method of Marano and Kaumann (1976). K_P was determined from the slope m of a weighted plot, which related equiactive concentrations of the full agonist A (5-HT) in the absence c(A) and presence c(A)* of the partial agonist P: $c(A) = m \cdot c(A)^* + b$, where b is the ordinate intercept. $pK_P = -\log_{10} K_P$ was calculated from the equation:

$\log (1/m - 1) = \log c(P) - \log K_P$.

All drug solutions were freshly prepared in distilled water with the following exceptions: ergoline derivatives were prepared in ethanol/water (50/50 v/v; stock solution 10 mM) by adding an equivalent of HCl. Compounds **5, 9** and **11** were dissolved in ethanol/water/DMSO (25/25/50 v/v/v; stock solution 5 mM) by adding an equivalent of HCl.

Results and Discussion

Oligomers of pergolide and terguride (compounds **3**, **4, 6a-e, 7, 8**) with a spacer linking the indole nitrogens were prepared as described previously (Křen et al. 2001, 2002). Compound **9** with a spacer linking the piperidine nitrogens was prepared analogously as described for compound **5** (Křen et al. 2001). Its structure was confirmed by both ^1H and ^{13}C NMR spectra, which were symmetrical. All signals except the aromatic part of the spacer had the same intensity. The indole part was intact since N-1-H coupled to H-2. This suggests that the spacer connects both N-6 of the depropylpergolide moieties. Moreover, both protons of the methylene groups attached to the aromatic spacer moiety coupled to H-5 and H-7. This is direct proof of the dimer structure. The mass spectrum corroborates the structure of compound **9**.

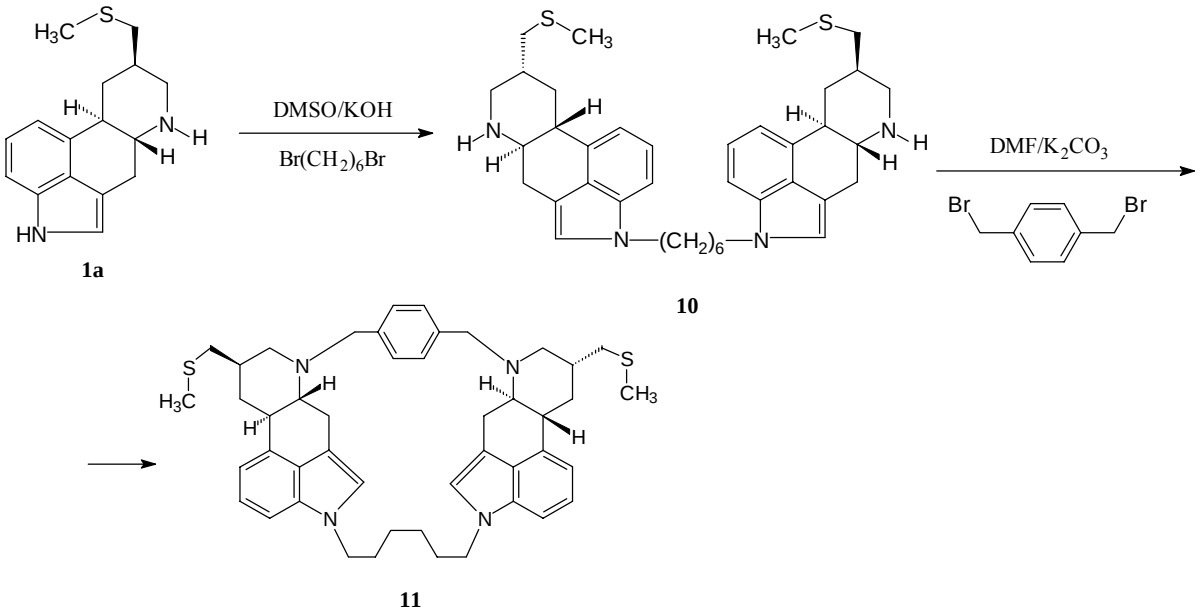


Fig. 4. Synthesis of cyclic pergolide dimer

For the preparation of compound **11**, which is the first cyclic ergoline dimer, the problem of selective substitution of two types of heterocyclic nitrogens of depropylpergolide (compound **1a**) had to be solved (Fig. 4). The indole hydrogen is slightly acidic, so that it can be abstracted by a strong base (NaOH, KOH, NaH or sodium in liquid ammonia, respectively). This deprotonized species gives rise to a green color of the reaction mixture (e.g., KOH/DMSO). In contrast, the piperidine nitrogen (N-6) is basic. By a rational choice of reaction conditions both hydrogens can be substituted independently. Strongly basic conditions

(KOH/DMSO) support fast reaction at N-1. The reactions with alkyl or aralkyl bromides are usually completed within 1 h. Weaker base (K_2CO_3/DMF) is not sufficient for inducing deprotonation of N-1 and, therefore, slower reaction at N-6 (10–20 h) takes place. Thus, depropylpergolide (compound **1a**) can be converted into a dimer either at N-6 (as in the case of compound **5**) or at N-1 creating dimer (compound **10**). The ^{1}H NMR spectrum of compound **10** lacked the indole N-H and was symmetric. Spacer attachment to N-1 was inferred from long-range coupling between H-2 and protons of the adjacent methylene group of the spacer and confirmed by HMBC. Compound **10** could undergo cyclization using different conditions to create the cyclic dimer (compound **11**). The reaction was conducted in very diluted solution to avoid polymerization. Elemental composition deduced from the observed $[M + H]^+$ peak m/z 729 was $C_{46}H_{57}N_4S_2$. Only 23 signals were found in HMQC (one among them of double intensity); also ^{1}H NMR spectrum reflected the expected symmetry. The final proof was again provided by HMBC.

Table 1. Contraction of rat tail artery in response to dimers of pergolide/terguride

Compound	*pEC_{50} ± S.E.M. (n)*	*Relative potency (95% c.l.)*	*E_{max} [%]*	*pK_P[a] ± S.E.M. (n)*	*μM*	*Antagonism by ketanserin[b] (n)*
*Pergolide (**1**)*	7.52 ± 0.06 (6)	412 (299 – 567)	55 ± 2	7.17 ± 0.06 (6)	3	9.38 ± 0.06 (4)
3	6.96 ± 0.04 (6)	87 (69 – 190)	55 ± 4	6.56 ± 0.13 (6)	3	9.05 ± 0.12 (6)
5	–	–	0	< 5.5[c] (8)	3	–
9	–	–	0	< 5.5[c] (4)	3	–
11	–	–	0	< 5.5[c] (4)	3	–
4[d]	Not determined		5 ± 1	6.82 ± 0.08 (4)	0.3	–
5-HT	6.95 ± 0.04 (34)	100	100	–		9.55 ± 0.03[e]

[a] –log K_P, calculated from the antagonism of the 5-HT response by compounds **3** and **4**, respectively. [b] pA_2 value at 3 nM ketanserin. [c] pA_2 value. [d] Hybrid molecule of pergolide and terguride. [e] Data from Pertz et al.(1999). Number of experiments in parentheses.

The interaction of the compounds with 5-HT_{2A} receptors was studied in rings of the isolated rat tail artery. The contractile effects of partial agonists were examined in the absence and presence of the selective 5-HT_{2A} receptor antagonist ketanserin (3 nM). Agonist potencies were expressed as pEC_{50} values and antagonist affinities as apparent pA_2 values. Partial agonists were additionally characterized by estimation of the equilibrium dissociation constant K_P (Marano and Kumann 1976), since these compounds were able to antagonize the contractile response to 5-HT. Those compounds which failed to contract rat tail arteries were tested for their ability to antagonize 5-HT-induced contractions (for details see the Methods).

The interaction of pergolide and dimers of pergolide with 5-HT_{2A} receptors of rat tail artery is summarized in Table 1. Pergolide contracted rat tail arteries with a pEC_{50} of 7.5 and an intrinsic activity of 0.55 with respect to 5-HT (Fig. 5). Pergolide was four times more potent than 5-HT as a constrictor of this tissue. The pEC_{50} value for pergolide (7.5) was in good agreement with its pK_P value (7.2) calculated from the antagonism of the 5-HT response by pergolide (Fig. 5). Moreover, the affinity value pK_P for pergolide was in the same concentration range as the affinity value pK_i (6.7) determined at 5-HT_{2A} receptors of the rat brain (Hagen et al. 1994). Figure 5 shows that the 5-HT_{2A} receptor-selective antagonist ketanserin (3 nM) caused a rightward shift of the CRC to pergolide. The apparent pA_2 value for ketanserin against pergolide (9.4) was close to a pA_2 value of 9.5 for ketanserin against 5-HT (Pertz et al. 1999). The similarity of blocking potency of ketanserin against pergolide and 5-HT is consistent with the interaction of the two drugs with the same receptor class (5-HT_{2A}) in this tissue. It is worth to mention that the ability of pergolide to contract rat tail arteries could explain why pergolide displays more 5-HT_{2A} receptor-mediated adverse effects compared to those antiparkinsonian drugs that lack 5-HT_{2A} receptor agonism (e.g. terguride, see below). Especially when taken in excess the activation of 5-HT_{2A} receptors by pergolide may contribute to unwanted psychic effects (e.g. hallucination, confusion) and digital vasospasm (Standaert and Young 1995).

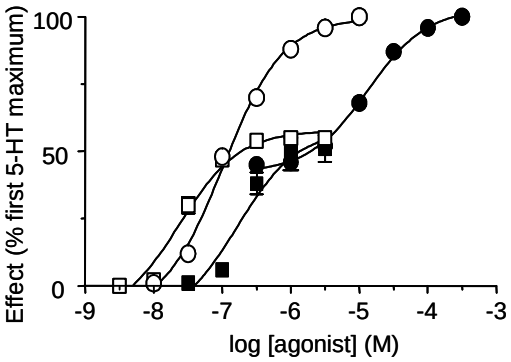


Fig. 5. Concentration-response curves to 5-HT in the absence (○) and presence of pergolide (●), and to pergolide in the absence (□) and presence of ketanserin (3 nM, ■). The 5-HT curve in the presence of pergolide (E_{max} 67 ± 3 %, not shown) was normalized to 100%. Points represent mean ± S.E.M. of the increase in force as percentage of the maximum response to 5-HT observed in the first curve. Number of experiments: n = 4-8.

Among the dimers of pergolide, in which two monomeric moieties are linked together through their indole nitrogen (N-1), compound **3** with a *p*-xylene spacer displayed appreciable partial agonism in rat tail artery (Fig. 6). Compound **3** was equipotent with 5-HT. The pK_P value (6.6) for compound **3** was in reasonable agreement with its pEC_{50} value (7.0). Figure 6 shows that the 5-HT$_{2A}$ receptor antagonist ketanserin (3 nM) shifted the concentration-response curve to compound **3** to the right. The apparent pA_2 value for ketanserin against compound **3** was 9.1 and argues for 5-HT$_{2A}$ receptor interaction.

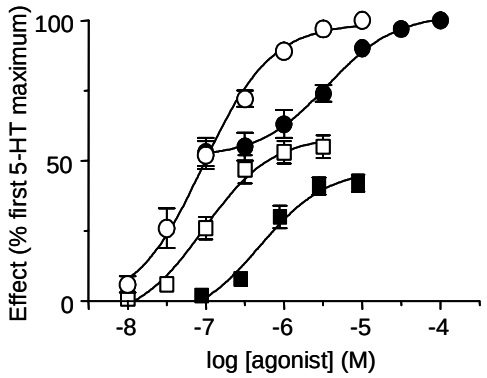


Fig. 6. Concentration-response curves to 5-HT in the absence (○) and presence of compound **3** (●), and to compound 3 in the absence (□) and presence of ketanserin (3 nM, ■). The 5-HT curve in the presence of compound 3 (E_{max} 83 ± 5 %, not shown) was normalized to 100 %. Points represent mean ± S.E.M. of the increase in force as percentage of the maximum response to 5-HT observed in the first curve. Number of experiments: n = 6 each.

In contrast, dimers of pergolide (compounds **5** and **9**), in which two monomeric moieties are linked together through their piperidine nitrogens (N-6) by an alkyl and an aralkyl spacer, respectively, had no contractile effect by themselves and possessed only low affinity for rat 5-HT$_{2A}$ receptors ($pA_2 < 5.5$). The same was true for the cyclic dimer of depropylpergolide (compound **11**). Obviously, the choice of the position of the spacer attachment to pergolide at N-6 seems to be detrimental for affinity and intrinsic activity. Compound **4**, a hybrid molecule of pergolide and terguride was a low efficacy partial agonist (i.a. 0.05) that exhibited moderate affinity for rat 5-HT$_{2A}$ receptors (pK_P 6.8).

Table 2. Insurmountable antagonism of 5-HT-induced contractions by terguride oligomers in rat tail artery

Compound	*n*	*Affinity* pA_2 ± *S.E.M.*	*Maximum 5-HT response (%)*
2	4	8.38 ± 0.13	63 ± 3
6a	4	6.67 ± 0.07	73 ± 1
6b	4	7.71 ± 0.05	62 ± 3
6c	6	7.18 ± 0.12	75 ± 3
6d	4	7.01 ± 0.05	84 ± 4
6e	4	6.67 ± 0.09	90 ± 1
7	4	6.67 ± 0.14	68 ± 3
8	4	7.47 ± 0.04	65 ± 1

Compound **2** – terguride

The interaction of terguride and oligomers of terguride with 5-HT$_{2A}$ receptors of rat tail artery is summarized in Table 2. In contrast to the oligomers of pergolide, terguride oligomers failed to contract rat tail arteries by themselves but insurmountably blocked the contractile response to 5-HT. Dimers and trimers of terguride showed moderate antagonist activity (pA_2 6.7-7.7) (Fig. 7). The reasons for the moderate affinity of the terguride oligomers are difficult to rationalize. Theoretically, the chemical structure of the spacer may be inappropriate or more probably the spacer attachment to the respective monomeric ligand may be located in an unfavorable position. For example, dimer **6a** in which two terguride moieties are linked through their indole nitrogen (N-1) by a methylene group, displayed the lowest antagonist affinity compared to its monomeric counterpart terguride (Table 2). The observation is markedly in contrast to previously reported findings that

ergoline derivatives, which contain a methyl substitution on the indole nitrogen, show higher affinities for rat 5-HT_{2A} receptors than their N-1-unsubstituted analogues (Hagen et al. 1994). The use a methylene linker (compound **6a**) may have caused that the distance between the bulky ergoline moieties is too short. The higher affinity of compound **6b** which has a n-hexyl linker favors this hypothesis (pA_2 7.7).

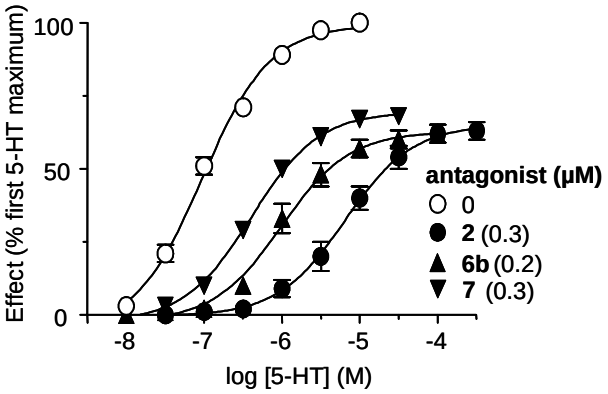


Fig. 7. Concentration-response curves to 5-HT (○, n = 12) in the absence and presence of antagonists. Points represent mean ± S.E.M. of the increase in force as percentage of the maximum response to 5-HT observed in the first curve. Number of experiments: n = 4 each.

Conclusions

The present study on the isolated rat tail artery shows that the mixed dopamine D_1 and D_2 receptor agonist pergolide is also a potent contractile partial 5-HT_{2A} receptor agonist. Stimulation of central 5-HT_{2A} receptors by pergolide may contribute to digital vasospasm and the psychic side effects (hallucinations) which have been observed as adverse effects of this drug in the treatment of Parkinson's disease. In contrast, the dopamine D_2 receptor partial agonist terguride is a silent 5-HT_{2A} receptor antagonist. Terguride shows nanomolar affinity for this site. Oligomerization of the monomeric counterparts fails to increase the affinity for 5-HT_{2A} receptors.

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Reprint requests

Vladimír Křen, Institute of Microbiology, Laboratory of Biotransformation, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ 142 20 Prague 4, Czech Republic, e-mail: kren@biomed.cas.cz