Naturally Occurring Clavines: Antagonism/Partial Agonism at 5-HT_{2A} Receptors and Antagonism at α_1-Adrenoceptors in Blood Vessels

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Abstract: The interaction of eight typical representatives of naturally occurring clavines (agroclavine, costaclavine, dihydrolysergol-I, elymoclavine, festuclavine, lysergene, lysergol, and pyroclavine) with 5-HT_{2A} receptors and α_1-adrenoceptors was studied in rat tail artery and aorta, respectively. Clavines antagonized 5-HT-induced contractions with calculated pK_B values (pK_P values for partial agonists) of 4.84–7.81 and (*R*)-phenylephrine-induced contractions with calculated pK_B values of 5.34–7.09. Specificity of clavines at 5-HT_{2A} receptors relative to α_1-adrenoceptors was rather low. Low affinity for costaclavine at both 5-HT_{2A} receptors ($pK_P = 4.84 \pm 0.06$) and α_1-adrenoceptors ($pK_B = 5.34 \pm 0.05$) indicates that the *trans*-junction of ring C and D of the ergoline pharmacophore is crucial for the binding of ergolines to these sites. Lysergol, lysergene, and costaclavine produced non-parallel displacements of the 5-HT concentration-response curve in the rat tail artery and caused small contractions by themselves. Lysergol contracted the rat tail artery with a pEC_{50} of 6.36 ± 0.04 and an intrinsic activity of 0.18 ± 0.03 with respect to 5-HT. Lysergol-induced contractile responses were surmountably antagonized by ketanserin (10 nM) with a pK_B of 9.1 which is consistent with an interaction of lysergol with 5-HT_{2A} receptors. The pK_P for the lysergol-5-HT_{2A} receptor complex calculated from concentration-response curves to lysergol was 6.88 ± 0.07 and did not match the pK_P of 7.66 ± 0.02 calculated from antagonism by lysergol of the contractile response to 5-HT. This suggests that lysergol and 5-HT possibly bind in two slightly different orientations at the 5-HT_{2A} receptor. It is concluded that partial agonism and pure antagonism at 5-HT_{2A} receptors on the one side and antagonism at α_1-adrenoceptors on the other side may contribute to the noxious effects of naturally occurring clavines.

Key words: Naturally occurring clavines, 5-HT_{2A} receptors, α_1-adrenoceptors, partial agonism, antagonism.

Introduction

Semisynthetic ergolines such as LY 53857, sergolexole, amesergide, and LY 215840 are among the most potent antagonists of 5-HT at vascular 5-HT_{2A} receptors with negligible α_1-adrenergic, histaminergic, and dopaminergic blocking properties (for review, see Refs. 1, 2). The compounds represent small ester and amide derivatives, respectively, of 9,10-dihydrolysergic acid and are substituted with an isopropyl group at the indole nitrogen (N-1). The existence of 9,10-dihydro derivatives is not common among naturally occurring ergot alkaloids. With the exception of 9,10-dihydroergosine, a naturally occurring representative of ergopeptine alkaloids which has been isolated from the fungus *Spacelia sorghi* (3), natural 9,10-dihydro derivatives have solely been identified within the class of clavine alkaloids (e.g. festuclavine, pyroclavine, costaclavine, and dihydrolysergol-I). In contrast to the derivatives of lysergic acid, naturally occurring clavines show a higher structural variability in the ring D of the ergoline pharmacophore. Clavines carry C-17 in a lower oxidation state than the lysergic acid derivatives. Alternatively, the double bond in ring D may be in the 8,9- or 9,10-position or may be lacking altogether as mentioned above. Furthermore, the junction between rings C and D may be *trans* or *cis*. In addition to a common occurrence in *Claviceps* species, clavines have been found in other fungal genera, e.g. *Aspergillus*, *Balansia*, *Penicillium*, *Phycomyces*, *Rhizopus* (4–6) and even in some genera of the higher plant family Convolvulaceae (7–9).

Semisynthetic clavine derivatives such as pergolide and lergotrile are clinically effective as anti-Parkinsonian drugs and inhibitors of prolactin release due to their dopaminergic D_2-like receptor stimulatory properties (10, 11). Another semisynthetic clavine derivative, nicergoline, is used in the treatment of hypertension and of poor peripheral and cerebral blood circulation partially due to the blockade of α_1-adrenoceptors (12). The effectiveness of such clavines have supported the conclusion that the pharmacological activities of ergot alkaloids are not dependent on the peptide or even an amide moiety (13).

The interaction of naturally occurring clavines with 5-HT receptors, which represent a heterogeneous family of at least 15 members (14), is less well documented. It has recently been reported that lysergol is a powerful ligand for human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors, showing the highest affinity among 20 non-ergoline and ergoline-based ligands tested at both sites (15). This is consistent with the agonist activity of lysergol at 5-HT_{1D}-like receptors of guinea-pig iliac artery where lysergol was equipotent with 5-HT (16). Further evidence for the conclusion that the side chain of the ergoline pharmacophore is not crucial for the pharmacological activities of ergot alkaloids was recently provided by the potent and competitive antagonism of 5-HT caused by 1-isopropylelymoclavine, 1-isopropylagro-

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clavine, and 1-isopropylfestuclavine, respectively, at vascular 5-HT_{2A} receptors (17).

The aim of the present study was to extend our knowledge about the interaction of naturally occurring clavines (Fig. **1**) with 5-HT_{2A} receptors in rat tail artery and α_1-adrenoceptors in rat aorta (18).

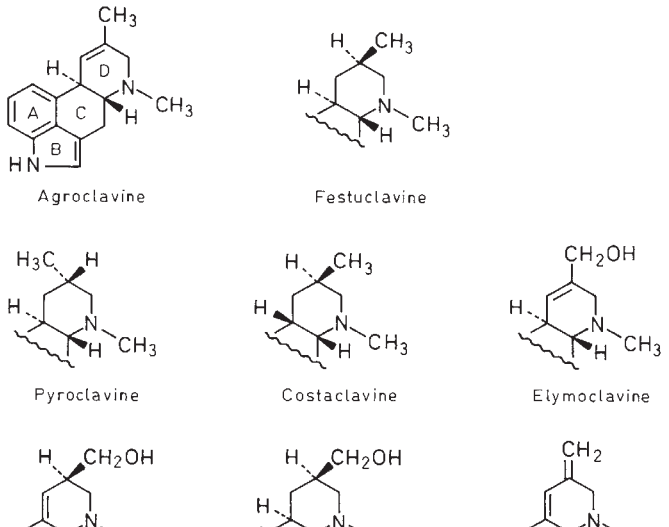

Fig. 1 Chemical structures of naturally occurring clavines.

Materials and Methods

Compounds

The following compounds were obtained as gifts from Prof. Dr. E. Eich (Freie Universität Berlin, Germany): Agroclavine, costaclavine, elymoclavine, lysergene, lysergol, and pyroclavine. Ketanserin tartrate and (*R*)-phenylephrine were gifts from Janssen (Beerse, Belgium) and Winthrop (Norderstedt, Germany), respectively. Cocaine hydrochloride was purchased from Merck (Darmstadt, Germany), 5-hydroxytryptamine creatinine sulfate from Janssen (Beerse, Belgium), prazosin hydrochloride from RBI (Natick, MA, USA), and (*R,S*)-propranolol from Sigma (St. Louis, MO, USA).

Since festuclavine and dihydrolysergol-I from natural sources (e.g. *Claviceps* species) were not available in house, they were synthesized as follows: Festuclavine was prepared from agroclavine by catalytic hydrogenation with Raney nickel in MeOH (19). Dihydrolysergol-I was prepared from lysergol by catalytic hydrogenation with Pd 10%/C in DMF/pyridine (100 : 2, v/v) at 5 bar (20). Both compounds were of analytical grade (HPLC purity > 98%).

5-HT_{2A} Antagonist activity (isolated rat tail artery)

Male Wistar rats (280–350 g) were killed by cervical dislocation. The ventral caudal artery was quickly dissected and cleared of connective tissue. A stainless steel wire (diam. 0.3 mm) was inserted into the artery to rub off the endothelium. Up to 24 cylindrical segments of 5–6 mm length were prepared from each artery and were horizontally suspended between two L-shaped stainless-steel hooks (diam. 0.15 mm) gently inserted into the lumen for the recording of contractile responses (21). Each preparation was mounted in a 20 ml organ bath containing 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 a constant temperature of 37 °C. Preparations were connected to a force displacement transducer attached to a TSE 4711 transducer coupler and a Siemens C 1016 compensograph for the continuous recording of isometric changes in tension. Resting force was adjusted to 5 mN at the beginning of each experiment. During an equilibration period of 120 min preparations were stimulated once (after 60 min) with 5-HT (1 μM).

In antagonist experiments two cumulative concentration-response curves to 5-HT were determined on each arterial segment at an interval of 60 min in the absence and presence of clavines. Antagonists were incubated 30 min before the second curve. It has previously been shown that successive concentration-response curves to 5-HT (control curves) are highly reproducible in rat tail artery (22). In experiments dealing with the agonism of lysergol three cumulative concentration-response curves were established at intervals of 60 min, the first curve for 5-HT, the second curve for lysergol in the absence of ketanserin, and the third curve for lysergol in the absence or presence of ketanserin (10 nM). Ketanserin was incubated 30 min before the third curve. In control experiments the third curve to lysergol showed a slight leftward shift of 0.2 log units and an increase in maximum response of up to 11% of the maximum response to 5-HT compared with the second curve to lysergol (n = 5). The shift to the right observed in the presence of ketanserin in the third curve was corrected individually using the sensitization measured for the respective control preparation in the absence of ketanserin. Prazosin (30 nM) and cocaine (6 μM) were present throughout the experiments to block α_1-adrenoceptors and neuronal uptake of 5-HT (23).

α_1-Adrenoceptor affinity (isolated rat thoracic aorta)

The thoracic aorta was removed from rats used for studies at 5-HT_{2A} receptors in the rat tail artery (see above). When cleared of connective tissue the aorta was cut into 6–12 rings of 4–6 mm length. Each cylindrical segment was rolled with a pair of tweezers to destroy the endothelium. The segments were horizontally suspended between two L-shaped stainless-steel holders (diam. 0.3 mm) (24). The organs were isometrically mounted as described for rat tail artery experiments (see above). The bath fluid (modified Krebs-Henseleit solution of the above composition at 37 °C, gassed with 95% O_2/5% CO_2) contained (*R,S*)-propranolol (1 μM) to block β-adrenoceptors. The applied resting force was 20 mN. During an equilibration period of 140 min the organs were stimulated twice with (*R*)-phenylephrine (100 nM). Two cumulative concentration-response curves for the contractile effect of (*R*)-phenylephrine were determined in the absence and presence of antagonist. Antagonists were incubated 30 min before the second curve.

Receptor mathematics

Agonist potencies were expressed as pEC_{50} values (negative logarithm of the molar concentration of agonist which pro-

duced 50% maximum contractile effect). Antagonist dissociation constants (K_B values) were calculated from (25):

$$K_B = c(B)/(CR - 1) \quad [1]$$

where c(B) is the concentration of antagonist used and CR (concentration ratio) is the ratio of agonist EC_{50} measured in the presence of antagonist over that measured in the absence of antagonist. The equilibrium dissociation constant K_P for the lysergol-5-HT$_{2A}$ receptor complex resulting from concentration-response curves to lysergol (method A) was calculated from (26):

$$c(5\text{-}HT) = m \cdot \frac{c(5\text{-}HT)}{c(P)} + b \quad [2]$$

$$\text{with } m = \frac{K_P}{\left(\frac{\varepsilon_P}{\varepsilon_{5\text{-}HT}} - 1\right)} \quad [3]$$

where c(5-HT) is the concentration of the full agonist 5-HT, c(P) is the concentration of the partial agonist P, m is the slope and b the intercept of the regression line of c(5-HT) on c(5-HT)/c(P). ε_A and ε_P represent the intrinsic efficacies of the full agonist and the partial agonist, respectively (26).

If $\varepsilon_P << \varepsilon_A$, K_P can be calculated as

$$K_P = -m \quad [4]$$

The K_P values resulting from antagonism of the 5-HT response by partial agonists (method B) were calculated from (26):

$$c(5\text{-}HT) = m \cdot c(5\text{-}HT)^* + b \quad [5]$$

$$\text{with } m = \frac{1}{1 + \left(1 - \frac{\varepsilon_P}{\varepsilon_P}\right) \cdot \frac{c(P)}{K_P}} \quad [6]$$

where c(5-HT) is the concentration of the full agonist 5-HT in the absence of the partial agonist P and c(5-HT)* is the concentration of the full agonist 5-HT in the presence of the partial agonist P. m is the slope and b the intercept of the weighted regression line of c(5-HT) on c(5-HT)*. Weights w_i were calculated from (27):

$$w_i = \{c(5\text{-}HT)_i^* + c(5\text{-}HT)^*_{50}\}^{-4} \quad [7]$$

$c(5\text{-}HT)^*_{50}$ is the concentration of the full agonist 5-HT which produced 50% maximum contractile effect after the administration of the partial agonist P.

b was calculated with the equation:

$$b = \frac{\left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i^*\right)\right) \cdot \left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i \cdot c(5-HT)_i^*\right)\right) - \left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i\right)\right) \cdot \left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i^{*2}\right)\right)}{\left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i^*\right)\right)^2 - \left(\sum_{i=1}^{N} w_i\right) \cdot \left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i^{*2}\right)\right)} \quad [8]$$

and m with the equation:

$$m = \frac{\left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i\right)\right) \cdot \left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i^*\right)\right) - \left(\sum_{i=1}^{N} w_i\right) \cdot \left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i \cdot c(5-HT)_i^*\right)\right)}{\left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i^*\right)\right)^2 - \left(\sum_{i=1}^{N} w_i\right) \cdot \left(\sum_{i=1}^{N}\left(w_i \cdot c(5-HT)_i^{*2}\right)\right)} \quad [9]$$

If $\varepsilon_P << \varepsilon_A$, K_P can be calculated as

$$K_P = c(P) \cdot \left(\frac{1}{1-m} - 1\right) \quad [10]$$

Statistics

Data are presented as means ± SEM or geometric means with 95% confidence limits.

	5-HT$_{2A}$ receptor affinity	E_{max}[a]	n	α_1-adrenoceptor affinity[b]	E_{max}[a]	n	Specificity 5-HT$_{2A}$/α_1
Agroclavine	7.40 ± 0.04[b]	89 ± 4	6	7.09 ± 0.06	100 ± 2	4	2
Elymoclavine	6.95 ± 0.07[b]	94 ± 3	4	6.37 ± 0.09	97 ± 1	4	4
Festuclavine	6.83 ± 0.06[b]	95 ± 2	4	6.85 ± 0.04	99 ± 2	4	1
Pyroclavine	7.19 ± 0.05[b]	95 ± 2	4	6.21 ± 0.07	107 ± 4	4	10
Costaclavine	4.84 ± 0.06[c]	93 ± 1	4	5.34 ± 0.05	106 ± 1	4	0.5
Lysergol	7.66 ± 0.02[c]	97 ± 1	8	6.35 ± 0.03	103 ± 1	4	20
Dihydrolysergol-I	6.27 ± 0.02[b]	100 ± 1	5	6.21 ± 0.06	101 ± 2	4	1
Lysergene	7.81 ± 0.04[c]	94 ± 2	8	6.74 ± 0.11	100 ± 1	4	12

Table 1 Effects of naturally occurring clavines on 5-HT-induced contractions of rat tail artery and (*R*)-phenylephrine-induced contractions of rat aorta.

Values are expressed as mean ± SEM from n individual vascular segments of at least two animals.

[a] E_{max} values are expressed as percentage of the maximum response to the agonist in the 2nd curve relative to the 1st curve.

[b] pK_B values for pure antagonists from single point analysis at 1–30 μM antagonist.

[c] pK_P values for partial agonists form single point analysis at 1–100 μM antagonist (method B).

Results

5-HT$_{2A}$ Receptor and α_1-adrenoceptor affinity of naturally occurring clavines

Naturally occurring clavines were investigated as antagonists of 5-HT in rat tail artery and as antagonists of (*R*)-phenylephrine in rat aorta. Affinity estimates are summarized in Table **1**. All compounds caused a rightward shift of the concentration-response curves to 5-HT (Fig. **2**) and (*R*)-phenylephrine, respectively, with little or no effect on maximum response. With the exception of costaclavine, which was clearly different from the other compounds due to its considerably lower affinity (pK_B = 4.84), clavines possessed moderate affinity at 5-HT$_{2A}$ receptors in rat tail artery with pK_B values (pK_P values for lysergene, lysergol, and costaclavine) of 6.27–7.81. Similar findings were obtained at α_1-adrenoceptors in rat aorta. Whereas costaclavine also behaved as an outlier in this tissue due to its low antagonist activity (pK_B = 5.34), pK_B values for the remaining clavines ranged from 6.21 to 7.09.

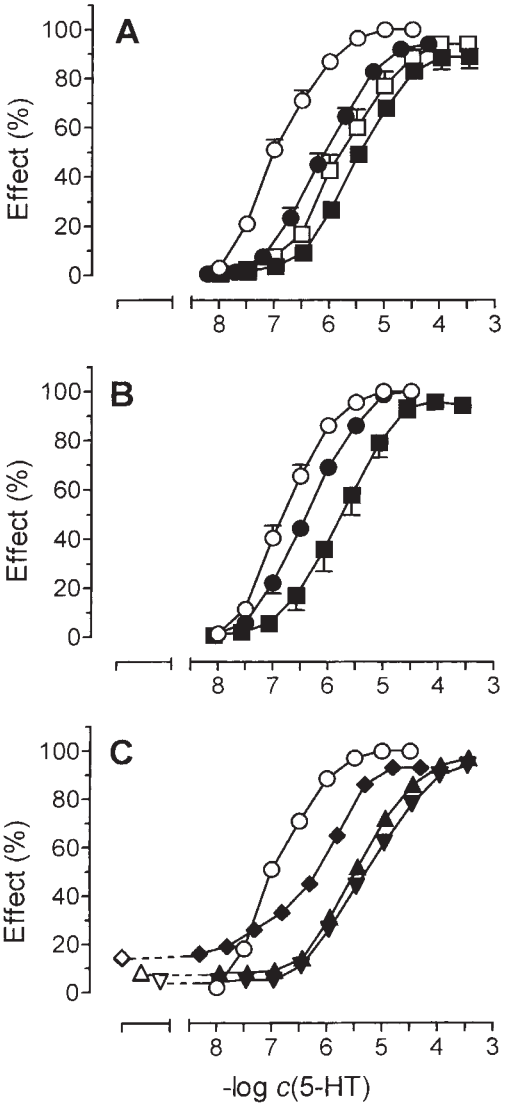

Fig. 2 Antagonism of 5-HT-induced contractions by naturally occurring clavines in rat tail artery. Shown are concentration-response curves to 5-HT in the absence (○, *n* = 9–20) and presence of 1 μM antagonist (costaclavine 100 μM). **A**: Festuclavine (●, *n* = 4), pyroclavine (□, *n* = 4), agroclavine (■, *n* = 6). **B**: Dihydrolysergol-I (●, *n* = 5), elymoclavine (■, *n* = 4). **C**: Costaclavine (◆, *n* = 4), lysergol (▲, *n* = 8), and lysergene (▼, *n* = 8). Stimulant effects elicited by costaclavine (100 μM), lysergol (1 μM), and lysergene (1 μM) amounted to 15 ± 2 (◇), 9 ± 1 (△) and 5 ± 1% (▽), respectively, of the maximum response to 5-HT. All values are mean ± SEM (vertical bars, only illustrated when larger than symbols).

Some differences were found concerning the mechanism of action of clavines at 5-HT$_{2A}$ receptors. Festuclavine, pyroclavine, agroclavine, dihydrolysergol-I, and elymoclavine were acting as pure 5-HT$_{2A}$ receptor antagonists at 1 μM (Figs. **2A–B**). On the other hand, costaclavine, lysergol, and lysergene produced non-parallel displacements of the 5-HT concentration-response curve and caused slight contractions by themselves (Fig. **2C**). Costaclavine, lysergol, and lysergene were acting as partial agonists in rat tail artery. Their affinity at 5-HT$_{2A}$ receptors was determined by estimation of equilibrium dissociation constants K_P for partial agonists. Stimulant effects elicited by costaclavine (100 μM), lysergol (1 μM), and lysergene (1 μM) amounted to 15 ± 2, 9 ± 1 and 5 ± 1%, respectively, of the maximum response to 5-HT. Ketanserin (3 nM) reduced the stimulant effect evoked by costaclavine (100 μM) to 7 ± 1% (n = 4, $P < 0.05$, not shown). The effect of ketanserin on the slight contractile response of 5 ± 1% evoked by lysergene (1 μM) was not examined in the study.

Characterization of lysergol as a partial agonist at 5-HT$_{2A}$ receptors

Lysergol contracted cylindrical segments of rat tail artery with a pEC_{50} of 6.63 ± 0.04 (n = 11) and an intrinsic activity of 0.18 ± 0.03 with respect to 5-HT. Potency ratio [ratio of the EC_{50} of lysergol (2nd curve) over that of 5-HT (1st curve)] was 1.34 (0.94 – 1.91). Ketanserin (10 nM) caused a 1.1 log unit rightward shift of the concentration-response curve to lysergol (n = 6, Fig. **3**). pK_P for the lysergol-5-HT$_{2A}$ receptor complex resulting from concentration-response curves to lysergol (method A) was calculated by comparison of equiactive concen-

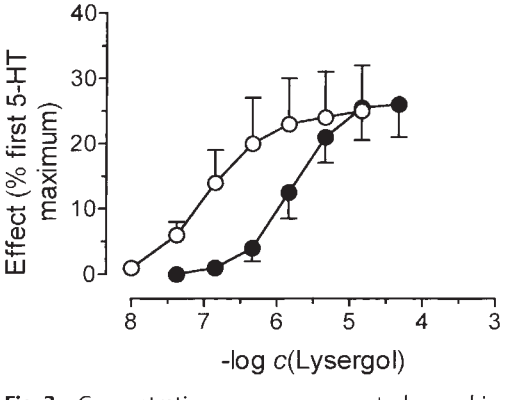

Fig. 3 Concentration-response curves to lysergol in rat tail artery. Shown are the third curves in the absence (○, *n* = 5) and presence of 10 nM ketanserin (●, *n* = 6). Effects are expressed as percentage of the maximum response to 5-HT observed in the first curve. Values are mean ± SEM (vertical bars, only illustrated when larger than symbols).

trations of the full agonist 5-HT and the partial agonist lysergol according to equation [2]–[4] (Fig. **4**). pK_P was 6.88 ± 0.07 (n = 11). pK_p for the lysergol-5-HT_{2A} receptor complex resulting from experiments where lysergol was tested as an antagonist of 5-HT-induced contractions (method B) was calculated by comparison of equiactive concentrations of the full agonist 5-HT in the absence and presence of the partial agonist lysergol according to equation [5]–[10] (Fig. **5**). pK_P was 7.66 ± 0.02 (n = 8).

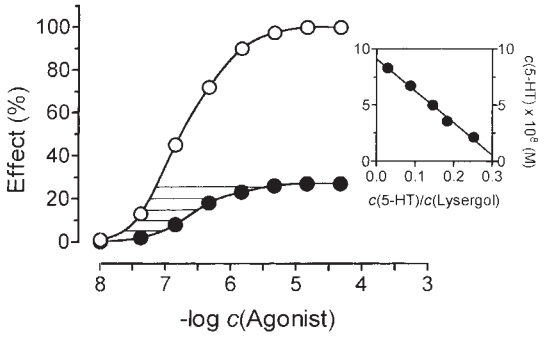

Fig. 4 Estimation of K_P for the partial agonist lysergol by comparison of equiactive concentrations of 5-HT and lysergol. Shown are typical concentration-response curves to 5-HT (○) and lysergol (●) of a single segment of rat tail artery. The *inset* represents the regression of c(5-HT) (ordinate) on the ratio of equiactive concentrations of 5-HT and lysergol (abscissa) according to equation [2]. Slope = -2.861×10^{-7}, intercept = 9.12×10^8, pK_P = 6.54.

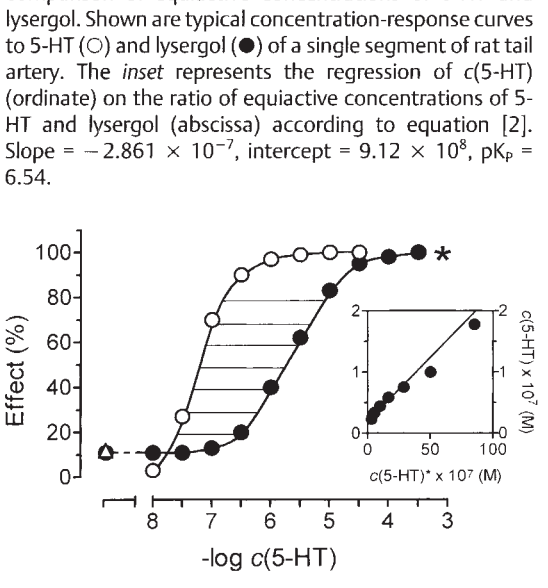

Fig. 5 Estimation of K_P from antagonism of the 5-HT response by lysergol. Shown are typical concentration-response curves to 5-HT in the absence (○) and presence (●) of lysergol (1 μM) of a single segment of rat tail artery. The *inset* represents the weighted regression of equiactive concentrations of 5-HT (27) in the absence (ordinate) and presence (abscissa) of lysergol. Slope = 0.0204, pK_P = 7.68.

Discussion

Since ergolines in general are highly effective in the cardiovascular system the present study was aimed at characterizing the interaction of typical representatives of naturally occurring clavines, which by contrast to semisynthetic ergolines (see "Introduction") have only been poorly studied, with 5-HT_{2A} receptors in rat tail artery and α_1-adrenoceptors in rat aorta. The main finding of the study is that clavines with the exception of costaclavine show moderate affinity at both 5-HT_{2A} receptors and α_1-adrenoceptors in the rat. Compared to the high antagonist activity of well-established 5-HT_{2A} receptor blocking drugs such as methysergide and LY 53857 (22) the antagonist activity of clavines at 5-HT_{2A} receptors was low. On the whole, 5-HT_{2A} receptor affinity was somewhat higher than α_1-adrenoceptor affinity for these clavines, although specificity for either site was relatively low. Specificity at 5-HT_{2A} receptors relative to α_1-adrenoceptors was highest with lysergol (20-fold) and lysergene (12-fold) which also had the highest 5-HT_{2A} receptor affinity (pK_P of 7.66 and 7.81) in this series. The markedly lower affinity for costaclavine at both 5-HT_{2A} receptors and α_1 adrenoceptors indicates that the *trans*-junction of ring C and D of the ergoline pharmacophore is crucial for the binding of ergolines to these sites.

Lysergol, lysergene, and costaclavine differed from the remaining clavines due to their partial agonist activity at 5-HT_{2A} receptors of rat tail artery. The potent 5-HT_{2A} receptor antagonist ketanserin (28) reduced at 3 nM the stimulant effect of 100 μM costaclavine from 15 ± 2 to 7 ± 1% and caused at 10 nM a 1.1 log unit rightward shift of the concentration-response curve to lysergol (Fig. **3**), consistent with a pK_B of 9.1 for ketanserin which suggests the interaction of lysergol with 5-HT_{2A} receptors. Figures **2C** and **5** clearly illustrate that concentration-response curves to 5-HT in the presence of a partial agonist were not parallel to the curve of 5-HT in its absence. Therefore, the estimation of the equilibrium dissociation constant K_P for the partial agonist-5-HT_{2A} receptor complex is more appropriate (27) than the estimation of the antagonist dissociation constant K_B (25). Table **2** summarizes the different pharmacological parameters for lysergol estimated in the present study. It is interesting to note that pK_P calculated from experiments where lysergol was tested as an agonist did not match pK_P calculated from experiments where lysergol was tested as an antagonist of 5-HT. The difference suggests that lysergol and 5-HT possibly bind in two slightly different orientations at the 5-HT_{2A} receptor.

Table 2 Pharmacological parameters of lysergol at 5-HT_{2A} receptors in rat tail artery.

		95% C. L.[a]	n	c[b] [μM]
pEC_{50}[c]	6.63 ± 0.04	6.53–6.73	11	0.01–10
pK_p[d]	6.88 ± 0.07	6.73–7.03	11	0.01–10
pK_P[e]	7.66 ± 0.02	7.60–7.72	8	1
pK_B[f,g]	7.47 ± 0.03	7.40–7.54	8	1

[a] 95% confidence limits.
[b] Concentration of lysergol.
[c,d] Calculated from concentration-response curves to lysergol (method A).
[e,f] Calculated from antagonism of the 5-HT response by lysergol (method B).
[g] Calculation inappropriate according to (27).

It has recently been shown (17) that the introduction of an isopropyl group at the indole nitrogen (N-1) of elymoclavine, agroclavine, and festuclavine enhances 5-HT_{2A} receptor affinity in rat tail artery resulting in an appreciably higher specificity at 5-HT_{2A} receptors relative to α_1-adrenoceptors (Table **3**) which is in marked contrast to the specificity (5-HT_{2A}/α_1) of the corresponding N-1-unsubstituted clavines (Table **1**). Recent data from radioligand binding studies have indicated that ergolines with an isopropyl group at N-1 show higher affinity at the rat *versus* the pig, monkey, and human 5-HT_{2A} receptor, whereas the corresponding N-1 unsubstituted compounds show higher

	5-HT_{2A} receptor $pA_2 \pm SEM$[a]	α_1-adrenoceptor $pK_B \pm SEM$[b]	Specificity 5-HT_{2A}/α_1
1-Isopropylelymoclavine	9.14 ± 0.11	5.69 ± 0.07	2,818
1-Isopropylagroclavine	8.84 ± 0.07	6.34 ± 0.05	316
1-Isopropylfestuclavine	8.50 ± 0.06	6.70 ± 0.03	63

Results are taken from (17).
[a] pA_2 from Schild plot (32).
[b] pK_B from single point analysis at 30 μM antagonist.

Table 3 Affinity estimates for the interaction of 1-isopropylelymoclavine, 1-isopropylagroclavine, and 1-isopropylfestuclavine with 5-HT_{2A} receptors and α_1-adrenoceptors of rat tail artery and aorta, respectively.

affinity at the pig, monkey, and human *versus* the rat 5-HT_{2A} receptor (29, 30). Because species differences exist among 5-HT_{2A} receptors, moderately active clavines of the present study may be even more potent in other species than the rat. Furthermore, it is worth mentioning that lysergol which produced partial agonism at 5-HT_{2A} receptors of rat tail artery [potency ratio of 1.34 (0.94–1.91)] was equipotent with 5-HT as a partial agonist at 5-HT_{1D}-like receptors of guinea-pig iliac artery (16) and possessed high affinity at human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors in radioligand binding studies (15). Finally, it should be noted that serotoninergic 5-HT_{2A}-mediated effects may amplify α_1-adrenergic-induced vasoconstriction (31). Compounds like clavines that block both 5-HT_{2A} receptors and α_1-adrenoceptors may have greater antihypertensive efficacy than compounds possessing only α_1-adrenergic receptor blocking properties. Thus, species variability, lack of selectivity at different 5-HT receptors, and lack of specificity (5-HT_{2A}/α_1) should be taken into account when the serotoninergic and adrenergic profile of naturally occurring clavines is quantified. This is particularly important since clavines may have an effect on a wide range of multicellular animals where 5-HT and norepinephrine are ubiquitous neurotransmitters.

In conclusion, the results from the present study demonstrate that naturally occurring clavines are acting as pure antagonists or partial agonists at 5-HT_{2A} receptors of rat tail artery and as antagonists at α_1-adrenoceptors of rat aorta showing relatively low specificity at either site. The interaction with 5-HT_{2A} receptors and α_1-adrenoceptors may contribute to the noxious effects of clavines which are major components or intermediate products in the biosynthesis of lysergic amides and ergopeptines of *Claviceps* species, other fungal genera, and some genera of the higher plant family Convolvulaceae.

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