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Simple O-Acylated Derivatives of Lysergol and Dihydrolysergol-I: Synthesis and Interaction with 5-HT_{2A}, 5-HT_{2C} and 5-HT_{1B} Receptors, and α_1 Adrenergic Receptors

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Abstract

A series of simple O-acylated derivatives of the naturally occurring clavine alkaloids lysergol and dihydrolysergol-I were synthesized and tested in-vitro for their ability to interact with 5-HT_{2A} receptors in rat tail artery, 5-HT_{2C} receptors in piglet choroid plexus, 5-HT_{1B} receptors in guinea-pig iliac artery and α_1-adrenergic receptors in rat aorta.

In contrast to the classical ergoline 5-HT_{2A} receptor antagonists methysergide and LY53857, the compounds produced competitive antagonism of the 5-HT response in rat tail artery. Affinities of ergolines **3**–**14** were higher (pA_2 values of 7·33–8·40) than those of the parent alcohols lysergol (**1**) and dihydrolysergol-I (**2**), respectively. The introduction of an isopropyl substituent at the N(1) position of the compounds failed to enhance 5-HT_{2A} receptor affinity. Compounds **3**–**14** exhibited lower affinities for α_1-adrenergic receptors than for 5-HT_{2A} receptors. In particular, those lysergol derivatives that had an isopropyl substituent at the N(1) position were highly specific 5-HT_{2A} receptor antagonists (ratio 5-HT_{2A}/α_1 = 302–3548).

Selected derivatives of lysergol (**3**–**5**, **9**–**11**) which were assayed for radioligand binding at 5-HT_{2C} receptors in piglet choroid plexus had affinities that were similar to those found in rat tail artery. Additionally, lysergol and its N(1)-unsubstituted derivatives were found to be partial agonists (α of 0·2–0·4) for 5-HT_{2C} receptor-mediated inositol phosphate accumulation in piglet choroid plexus. On the other hand, analogues with an isopropyl substituent at N(1) showed no measurable agonist activity. The observation that N(1)-unsubstituted derivatives of lysergol possessed agonist properties at 5-HT_{2C} receptors whereas their agonist activity at 5-HT_{2A} receptors was marginal (α of 0·05 for compound **3** at 1 μM) or not measurable, suggests that these compounds have different abilities to cause conformational change at the two receptor types. Selected derivatives of lysergol (**3**–**5**, **9**–**11**) which were examined as ligands for 5-HT_{1B} receptors in guinea-pig iliac artery caused insurmountable blockade of the contractile effect of 5-HT. N(1)-isopropyl derivatives had 30–50-fold lower affinities for 5-HT_{1B} receptors of this tissue than their N(1)-unsubstituted analogues.

It is concluded that O-acylated derivatives of the clavine alkaloids lysergol and dihydrolysergol-I mimic therapeutically relevant ergolines due to the complexity of their pharmacological profile as partial agonists and antagonists at 5-HT_{2A}, 5-HT_{2C} and 5-HT_{1B} receptors, and at α_1-adrenergic receptors.

A number of natural ergot alkaloids and related synthetic compounds are known to be highly active in the cardiovascular system. For example, small amide derivatives of lysergic acid such as methysergide and lysergic acid diethylamide (LSD) are among the most potent antagonists of the 5-HT_{2A} receptor-mediated contractile effect of 5-HT in the vasculature (for review, see Kaumann 1989). In addition, it has been shown that ester derivatives of dihydrolysergic acid such as LY53857 and sergolexole potently block 5-HT_{2A} receptors on both blood vessels and platelets, therefore being candi-

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dates for the treatment of ischaemic heart disease and other vascular disorders (for review, see Audia & Cohen 1991). Although compounds such as LY53857 show negligible α_1-adrenergic, histaminergic and dopaminergic receptor blocking properties, they are non-selective ligands because they fail to discriminate between the subtypes within the 5-HT_2 family of receptors (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}) (Baxter et al 1995).

The chemical structures of the highly potent 5-HT_{2A} receptor antagonists methysergide, LY53857 and sergolexole (Figure 1) unequivocally illustrate that both functionalization of the carboxyl group at C-8 to create amides or esters and N(1)-alkylation play a pivotal role with regard to structural modifications of the ergoline molecule. Recently, we reported that naturally occurring clavines, which have either methyl (e.g. agroclavine, costaclavine, festuclavine, pyroclavine), methylene (e.g. lysergene) or hydroxymethyl (e.g. elymoclavine, lysergol, dihydrolysergol-I) at C-8 of the tetracyclic ergoline ring system, showed moderate affinity for both 5-HT_{2A} and α_1-adrenergic receptors in rat blood vessels (Pertz 1996). Among the clavines lysergol justifies special mention as a powerful non-selective ligand for human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors (Weinshank et al 1992) which are now termed 5-HT_{1D} and 5-HT_{1B}, respectively (Hartig et al 1996). The observation is consistent with the agonist activity of lysergol at 5-HT_{1B} receptors of guinea-pig iliac artery where lysergol was equipotent with 5-HT (Pertz 1993).

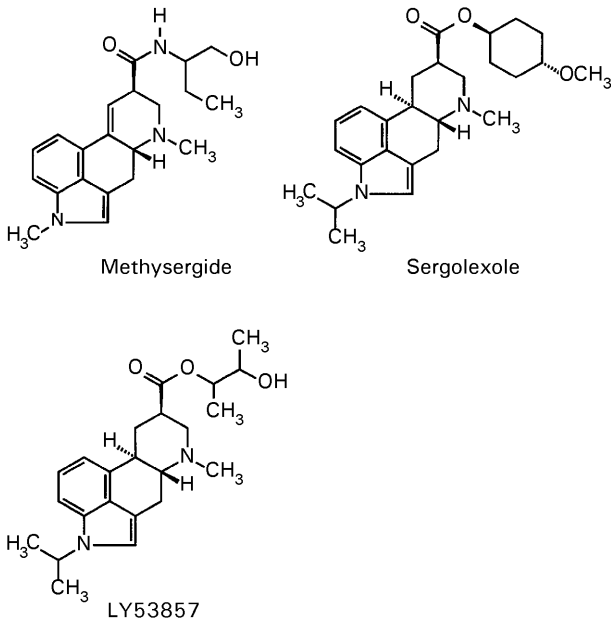


Figure 1. Chemical structures of ergolines that show potent antagonist activity at 5-HT_{2A} receptors.

The aim of the present study was to clarify the question of whether O-acylation of the hydroxymethyl group at C-8 of lysergol and dihydrolysergol-I, respectively, and N(1)-isopropyl substitution would lead to compounds with substantially enhanced antagonist activity at vascular 5-HT_{2A} receptors of the rat. It has recently been shown by Nelson et al (1993) that several ester and amide derivatives of dihydrolysergic acid that had an N(1)-isopropyl substituent showed higher affinity for rat 5-HT_{2A} receptors than their N(1)-unsubstituted analogues. In order to gain information about the selectivity of the new clavine derivatives within the 5-HT_2 family of receptors, we additionally examined the interaction of the compounds with 5-HT_{2C} receptors. Since ergoline-based compounds generally show high affinity for vascular 5-HT_{1B} receptors and α_1-adrenergic receptors, we examined further the effects of the new esters at these sites. The interactions of the compounds with 5-HT_{2A}, 5-HT_{2C} and 5-HT_{1B} receptors and α_1-adrenergic receptors were studied in various functional in-vitro assays. Selected compounds (**3**–**5**, **9**–**11**) were assayed for radioligand binding at 5-HT_{2C} receptors. A preliminary report of some of these data has been published (Brown et al 1993).

Materials and Methods

Materials

Chemicals used for synthesis were reagent-grade from Sigma-Aldrich, Acros or Merck. The following compounds used for biological experiments were obtained as gifts: cimetidine (SmithKline Beecham, Welwyn, UK), dinoprost tromethamine ($PGF_{2\alpha}$; Upjohn, Heppenheim, Germany), ketanserin tartrate (Janssen, Beerse, Belgium), LY53857 (Eli Lilly and Company, Indianapolis, IN), mesulergine (Novartis, Basle, Switzerland) and (*R*)-phenylephrine (Winthrop, Nordenstedt, Germany). The following compounds were purchased: carbachol (Sigma), cocaine hydrochloride (Merck), 5-hydroxytryptamine creatinine sulphate (Sigma), mepyramine hydrogen maleate (Sigma), [^{3}H]mesulergine (Amersham International), methiothepin mesylate (RBI, Natick, MA), [^{3}H]-*myo*-inositol (New England Nuclear), pargyline (Sigma), phenoxybenzamine hydrochloride (RBI), and (*R*,*S*)-propranolol (Sigma).

Syntheses

^{1}H NMR spectra were recorded on a Bruker AC 300 or AC 400 spectrometer. Chemical shifts are

given in ppm (δ) downfield from TMS. Electron impact mass spectra (EIMS) were obtained using a MAT-711 spectrometer operating at 70 eV. Elemental analyses (C, H, N) for novel compounds were determined with a Perkin-Elmer 240C instrument. Melting points were taken on a Büchi 530 melting point apparatus and are uncorrected. Chemical purifications on a preparative scale were performed by radial centrifugal chrom-atography with a Chromatotron 7924 (Harrison Research, Palo Alto, CA) using glass rotors with 1-, 2- or 4-mm layers of silica gel 60 PF254 containing gypsum (Merck) and appropriate eluents. The Chromatotron chamber was continuously purged with dry nitrogen and protected from light. All experiments were monitored by thin layer chromatography (TLC) using aluminium sheets coated with a 0·2-mm layer of silica gel 60 F254 (Merck) and appropriate eluents. Detection of compounds on TLC was additionally achieved with van Urk's reagent. All reactions were carried out in the dark under an inert atmosphere of dry nitrogen or argon using glassware that had been carefully cleaned and dried overnight in a 120°C oven.

*General procedure for the preparation of O-acylated derivatives of lysergol (**3–5**) and dihydrolysergol-I (**6–8**)*

9,10-Didehydro-6-methyl-8β-ergolinylmethyl R,S-*2-methylbutyrate (**3**)*. To a cooled (0°C) and stirred solution of **1** (1·0 g, 3·93 mmol) in dry pyridine (60 mL) was added dropwise, and in the presence of catalytic amounts of 4-dimethylaminopyridine(4-DMAP), a solution of freshly distilled (*R,S*)-(±)-2-methylbutyrochloride (0·943 g, 7·86 mmol) in $CHCl_3$ (15 mL) over 160 min. After the addition was finished, the reaction mixture was allowed to stand at room temperature overnight. The resulting solution was evaporated to dryness and the residue partitioned between $CHCl_3$ and a saturated solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4 and the solvent removed under vacuum. Radial centrifugal chromatography (Chromatotron instrument, eluent CH_2Cl_2/cyclohexane/MeOH, 100/20/2-100/10/2 (v/v/v)) of the residue afforded a yellow oil. The hydrogen maleate salt of **3** was precipitated from THF/Et_2O to give a white powder: yield 1·27 g (71%); mp 196–197°C (decomposition); ^{1}H NMR (pyridine-d5) δ 0·87, 0·88 (2 t, J = 7·5 Hz, 3H), 1·14, 1·15 (2 d, J = 7·0 Hz, 3H), 1·45 (m, 1H), 1·71 (m, 1H), 2·43 (quasi sext, J = 7·0 Hz, 1H), 2·66 (quasi t, J = 10·0 Hz, 1H, H-7α, 2·74 (s, 3H, NCH_3), 3·05 (quasi t, J = 14·5 Hz, 1H, H-4α), 3·34–3·41 (m, 2H, H-8, H-7α), 3·60 (m, 1H, H-5), 3·69 (dd, J = 14·5, 5·5 Hz, 1H, H-4β), 4·27–4·35 (dd, J = 11·0, 7·0 Hz, 1H, H-17β, dd, J = 11·0, 5·5 Hz, 1H, H-17α, superimposed), 6·59 (br s, 1H, H-9), 6·64 (s, 2H, maleate CH = CH), 7·25 (s, 1H, H-2), 7·32 (t, J = 8·0 Hz, 1H, H-13), 7·39 (d, J = 8·0 Hz, 1H, H-12 or H-14), 7·44 (d, J = 8·0 Hz, 1H, H-12 or H-14), 11·77 (br s, 1H, NH); MS (m/z) 338 ($M^{+\cdot}$, 100). Anal. ($C_{25}H_{30}N_2O_6$) calcd: C 66·1, H 6·7, N 6·2; found: C 66·0, H 6·8, N 6·1.

9,10-Didehydro-6-methyl-8β-ergolinylmethyl S-*2-methylbutyrate (**4**)*. Using the general procedure given above, a 67% yield of **4** as hydrogen maleate was achieved by O-acylation of **1** with (*S*)-(+)-2-methylbutyrochloride; mp 183–185°C (decomposition); ^{1}H-NMR (pyridine-d_5) δ 0·87 (t, J = 7·5 Hz, 3H), 1,16 (d, J = 7·0 Hz, 3H), 1·46 (m, 1H), 1·69 (m, 1H), 2·43 (quasi sext, J = 7·0 Hz, 1H), 2·58 (m, 1H, H-7β), 2·68 (s, 3H, NCH_3), 3·01 (quasi t, J = 14·5 Hz, 1H, H-4α), 3·30–3·40 (m, 2H H-8, H-7α), 3·51 (m, 1H, 5-H), 3·68 (dd, J = 14·5, 5·5 Hz, 1H, H-4β), 4·24–4·34 (dd, J = 11·0, 7·0 Hz, 1H, H-17β, dd, J = 11·0, 5·5 Hz, 1H, H-17α, superimposed), 6·59 (br s, 1H, H-9), 6·65 (s, 2H, maleate CH = CH), 7·25 (s, 1H, H-2), 7·32 (t, J = 8·0 Hz, 1H, H-13), 7·39 (d, J = 8·0 Hz, 1H, H-12 or H-14), 7·44 (d, J = 8·0 Hz, 1H, H-12 or H-14), 11,75 (br s, 1H, NH); MS (m/z) 338 ($M^{+\cdot}$, 100). Anal. ($C_{25}H_{30}N_2O_6$) calcd: C 66·1, H 6·7, N 6·2; found: C 65·7, H 6·7, N 6·1.

*9,10-Didehydro-6-methyl-8β-ergolinylmethyl-2-ethylbutyrate (**5**)*. Using the general procedure above, a 76% yield of **5** as hydrogen maleate was achieved by O-acylation of **1** with 2-ethylbutyrochloride; mp 162–164°C (decomposition); ^{1}H-NMR (pyridine-d_5) δ 0·88 (t, J = 7·5 Hz, 3H), 0·90 (t, J = 7·5 Hz, 3H), 1·45–1·53 (m, 2H), 1·61–1·73 (m, 2H), 2·29 (m, 1H), 2·64 (quasi t, J = 10·0 Hz, 1H, H-7β), 2·72 (s, 3H, NCH_3), 3·04 (quasi t, J = 14·5 Hz, 1H, H-4α), 3·34–3·43 (m, 2H, H-8, H-7α), 3·58 (m, 1H, H-5), 3·69 (dd, J = 14·5, 5·5 Hz, 1H, H-4β), 4·29–4·36 (dd, J = 11·0, 7·0 Hz, 1H, H-17β, dd, J = 11·0, 5·5 Hz, 1H, H-17α, superimposed), 6·60 (br s, 1H, H-9), 6·64 (s, 2H, maleate CH = CH), 7·25 (s, 1H), 7·31 (t, J = 8·0 Hz, 1H, H-13), 7·39 (d, J = 8·0 Hz, 1H, H-12 or H-14), 7·45 (d, J = 8·0 Hz, 1H, H-12 or H-14), 11·79 (br s, 1H, NH); MS (m/z) 352 ($M^{+\cdot}$, 100). Anal. ($C_{26}H_{32}N_2O_6$) calcd: C 66·6, H 6·9, N 6·0; found: C 66·6, H 6·8, N 5·9.

6-Methyl-8β-ergolinylmethyl R,S-*2-methylbutyrate (**6**)*. Using the general procedure above, a 64% yield of **6** as hydrogen maleate was achieved by O-

acylation of **2** with (*R*,*S*)-(±)-2-methylbutyrochloride; mp 184–185°C (decomposition); ^{1}H-NMR (pyridine-d_5) δ 0·88, 0·89 (2 t, J = 7·5 Hz, 3H), 1·16, 1·17 (2 d, J = 7·0 Hz, 3H), 1·37 (q, J = 12·0 Hz, 1H, H-9β), 1·47 (m, 1H), 1·73 (m, 1H), 2·46 (quasi sext, J = 7·0 Hz, 1H), 2·53 (quasi t, J = 11·5 Hz, 1H, H-7β), 2·62 (m, 1H, H-8), 2·70–2·76 (m, partially superimposed, 2H, H-9α, H-5), 2·76 (s, 3H, NCH_3), 3·07 (quasi t, J = 14·5 Hz, 1H, H-4α), 3·39 (m, 1H, H-10), 3·52–3·58 (d, J = 11·5 Hz, 1H, H-7α, dd, J = 14·5, 4·5 Hz, H-4β, superimposed), 4·13 (dd, J = 11·0, 7·0 Hz, 1H, H-17β), 4·29 (dd, J = 11·0, 5·0 Hz, 1H, H-17α), 6·64 (s, 2H, maleate CH = CH), 7·05 (d, J = 7·0 Hz, 1H, H-12 or H-14), 7·23 (s, 1H, H-2), 7·33 (t, J = 8·0 Hz, 1H, H-13), 7·44 (d, J = 8·0 Hz, 1H, H-12 or H-14), 11·98 (br s, 1H, NH); MS (m/z) 340 ($M^{+\cdot}$, 100). Anal. ($C_{25}H_{32}N_2O_6$) calcd: C 65·8, H 7·1, N 6·1; found: C 65·4, H 7·1, N 6·0.

6-Methyl-8β-ergolinylmethyl S-*2-methylbutyrate* (***7***). Using the general procedure above, a 75% yield of **7** as hydrogen maleate was achieved by O-acylation of **2** with (*S*)-(+)-2-methylbutyrochloride; mp 189–191°C (decomposition); ^{1}H-NMR (pyridine-d_5) δ 0·88 (t, J = 7·5 Hz, 3H), 1·16 (d, J = 7·0 Hz, 3H), 1·36 (q, J = 12·0 Hz, partially superimposed, 1H, H-9β), 1·45 (m, 1H), 1·71 (m, 1H), 2·44 (quasi sext, J = 7·0 Hz, 1H), 2·54 (quasi t, J = 11·5 Hz, 1H, H-7β), 2·62 (m, 1H, H-8), 2·72–2·78 (m, partially superimposed, 2H, H-9α, H-5), 2·78 (s, 3H, NCH_3), 3·08 (quasi t, J = 14·5 Hz, 1H, H-4α), 3·41 (m, 1H, H-10), 3·53–3·58 (d, J = 11·5 Hz, 1H, H-7α, dd, J = 14·5, 4·5 Hz, H-4β, superimposed), 4·13 (dd, J = 11·0, 7·0 Hz, 1H, H-17β), 4·29 (dd, J = 11·0, 5·0 Hz, 1H, H-17α), 6·63 (s, 2H, maleate CH = CH), 7·04 (d, J = 7·0 Hz, 1H, H-12 or H-14), 7·23 (s, 1H, H-2), 7·32 (t, J = 8·0 Hz, 1H, H-13), 7·44 (d, J = 8·0 Hz, 1H, H-12 or H-14), 11·79 (br s, 1H, NH); MS (m/z) 340 ($M^{+\cdot}$, 100). Anal. $C_{25}H_{32}N_2O_6$) calcd: C 65·8, H 7·1, N 6·1; found: C 65·7, H 7·0, N 6·1.

6-Methyl-8β-ergolinylmethyl-2-ethylbutyrate (***8***). Using the general procedure above, a 75% yield of **8** as hydrogen maleate was achieved by O-acylation of **2** with 2-ethylbutyrochloride; mp 167–168°C (decomposition); ^{1}H-NMR (pyridine-d_5) δ 0·89 (t, J = 7·5 Hz, 3H), 0·92 (t, J = 7·5 Hz, 3H), 1·38 (q, J = 12·0 Hz, 1H, H-9β), 1·46–1·57 (m, 2H), 1·64–1·75 (m, 2H), 2·31 (m, 1H), 2·55 (quasi t, J = 11·5 Hz, 1H, H-7β), 2·64 (m, 1H, H-8), 2·74–2·78 (m, partially superimposed, 2H, H-9α, H-5), 2·78 (s, 3H, NCH_3), 3·08 (quasi t, J = 14·5 Hz, 1H, H-4α), 3·41 (m, 1H, H-10), 3·54–3·59 (d, J = 11·5 Hz, 1H, H-7α, dd, J = 14·5, 4·5 Hz, H-4β, superimposed), 4·15 (dd, J = 11·0, 7·0 Hz, 1H, H-17β), 4·33 (dd, J = 11·0, 5·0 Hz, 1H, H-17α), 6·63 (s, 2H, maleate, CH = CH), 7·05 (d, J = 7·0 Hz, 1H, H-12 or H-14), 7·23 (s, 1H, H-2), 7·32 (t, J = 8·0 Hz, 1H, H-13), 7·44 (d, J = 8·0 Hz, 1H, H-12 or H-14), 11·76 (br s, 1H, NH); MS (m/z) 354 ($M^{+\cdot}$, 100). Anal. ($C_{26}H_{34}N_2O_6$) calcd: C 66·4, H 7·3, N 6·0; found: C 66·3, H 7·3, N 5·8.

General procedure for the preparation of O-acylated N(1)-isopropyl derivatives of lysergol (***9–11***) *and dihydrolysergol-I* (***12–14***)

9,10-Didehydro-1-isopropyl-6-methyl-8β-ergolinylmethyl R,S-*2-methylbutyrate* (***9***). To a stirred solution of the base of compound **3** (0·18 g, 0·53 mmol) in dry THF (10 mL) was added 18-crown-6 (0·1 g, 0·38 mmol), powdered KOH (0·4 g, 7·13 mmol), and 2-iodopropane (0·52 mL, 5·3 mmol). The mixture was stirred for 1 h, diluted with CH_2Cl_2 and filtered to remove solids. After the filtrate was evaporated to dryness, the residue was partitioned between CH_2Cl_2 and a saturated solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4 and the solvent removed under vacuum. Radial centrifugal chromatography (Chromatotron instrument, eluent CH_2Cl_2/cyclohexane/MeOH, 100/20/2 (v/v/v)) of the residue afforded a yellow oil. The hydrogen maleate salt of **9** was precipitated from THF/Et_2O to give a white powder: yield 0·18 g (69%); mp 176–177°C (decomposition); ^{1}H-NMR (pyridine-d5) δ 0·86, 0·87 (2 t, J = 7·5 Hz, 3H), 1·13, 1·14 (2 d, J = 7·0 Hz, 3H), 1·36 (2 d, J = 6·5 Hz, 6H), 1·45 (m, 1H), 1·70 (m, 1H), 2·42 (quasi sext, J = 7·0 Hz, 1H), 2·63 (quasi t, J = 10·0 Hz, 1H, H-7β), 2·73 (s, 3H, NCH_3), 2·99 (quasi t, J = 14·5 Hz, 1H, H-4α), 3·28–3·39 (m, 2H, H-8, H-7α), 3·54 (m, 1H, H-5), 3·64 (dd, J = 14·5, 5·5 Hz, 1H, H-4β), 4·25–4·33 (dd, J = 11·0, 7·0 Hz, 1H, H-17β, dd, J = 11·0, 5·5 Hz, 1H, H-17α, superimposed), 4·54 (sept, J = 6·5 Hz, 1H), 6·58 (br s, 1H), 6·63 (s, 2H, maleate CH = CH), 7·04 (s, 1H, H-2), 7·30–7·40 (m, 3H, H-12, H-13, H-14); MS (m/z) 380 ($M^{+\cdot}$, 100). Anal. ($C_{28}H_{36}N_2O_6$) calcd: C 67·7, H 7·3, N 5·6; found: C 67·6, H 7·3, N 5·6.

9,10-Didehydro-1-isopropyl-6-methyl-8β-ergolinylmethyl S-*2-methylbutyrate* (***10***). Using the general procedure above, a 59% yield of **10** as hydrogen maleate was achieved by the N(1)-alkylation of **4** with 2-iodopropane; mp 180–181°C (decomposition); ^{1}H-NMR (pyridine-d_5) δ 0·87 (t, J = 7·5 Hz, 3H), 1·14 (d, J = 7·0 Hz, 3H), 1·36 (2 d, J = 6·5 Hz, 6H), 1·45 (m, 1H), 1·70 (m, 1H), 2·43

(quasi sext, J = 7·0 Hz, 1H), 2·59 (m, 1H, H-7β), 2·70 (s, 3H, NCH_3), 2·97 (quasi t, J = 14·5 Hz, 1H, H-4α), 3·28–3·37 (m, 2H, H-8, H-7α), 3·49 (m, 1H, H-5), 3·63 (dd, J = 14·5, 5·5 Hz, 1H, H-4β), 4·26–4·44 (dd, J = 11·0, 7·0 Hz, 1H, H-17β, dd, J = 11·0, 5·5 Hz, 1H, H-17α, superimposed), 4·53 (sept, J = 6·5 Hz, 1H), 6·57 (br s, 1H, H-9), 6·64 (s, 2H, maleate CH = CH), 7·04 (s, 1H, H-2), 7·30–7·42 (m, 3H, H-12, H-13, H-14); MS (m/z) 380 ($M^{+\cdot}$, 100). Anal. ($C_{28}H_{36}N_2O_6$) calcd: C 67·7, H 7·3, N 5·6; found: C 67·7, H 7·3, N 5·5.

*9,10-Didehydro-1-isopropyl-6-methyl-8β-ergolinyl-methyl-2-ethylbutyrate (**11**)*. Using the general procedure above, a 61% yield of **11** as hydrogen maleate was achieved by the N(1)-alkylation of **5** with 2-iodopropane; mp 187–188°C (decomposition); ^{1}H-NMR (pyridine-d_5) δ 0·89 (t, J = 7·5 Hz, 3H), 0·90 (t, J = 7·5 Hz, 3H), 1·36 (2 d, J = 6·5 Hz, 6H), 1·45–1·53 (m, 2H), 1·61–1·73 (m, 2H), 2·29 (m, 1H), 2·56 (quasi t, J = 12·0 Hz, 1H, H-7β), 2·67 (s, 3H, NCH_3), 2·95 (quasi t, J = 14·5 Hz, 1H, H-4α), 3·25–3·34 (m, 2H, H-8, H-7α), 3·46 (m, 1H, H-5), 3·63 (dd, J = 14·5, 5·5 Hz, 1H, H-4β), 4·27–4·33 (dd, J = 11·0, 7·0 Hz, 1H, H-17β, dd, J = 11·0, 5·5 Hz, 1H, H-17α, superimposed), 4·52 (sept, J = 6·5 Hz, 1H), 6·58 (br s, 1H, H-9), 6·65 (s, 2H, maleate CH = CH), 7·04 (s, 1H, H-2), 7·31–7·40 (m, 3H, H-12, H-13, H-14); MS (m/z) 394 ($M^{+\cdot}$, 100). Anal. ($C_{29}H_{38}N_2O_6$) calcd: C 68·2, H 7·5, N 5·5; found: C 68·1, H 7·5, N 5·3.

1-Isopropyl-6-methyl-8β-ergolinylmethyl R,S-*2-methylbutyrate (**12**)*. Using the general procedure above, a 68% yield of **12** as hydrogen maleate was achieved by the N(1)-alkylation of **6** with 2-iodopropane; mp 194–195°C (decomposition); ^{1}H-NMR (pyridine-d_5) δ 0·87, 0·90 (2 t, J = 7·5 Hz, 3H), 1·15, 1·16 (2 d, J = 7·0 Hz, 3H), 1·34 (q, J = 12·0 Hz, partially superimposed, 1H, H-9β), 1·37 (2 d, J = 6·5 Hz, 6H), 1·47 (m, 1H), 1·72 (m, 1H), 2·40–2·47 (m, 2H, *incl*. H-7β), 2·59–2·68 (m, 2H, H-8, H-5), 2·70–2·74 (m, partially superimposed, 1H, H-9α), 2·74 (s, 3H, NCH_3), 3·00 (m, 1H, H-4α), 3·33 (m, 1H, H-10), 3·47–3·52 (d, J = 11·5 Hz, 1H , H-7α, dd, J = 14·5, 4·5 Hz, H-4β, superimposed), 4·11 (dd, J = 11·0, 7·0 Hz, 1H, H-17β), 4·27 (dd, J = 11·0, 5·0 Hz, 1H, H-17α), 4·55 (sept, J = 6·5 Hz, 1H), 6·63 (s, 2H, maleate CH = CH), 7·02 (m, 2H), 7·30–7·40 (m, 2H); MS (m/z) 382 ($M^{+\cdot}$, 100). Anal. ($C_{28}H_{38}N_2O_6$) calcd: C 67·4, H 7·7, N 5·6; found: C 67·1, H 7·7, N 5·5.

1-Isopropyl-6-methyl-8β-ergolinylmethyl S-*2-methyl-butyrate (**13**)*. Using the general procedure above, a 70% yield of **13** as hydrogen maleate was achieved by the N(1)-alkylation of **7** with 2-iodopropane; mp 188–190°C (decomposition); ^{1}H-NMR (pyridine-d_5) δ 0·88 (t, J = 7·5 Hz, 3H), 1·16 (d, J = 7·0 Hz, 3H), 1·36 (q, J = 12·0 Hz, partially superimposed, 1H, H-9β), 1·39 (2d, J = 6·5 Hz, 6H), 1·45 (m, 1H), 1·72 (m, 1H), 2·41–2·51 (m, 2H, *incl*. H-7β), 2·57–2·68 (m, 2H, H-8, H-5), 2·71–2·76 (m, partially superimposed, 1H, H-9α), 2·76 (s, 3H, NCH_3), 3·01 (m, 1H, H-4α), 3·34 (m, 1H, H-10), 3·46–3·56 (d, J = 11·5 Hz, 1H , H-7α, dd, J = 14·5, 4·5 Hz, H-4β, superimposed), 4·13 (dd, J = 11·0, 7·0 Hz, 1H, H-17α), 4·27 (dd, J = 11·0, 5·0 Hz, 1H, H-17β), 4·55 (sept, J = 6·5 Hz, 1H), 6·63 (s, 2H, maleate CH = CH), 7·01 (m, 2H), 7·20–7·34 (m, 2H); MS (m/z) 382 ($M^{+\cdot}$, 100). Anal. ($C_{28}H_{38}N_2O_6$) calcd: C 67·4, H 7·7, N 5·6; found: C 67·1, H 7·7, N 5·5.

*1-Isopropyl-6-methyl-8β-ergolinylmethyl-2-ethyl-butyrate (**14**)*. Using the general procedure above, a 60% yield of **14** as hydrogen maleate was achieved by the N(1)-alkylation of **8** with 2-iodopropane. mp 195–196°C (decomposition); ^{1}H-NMR (pyridine-d_5) δ 0·90 (t, J = 7·5 Hz, 3H), 0·92 (t, J = 7·5 Hz, 3H), 1·37 (q, J = 12·0 Hz, partially superimposed, 1H, H-9β), 1·39 (2 d, J = 6·5 Hz, 6H), 1·47–1·57 (m, 2H), 1·64–1·75 (m, 2H), 2·31 (m, 1H), 2·52 (quasi t, J = 11·5 Hz, 1H, H-7β), 2·55 (m, 1H, H-8), 2·72–2·78 (m, partially superimposed, 2H, H-9α, H-5), 2·78 (s, 3H, NCH_3), 3·03 (m, 1H, H-4α), 3·36 (m, 1H, H-10), 3·48–3·56 (d, J = 11·5 Hz, 1H, H-7α, dd, J = 14·5, 4·5 Hz, H-4β, superimposed), 4·14 (dd, J = 11·0, 7·0 Hz, 1H, H-17β), 4·33 (dd, J = 11·0, 5·0 Hz, 1H, H-17α), 4·56 (sept, J = 6·5 Hz, 1H), 6·63 (s, 2H, maleate CH = CH), 7·02 (m, 2H), 7·30–7·41 (m, 2H); MS (m/z) 396 ($M^{+\cdot}$, 100). Anal. ($C_{29}H_{40}N_2O_6$) calcd: C 67·9, H 7·9, N 5·5; found: C 67·9, H 7·8, N 5·4.

Biological experiments

Functional 5-HT$_{2A}$ receptor assay (rat tail artery). Male Wistar rats (280–350 g) were killed by cervical dislocation. 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 8 cylindrical segments of 4–5 mm length were prepared from each artery and were horizontally suspended between two L-shaped stainless-steel hooks (diameter 0·15 mm) gently inserted into the lumen for the recording of contractile responses. 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 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). Three cumulative concentration effect curves for 5-HT were determined on each arterial segment at intervals of 60 min. Antagonists were incubated for 30 min. The blocking effect of a given concentration of antagonist was the same irrespective of whether it was determined in a second or a third concentration–effect curve for 5-HT. It could be shown that 3 successive control curves (in the absence of antagonist) were nearly superimposable. The pEC50 values (negative logarithm of the molar concentration of agonist which produces 50% maximum contractile effect) for 5-HT were $6{\cdot}61 \pm 0{\cdot}06$, $6{\cdot}54 \pm 0{\cdot}06$ and $6{\cdot}56 \pm 0{\cdot}06$. ($E_{max}$ values (maximum effects, expressed as percentage of the maximum effect of 3-HT in the first curve) were 100, 100 ± 1 and $101 \pm 1\%$ (n = 25).

5-HT_{2C} receptor assays (piglet choroid plexus): radioligand binding studies. Binding studies were carried out essentially as described previously (Brown et al 1991). Membranes prepared from piglet choroid plexus were initially incubated at 37°C for 30 min in a medium containing 50 mM Tris–Cl (pH 7·7 at room temperature), 4 mM $CaCl_2$, 10 μM pargyline, 0·1% ascorbic acid, and in a total volume of 1 mL. For binding inhibition studies, drugs were added in 250-μL amounts, [^{3}H]mesulergine (final concentration about 1 nM) in 250-μL amounts and membranes in 500-μL amounts (30–75 μg protein/assay tube), with all incubation tubes on ice. The incubations were started by transfer of the tubes to a water bath at 37°C and terminated after 30 min by rapid filtration through Whatman GF/C filters on a Brandel Harvester followed by three rapid washes with 3 mL of ice-cold 50 mM Tris–Cl (pH 7·4, room temperature), 4 mM $CaCl_2$. The filters were then counted for radioactivity using scintillation spectrometry. Protein was determined according to Bradford (1976).

The saturation binding isotherm for [^{3}H]mesulergine was determined using 0·05–80 nM [^{3}H]mesulergine. 1 μM mesulergine was used to define non-specific binding. At 1 nM [^{3}H]mesulergine, as used in the binding inhibition experiments, approximately 60% of the total [^{3}H] binding was specific. For all binding experiments, data were analysed by non-linear regression using GraFit (Erithacus Software Ltd., Staines, UK). Saturation binding and binding inhibition data were well fitted by equations assuming interactions at a single site and yielding a value for the binding equilibrium constant, K_D.

Functional studies: measurement of inositol phosphate accumulation. The method for measuring accumulation of inositol phosphates was carried out essentially as described previously (Brown et al 1991). Piglets (Camborough Hi-Bred, 5–9 days old) from a local farm were anaesthetised with halothane and killed by removal of the heart (Kaumann 1990). The skull was opened, the brain removed and the choroid plexus rapidly dissected and transferred to Na-Krebs medium at 37°C gassed with 95% O_2/5% CO_2. The Na-Krebs medium contained (mM): Na^+ 149; K^+ 6·4; Mg^{2+} 1·3; Ca^{2+} 0·8; Cl^- 128; HCO_3^- 26; phosphate 1·4; SO_4^{2-} 1·3; D-glucose 10. For each experiment, plexi were collected from 9 piglets (total time less than 2 h) and pooled. The choroid plexi were cross-chopped on a McIlwain tissue chopper set at 300 μm. The slices were washed twice by allowing them to settle under gravity, removing the supernatant and then resuspending them in fresh, gassed Na-Krebs at 37°C. After each resuspension, the slices were incubated for about 10 min before further processing, with gassing over the surface of the suspension. The slices were finally suspended in 6 mL of fresh Na-Krebs and 50 μCi of [^{3}H]-*myo*-inositol (10–20 Ci mmol^{-1}; stock solution dried under nitrogen and redissolved in Na-Krebs) was added. The slices were then incubated for 1·5–2 h at 37°C with constant gassing directed as a jet on to the surface of the suspension to provide good mixing.

After this incubation, 40 mL of Li-Krebs (as Na-Krebs but with 10 mM NaCl replaced by 10 mM LiCl) was added. The slices were mixed with a plastic Pasteur pipette and allowed to settle. The supernatant was discarded and replaced with fresh Li-Krebs and the slices incubated for 5 min at 37°C. This process was repeated twice more and the slices were finally respended in an appropriate volume of Li-Krebs containing (final concentrations in incubation vials) 100 μM pargyline (to inhibit monoamine oxidase), 6 μM cocaine (to inhibit possible tissue 5-HT uptake) and 0·2 mM ascorbic acid (to inhibit oxidation of 5-HT) and stirred gently with

magnetic stirring ready for distribution to the incubation vials.

Incubations were carried out in Beckman Bio-vials in Li-Krebs for 1 h at 37°C in a shaking water bath. The total incubation volume was 300 μL. Li was included in the incubation medium to prevent breakdown of inositol monophosphate and hence cause the inositol phosphates to accumulate over the incubation period. Drugs were added in a total volume of 20 μL. Agonists (5-HT, ergots) were dissolved in 2 mM ascorbic acid and added in 10-μL quantities. Mesulergine was dissolved in Li-Krebs containing 0·2 mM ascorbic acid and 10 μL was added. Incubations were started by addition of 280 μL of the slice suspension. The vial contents were gassed and mixed with a jet of 95% O_2/5% CO_2 and sealed. Incubations were terminated by addition of 300 μL of 7% perchloric acid after which the vials were placed on ice for 15 min. The contents of the vials were then centrifuged and 0·55 mL of the supernatant mixed well with 0·625 mL of (trioctylamine:1,1,2-trichlorotrifluoroethane, 1 : 1) to extract the perchloric acid. After centrifugation, 0·4 mL of the top (aqueous) phase was pipetted onto columns containing ion-exchange resin (Biorad AG 1-X8, 200–400 mesh, formate form) in 5 mL Milli-Q purified water. The columns were washed with a further 20 mL water to remove inositol. The inositol phosphates were then eluted into scintillation vials with 10 mL of a solution containing 1·05 M ammonium formate and 0·1 M formic acid. ^{3}H content of each vial was then determined by scintillation counting.

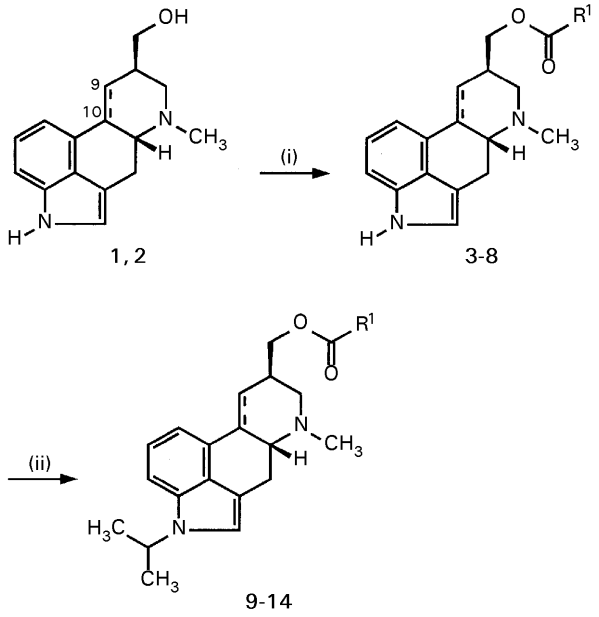
Functional 5-HT$_{1B}$ receptor assay (guinea-pig iliac artery). Guinea-pigs of either sex, 300–450 g, were stunned by a blow on the neck and bled. The abdominal aorta and the right and left common iliac arteries were removed and cleared of adhering connective tissue. Two or three cylindrical segments of 1–2 mm length from each iliac artery were horizontally suspended between two L-shaped stainless-steel hooks (diameter 0·15 mm) and isometrically mounted as described for rat tail artery experiments (see above). The bath fluid (modified Krebs-Henseleit solution with $CaCl_2$ 1·25 mM and D-glucose 11·5 mM at 37°C, gassed with 95% O_2/5% CO_2) contained ketanserin (1 μM), mepyramine (0·3 μM), cimetidine (30 μM), and cocaine (30 μM) to block 5-HT$_{2A}$ receptors, α_1-adrenergic receptors, histamine H_1 receptors, histamine H_2 receptors, and neuronal uptake of 5-HT. The applied resting force was 5 mN. During a stabilization period of 30 min, the organs were exposed to the irreversible α_1-adrenergic-receptor blocking drug phenoxybenzamine (0·3 μM). After wash-out the organs were allowed to equilibrate for a further 4 h. During this period the organs were stimulated after 100 min with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 30 μM). Relaxation was achieved by subsequent addition of carbachol (10 μM). After 175 min the organs were moderately precontracted with a concentration of $PGF_{2\alpha}$ which produced 10–20% effect of the inital $PGF_{2\alpha}$ stimulation of 30μM (50–500μM). Subsequently, the organs were stimulate with 5-HT (0·3μM). Two cumulative concentration–effect curves for 5-HT were determined at an interval of 80 min in the absence and presence of **3**–**5** and **9**–**11**, respectively, on organs precontracted with an EC10-EC20 of $PGF_{2\alpha}$ as above. Compounds **3**–**5** and **9**–**11** were incubated for 45 min and failed to produce contractile effects when added before the $PGF_{2\alpha}$-induced precontraction.

Functional α_1-adrenergic receptor assay (rat aorta). The thoracic aorta was removed from rats used for studies at 5-HT$_{2A}$ receptors in 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 (diameter 0·3 mm). The organs were isometrically mounted as described for rat tail artery experiments (see above). The bath fluid (modified Krebs-Henseleit solution as in rat tail artery experiments at 37°C, gassed with 95% O_2/5% CO_2) contained (*R*,*S*)-propranolol (1 μM) to block β-adrenergic receptors. 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.

Results

Chemistry

LY53857 is the prototype in a series of 6-methylergoline-8β-carboxylic acid esters (Marzoni et al 1987; Garbrecht et al 1988) that shows potent 5-HT$_{2A}$ receptor antagonist activity (Cohen et al 1983). We intended to synthesize structurally related O-acylated derivatives of 9,10-didehydro-8β-hydroxymethyl-6-methylergoline (lysergol) and



Compounds		Substituent
C-9–C10-double bond	C-9–C10-single bond, 10α-H	R^1
1 (Lysergol)	2	–
3	6	CH_3
9	12	CH_3
4	7	H_3C H
10	13	CH_3
5	8	CH_2CH_3
11	14	CH_3

Figure 2. Synthesis of O-acylated derivatives of lysergol (**1**) and dihydrolysergol-I (**2**). Reagents and experimental conditions: i. pyridine, 4-DMAP, addition of the respective acid chloride at 0°C within 160 min, then stirring for 12 h at room temperature; ii. dry THF, 18-crown-6, powdered KOH, isopropyl iodide, stirring for 1 h at room temperature.

8β-hydroxymethyl-6-methylergoline (dihydrolysergol-I), so-called ergoline reverse esters, which share with LY53857 an isopropyl substituent at N(1) and an aliphatic branched-chain substituent at the C-8β position. Lysergol (**1**) and dihydrolysergol-I (**2**) served as starting points for the preparation of the esters. While **1** was prepared from the naturally occurring major clavine alkaloid elymoclavine by heating on activated Al_2O_3 in pyridine (Eich 1975), **2** was prepared from **1** by catalytic hydrogenation with Pd 10%/C in DMF/pyridine (100 : 2) at 5 bar (Polgar et al 1990). O-Acylation of **1** and **2** to afford **3–8** was achieved by the Einhorn reaction using the respective aliphatic branched-chain acid chloride in pyridine in the presence of 4-DMAP. Introduction of the isopropyl substituent at N(1) to afford **9–14** was achieved by addition of isopropyl iodide to a solution of the corresponding O-acylated lysergol or dihydrolysergol-I derivative in THF using as base powdered KOH in the presence of 18-crown-6 (Figure 2). After work-up, all compounds were purified and separated by radial chromatography under an atmosphere of nitrogen or argon.

Biology

The effectiveness of the compounds in blocking 5-HT_{2A} receptor-mediated contractions by 5-HT was determined in cylindrical segments of the isolated rat tail artery. 5-HT_{2C} receptor binding affinities and stimulation of 5-HT_{2C} receptor-linked phospholipase C (phosphoinositide hydrolysis) of selected compounds (**3–5**, **9–11**) were studied in piglet choroid plexus membranes. The radioligand used was 1 nM [^{3}H]mesulergine. Selected compounds (**3–5**, **9–11**) were examined as antagonists of 5-HT-induced contractions mediated by 5-HT_{1B} receptors in cylindrical segments of the isolated guinea-pig iliac artery, moderately precontracted by $PGF_{2\alpha}$ (50–500 nM). The ability of the compounds to block α_1-adrenergic receptor-mediated contractions by (*R*)-phenylephrine was studied in cylindrical segments of the isolated rat aorta.

The effects of compounds **3–14** on 5-HT_{2A} and α_1-adrenergic receptors are summarized in Table 1; the effects of compounds **3–5** and **9–11** on 5-HT_{2C} and 5-HT_{1B} receptors are summarized in Tables 2 and 3, respectively. Affinities were estimated using apparent pA_2 values for those compounds that antagonized the effects of the agonist at a single concencentration of antagonist. Affinities of competitive antagonists were estimated using full pA_2 values according to the method of Arunlakshana & Schild (1959). For the calculation of full pA_2 values, the slope of the Schild plot was constrained to unity unless it was significantly different from unity. Agonist potencies of partial agonists were determined according to their respective pEC50 values. Since several ergolines with partial agonist properties were able to antagonize the effect of 5-HT at the 5-HT_{2C} receptor site, it was possible to determine the affinity values (pK_P) using the method of Marano & Kaumannn (1976). The

Table 1. Effects of O-acylated derivatives of lysergol (**3**–**5**, **9**–**11**) and dihydrolysergol-I (**6**–**8**, **12**–**14**) on 5-HT-induced contractions of rat tail artery and (*R*)-phenylephrine-induced contractions of rat aorta.

	5-HT_{2A} receptor		α_1-Adrenoceptor		Specificity	
Compound	Affinity[a]	m[b]	n	Affinity[c]	n	5-HT_{2A/α_1}
1[e]	7·66 ± 0·02[f]	–	8	6·35 ± 0·03	4	20
2[e]	6·27 ± 0·02[c]	–	5	6·21 ± 0·06	4	1
3	8·40 ± 0·07	0·97 ± 0·05	17	5·47 ± 0·06	4	851
4	8·34 ± 0·10	0·98 ± 0·04	17	5·26 ± 0·12	4	1202
5	8·07 ± 0·09	1·19 ± 0·12	17	6·26 ± 0·12	4	65
6	7·82 ± 0·07	0·92 ± 0·05	17	n.d.	–	–
7	8·10 ± 0·04	0·97 ± 0·03	23	6·13 ± 0·10	4	93
8	7·65 ± 0·04	0·98 ± 0·04	18	6·71 ± 0·08	4	9
9	8·31 ± 0·09	0·97 ± 0·07	16	4·76 ± 0·08	4	3548
10	8·13 ± 0·08	1·09 ± 0·09	25	4·77 ± 0·13	4	2290
11	7·99 ± 0·10	1·00 ± 0·10	17	5·51 ± 0·08	4	302
12	7·88 ± 0·07	1·08 ± 0·07	20	n.d.	–	–
13	7·90 ± 0·06	1·00 ± 0·05	22	4·78 ± 0·04	4	1318
14	7·33 ± 0·06	1·08 ± 0·08	16	6·15 ± 0·05	4	15

Values are expressed as mean ± s.e.m. from n individual vascular segments. [a]Full pA_2 values according to the method of Arunlakshana & Schild (1959). [b]Slopes of the regression lines of Schild plots. [c]Apparent pA_2 values for antagonists from single point analysis at 1–30 μM antagonist. [d]K_B (α_1)/K_B (5-HT_{2A}). [e]Data from Pertz (1996). [f]pK_P value, calculated from the antagonism of the 5-HT response by **1** (1 μM). n.d. = not determined.

Table 2. Effects of 5-HT, lysergol (**1**) and O-acylated derivatives of lysergol (**3**–**5**, **9**–**11**) on inositol phosphate accumulation and [^{3}H]mesulergine binding in piglet choroid plexus.

Compound	5-HT_{2C} receptor-mediated inositol phosphate accumulation				5-HT_{2C} pK_D[d]	5-HT_{2A} pA_2
	pEC50	α[a]	log r[b]	pK_P[c]		
5-HT	7·7	1·0	1·8	–	8·1	–
1	7·0	0·4	1·8	7·4	7·7	–
3	7·2	0·24	2·0	7·0	n.d.	8·4
4	7·1	0·20	1·8	7·0	7·9	8·3
5	6·7	0·25	1·8	6·9	8·4	8·1
9	–	n.a.	–	–	8·3	8·3
10	–	n.a.	–	–	8·9	8·1
11	–	n.a.	–	–	8·4	8·0

[a]Intrinsic activity relative to 5-HT. [b]Concentration ratio for antagonism by mesulergine (100 nM). [c]−Log K_P, calculated from antagonism of the 5-HT response by **1** and **3**–**5**. [d]−Log K_D, from inhibition of [^{3}H]mesulergine binding (1 nM). [e]From blockade of 5-HT_{2A} receptor-mediated responses in rat tail artery (data from Table 1). n.a. = not measurable activity. n.d. = not determined.

Table 3. Effects of O-acylated derivatives of lysergol (**3**–**5**, **9**–**11**) on 5-HT-induced contractions in guinea-pig iliac artery moderately precontracted by $PGF_{2\alpha}$ (50–500 nM).

Compound	5-HT_{1B} receptor			Selectivity 5-HT_{1B}/5-HT_{2A}[c]
	Log r[a]	E_{max}[b]	n	
3	0·78 ± 0·11[d]	74 ± 5	6	2
4	0·67 ± 0·09[d]	82 ± 6	5	2
5	0·73 ± 0·05[d]	76 ± 7	5	0·5
9	1·04 ± 0·04[e]	66 ± 5	5	65
10	1·08 ± 0·05[e]	59 ± 3	5	33
11	1·11 ± 0·11[e]	67 ± 5	7	28

[a]Log r (concentration ratio) values (calculation of pA_2 values not appropriate for partial agonists). [b]E_{max} values are expressed as percentage of the maximum response to 5-HT in the first curve. [c]K_B (5-HT_{2A})/K_B (5-HT_{1B}). [d]Concentration ratio for antagonism of the 5-HT response by the respective compound at 30 nM. [e]Concentration ratio for antagonism of the 5-HT response by the respective compound at 3 μM.

insurmountable blockade by **3**–**5** and **9**–**11** of the contractile effect of 5-HT in guinea-pig iliac artery was quantified by estimation of log *r* (concentration ratio) values instead of pA_2 values (calculation of pA_2 values not appropriate for partial agonists) at a concentration of antagonist of 30 nM for compounds **3**–**5** and 3 μM for **9**–**11**. The methods used in functional and binding experiments of the present study have also been described in earlier reports (Brown et al 1991; Pertz & Eich 1992; Pertz 1993).

Discussion

In previous reports, we showed that the naturally occurring clavine alkaloid lysergol (**1**) behaved as a partial agonist at vascular 5-HT_{1B} receptors and 5-HT_{2A} receptors, while its 9,10-dihydro analogue dihydrolysergol-I (**2**) acted as a silent but weak 5-HT_{2A} receptor antagonist. In addition, we found that **1** had 20-fold 5-HT_{2A} versus α_1-adrenergic receptor affinity, while **2** was equipotent at both sites (Pertz 1993, 1996). Since ergoline esters such as LY53857 and sergolexole were shown to block potently smooth muscle contractile 5-HT_{2A} receptors without blocking α_1-adrenergic receptors (Cohen et al 1983, 1988; Garbrecht et al 1988), the present study was aimed at synthesizing so-called ergoline reverse esters by O-acylation of **1** and **2** of which an antagonist profile similar to that of LY53857 and sergolexole could be expected.

Esterfication of aliphatic branched-chain carboxylic acids with **1** resulted in compounds **3**–**5** and **9**–**11** with decreased agonist activity but enhanced antagonist activity at 5-HT_{2A} receptors. Only **3** at 1 μM produced a slight contraction of $5 \pm 1\%$ (n = 14, not shown) by itself that was abolished by 0·1 μM LY53857 (n = 3, not shown). O-Acylated derivatives of lysergol **3**–**5** and **9**–**11** exhibited 2- to 6-fold higher affinity for 5-HT_{2A} receptors than the parent alcohol **1**. Similarly, conversion of **2** into the corresponding esters **6**–**8** and **12**–**14** afforded ligands with enhanced 5-HT_{2A} receptor antagonist activity. O-Acylated derivatives of dihydrolysergol-I (**6**–**8**, **12**–**14**) exhibited 12- to 68-fold higher affinity for 5-HT_{2A} receptors than the parent alcohol **2**. In contrast to the classical ergoline methysergide and LY53857 which acted as insurmountable 5-HT_{2A} receptor antagonists (Frenken & Kaumann 1987; Kaumannn 1989; Pertz & Eich 1992), compounds **3**–**14** exhibited competitive antagonism of the 5-HT_{2A} receptor-mediated contractile response of 5-HT in rat tail artery with calculated pA_2 values of 7·33–8·40 (Table 1). It has previously been shown that the most potent compound in this series, **3**, acted as an allosteric activator of the 5-HT_{2A} receptor system, since it was able to reverse the depressant effect of the insurmountable 5-HT_{2A} receptor antagonist methysergide (Pertz & Eich 1992). Analysis of the affinities of **3**–**14** shows that esters with a C-9 to C-10 double bond (**3**–**5**, **9**–**11**) seem to be more potent 5-HT_{2A} receptor antagonists than the corresponding esters without a double bond (**6**–**8**, **12**–**14**). In contrast to the ergoline esters examined as 5-HT_{2A} receptor antagonists by Nelson et al (1993), the introduction of an isopropyl substituent at the N(1)-position of **3**–**8** to give **9**–**14** failed to enhance 5-HT_{2A} receptor affinity in the rat. On the other hand, substitution with isopropyl at N(1) resulted in lower affinity for α_1-adrenergic receptors. Especially **9**–**11** seem to be highly specific antagonists at 5-HT_{2A} receptors (see ratio 5-HT_{2A}/α_1 of 302–3548 for **9**–**11** in Table 1). Furthermore, it is interesting to note that replacement of the racemic acyl portion of **3** and **9** and **6** and **12**, respectively, by the respective (*S*)-form to give **4** and **10** and **7** and **13**, respectively, resulted in only small differences in 5-HT_{2A} receptor affinities. Similar findings with the four diastereomers of LY53857, which were nearly equipotent as antagonists of 5-HT at 5-HT_{2A} receptors, were obtained by Cohen et al (1985). Thus, the current results provide further evidence that the stereochemical configuration of the substituent at C-8 of simple ergolines is not crucial for binding to the 5-HT_{2A} receptor site (Cohen et al 1985).

Further experiments were performed to find out whether the ergolines **3**–**14** were able to discriminate between 5-HT_{2A} and 5-HT_{2C} receptors. It has recently been shown that ergotamine and dihydroergotamine were among the most potent full agonists reported so far at 5-HT_{2C} receptors in piglet choroid plexus membranes (Brown et al 1991), whereas dihydroergocristine, dihydroergotoxine, ergometrine and bromocriptine were partial agonists in this preparation (Brown et al 1992). Therefore, it was of special interest to examine the stimulant properties of **3**–**14** on 5-HT_{2C} receptor-linked phospholipase C in piglet choroid plexus membranes. Lysergol (**1**) and its N(1)-unsubstituted derivatives **3**–**5** were partial agonists for inositol phosphate accumulation (α of 0·2–0·4, Table 2). Mesulergine (100 nM) caused surmountable blockade of the stimulant effects of 5-HT, **1** and **3**–**5**, shifting the concentration–effect curves to the right in a nearly parallel manner and to similar extent. The similarity of the concentration ratios for these agonists (Table 2) is consistent with 5-HT_{2C} receptor interaction. In contrast to **3**–**5**, derivatives **9**–**11** which are structurally characterized by an isopropyl substituent at N(1),

showed no measurable activity as activators of 5-HT_{2C} receptor-linked phospholipase C in piglet choroid plexus membranes. In addition, O-acylated derivatives of dihydrolysergol-I (**6**–**8**, **12**–**14**) had marginal activity in stimulating phosphoinositide hydrolysis (α of 0·02–0·06, not shown). The partial agonists **1** and **3**–**5** were further examined as blockers of the response to 5-HT on inositol phosphate accumulation. Compounds **1** and **3**–**5** caused typical rightward shifts of the concentration–effect curve to 5-HT. The effects were characterized by estimation of the equilibrium dissociation constant pK_P for the ergoline-5-HT_{2C} receptor complex (Marano & Kaumann 1976). pK_P values calculated from the antagonism of the 5-HT effects by **1** and **3**–**5** were in good agreement with the respective pEC50 values calculated from the stimulant effects of these compounds on inositol phosphate accumulation (Table 2). To further investigate the SAR of O-acylation, derivatives of lysergol (**1**) were examined as inhibitors of [^{3}H]mesulergine binding in piglet choroid plexus membranes. As compared with the parent drug **1**, O-acylation caused a 2- to 16-fold increase in affinity for the 5-HT_{2C} receptor binding site. The observation that lysergol and its O-acylated derivatives showed similar affinities for both 5-HT_{2C} and 5-HT_{2A} receptors reflects the pharmacological commonality of these sites as members of the 5-HT_2 family of 5-HT receptors (Baxter et al 1994; Bonhaus et al 1995). The most potent compound in the series was **10** ($pK_D = 8{\cdot}9$), which did not show any stimulant effect on inositol phosphate accumulation and had a 6-fold lower affinity for 5-HT_{2A} receptors ($pA_2 = 8{\cdot}13$). The latter finding needs to be interpreted with caution because it is critical to compare pK_D values with pA_2 values. Furthermore, it cannot be excluded that species differences (rat vs pig) might account for the difference in affinity for both sites. For the sake of completeness it should be mentioned that there was no good agreement between the pK_D values of partial agonists **4** and **5** determined from radioligand binding studies and the respective pK_P values from antagonism of the 5-HT response by these compounds in phosphoinositide hydrolysis (Table 2). The reasons for this dichotomy are difficult to rationalize. Similar observations with partial agonists in pig choroid plexus were made by Hoyer et al (1989). A potential explanation may be that partial agonists (e.g. **4** and **5**) and 5-HT are able to bind at different sites on the 5-HT_{2C} receptor.

In preliminary experiments it was shown that **3** behaved as a partial agonist at 5-HT_{1B} receptors of guinea-pig iliac artery moderately precontracted by $PGF_{2\alpha}$. The flat concentration–effect curve and the low pA_2 value of 7·8 for the antagonism by methiothepin (0·1 μM) of the contractile response of **3** makes it doubtful that the compound interacted with a single type of 5-HT receptor. Similar observations with methysergide and metergoline in guinea-pig iliac artery moderately precontracted by $PGF_{2\alpha}$ were made by Schoeffter & Sahin-Erdemli (1992). On the other hand, these authors demonstrated in the same study that partial agonists could be used as antagonists since they failed to contract the artery when added before the $PGF_{2\alpha}$-induced precontraction. A similar pharmacological profile could be demonstrated in the present study for selected compounds **3**–**5** and **9**–**11**. When incubated before the $PGF_{2\alpha}$-induced precontraction, **3**–**5** and **9**–**11** did not induce contractions by themselves but insurmountably blocked the contractile effect of 5-HT. Concentration–effect curves to 5-HT were concentration-dependently shifted to the right by **3**–**5** and **9**–**11** with a depression of the maximum responses of 18–41% (Table 3). Compounds **9**–**11** with isopropyl at N(1) showed a 30- to 50-fold lower affinity for 5-HT_{1B} receptors than their corresponding N(1)-unsubstituted analogues **3**–**5**. Thus, O-acylated derivatives of lysergol with an N(1)-isopropyl substitution did not only show high specificity (ratio 5-HT_{2A}/α_1 of 302–3548) in the rat but also sufficient selectivity (ratio 5-HT_{2A}/5-HT_{1B} of 28–65) at vascular 5-HT receptors of rat and guinea-pig.

In summary, a series of O-acylated derivatives of the ergolines lysergol and dihydrolysergol-I were synthesized and found to be more potent 5-HT_{2A} receptor antagonists than their naturally occurring parent drugs. Additionally, the compounds showed similar affinities for 5-HT_{2C} receptors. The similarities in the selectivity of 5-HT_{2A} and 5-HT_{2C} receptors for the compounds tested demonstrated that they are not suitable tools to discriminate between these closely related subtypes within the family of 5-HT_2 receptors. The difference that N(1)-unsubstituted derivatives of lysergol had partial agonist properties at 5-HT_{2C} receptors whereas their agonist activity was marginal or not measurable at 5-HT_{2A} receptors, suggests that these compounds have different abilities to cause conformational change at the two receptor types. On the other hand, derivatives of lysergol that had an N(1)-isopropyl substituent were potent 5-$HT_{2A/C}$ receptor antagonists which showed no measurable agonist activity at both sites. Furthermore, O-acylated derivatives of lysergol with an N(1)-isopropyl substitution showed lower affinity for 5-HT_{1B} receptors and negligible affinity for α_1-adrenergic receptors.

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