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ACTIVITY OF SEROTONIN (5-HT) RECEPTOR AGONISTS, PARTIAL AGONISTS AND ANTAGONISTS AT CLONED HUMAN 5-HT_{1A} RECEPTORS THAT ARE NEGATIVELY COUPLED TO ADENYLATE CYCLASE IN PERMANENTLY TRANSFECTED HELA CELLS

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Abstract—The activity of serotonin (5-HT) receptor agonists, partial agonists and antagonists, and various other neurotransmitter receptor antagonists at human 5-HT_{1A} receptors that are negatively coupled to adenylate cyclase in permanently transfected HeLa cells was investigated. 5-HT_{1A} receptor-mediated inhibition of adenylate cyclase was studied by measuring inhibition of cAMP accumulation, induced by forskolin. At 100 μM forskolin produced a 100-fold increase in cAMP formation: 5-HT concentration dependently inhibited the cAMP formation; maximal inhibition was attained at 1 μM 5-HT and represented 90% of the stimulated cAMP formation. Full inhibition was observed with 5-HT_{1A} receptor agonists: *N,N*-dipropyl-8-hydroxy-2-aminotetralin (8-OH-DPAT) and flesinoxan, and non-selective 5-HT receptor agonists: *d*-lysergic acid diethylamide (d-LSD), RU 24,969, bufotenine, methysergide and tryptamine. The rank order of potency of the compounds for inhibiting the cAMP formation corresponded to the rank order of the binding affinities of the drugs for the 5-HT_{1A} receptor. Partial inhibition was obtained with submicromolar concentrations of buspirone, spiroxatrine and ipsapirone. A slight inhibition was observed with 1 μM 5-HT receptor agonist CP 93129 and 1 μM 5-HT receptor antagonists mesulergine and BW-501. No inhibition was found with: the 5-HT receptor agonists quipazine, sumatriptan and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM); the 5-HT receptor antagonist ICS-205,930; and other neurotransmitter receptor antagonists such as pindolol, CGP 20712-A, prazosin, sulpiride and pyrilamine. Spiperone and pindolol fully antagonized the agonist-mediated inhibition of forskolin-stimulated cAMP formation. Partial inhibition of the agonist-mediated inhibition of forskolin-stimulated cAMP formation was apparent with 1 μM ocaperidone and 1 μM ipsapirone. It can be concluded that HeLa cells, permanently expressing human 5-HT_{1A} receptors, are a valid cellular system for studying the negative coupling of 5-HT_{1A} receptors to adenylate cyclase and the action of compounds thereupon.

Serotonin (5-HT†) receptors have been classified, mainly based on radioligand binding properties, signal transduction mechanisms and deduced amino acid sequences, into at least seven subtypes [1–3]. 5-HT_1, 5-HT_2 and 5-HT_4 receptors transduce extracellular signals by activating G-proteins and mediate slow modulatory responses via second messenger signalling pathways. 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors are negatively coupled to adenylate cyclase [4], whereas 5-HT_4 receptors are positively coupled to adenylate cyclase [5]. 5-HT_{1C} and 5-HT_2 receptors operate through stimulation of the turnover of phosphatidyl inositol, in which diacylglycerol and inositol trisphosphate have second messenger functions [6–8]. In contrast, the 5-HT_3 receptor is a ligand-gated ion channel, which when activated causes fast depolarizing responses [9, 10].

The measurement of the negative coupling of 5-HT receptors to adenylate cyclase in rodent and calf brain membrane preparations, as well as in neuronal cultures, has always been difficult. The inhibition of adenylate cyclase mediated by 5-HT_{1A} receptors in these preparations was never very pronounced; 5-HT receptor agonists inhibited maximally 30% of stimulated cAMP formation [11–14]. The availability of mammalian cell lines which permanently express cloned 5-HT_{1A} receptors is a major advance for the measurement of the effector coupling mechanism of these receptors. The cloned rat 5-HT_{1A} receptor in GH4C1 pituitary cells is negatively coupled to adenylate cyclase and reduces basal (by 30–60%) as well as stimulated (by 60–80%) cAMP accumulation [15]. In HeLa cells permanently expressing the human 5-HT_{1A} receptor gene, nanomolar concentrations of 5-HT mediate 90% inhibition of forskolin-stimulated cAMP formation [16]. This 5-HT effect was antagonized by the non-selective antagonists metitepine and spiperone. In these transfected cells, micromolar concentrations of 5-HT also stimulate phospholipase C [16]. G_i proteins, preferentially G_{i3}, mediate the effects of 5-HT both to inhibit adenylate cyclase and to stimulate phospholipase C in HeLa cells [17]. Dual coupling of the cloned rat 5-HT_{1A}

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† Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; CSS, controlled salt solution; d-LSD, *d*-lysergic acid diethylamide.

receptor to adenylate cyclase and phospholipase C has also been shown in stably transfected mouse Ltk$^-$ fibroblasts [18].

Until now, characterization of the negative coupling of the cloned human 5-HT_{1A} receptor to adenylate cyclase in terms of activities of various 5-HT receptor agonists, partial agonists and antagonists is poorly investigated. We have now undertaken such a study. We measured the human 5-HT_{1A} receptor-mediated inhibition of adenylate cyclase in the clonal HeLa cell line HA7 [16]. Stimulation of adenylate cyclase was induced by using forskolin and its inhibition was measured with 5-HT, various 5-HT receptor agonists and antagonists, and the partial 5-HT_{1A} receptor agonists buspirone, spiroxatrine and ipsapirone. The antagonism of 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated cAMP formation was studied in the presence of spiperone and a series of 5-HT receptor and other neurotransmitter receptor antagonists. The activities of the compounds on inhibition of forskolin-induced cAMP formation were compared with their binding affinities for human 5-HT_{1A} receptor sites in an HA7 cell membrane preparation using [^{3}H]8-OH-DPAT as radioligand.

MATERIALS AND METHODS

Cell culture. The clonal HeLa cell line HA7, permanently expressing a human 5-HT_{1A} receptor gene as described by Fargin *et al.* [16], was cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM glutamine, 1 mM pyruvate and 10% heat-inactivated foetal calf serum. Subcultures were made by using 0.025% trypsin in phosphate-buffered saline (PBS: 137 mM NaCl, 2.68 mM KCl, 1.47 mM KH_2PO_4, 8 mM Na_2HPO_4, pH 7.4). The split rate was 1–10. Cells were not subcultured more than 10 times. Cultures were maintained at 37° in an air/CO_2 (95:5) water-saturated atmosphere.

Binding experiments were performed with cultures grown for 3–4 days in tissue culture flasks (Falcon, 175 cm^2). cAMP experiments were carried out with cultures in 24-well culture plates (Nunc) with 1.0 mL medium/well (surface: 1.8 cm^2). After 3–4 days, confluent cultures (about 0.2×10^6 cells/well) were washed twice with 1.0 mL controlled salt solution (CSS: 120 mM NaCl, 5.4 mM KCl, 0.8 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM glucose, 25 mM Tris–HCl, pH 7.4) and used for cAMP experiments.

Binding assay to 5-HT_{1A} receptor sites in membrane preparation of HA7 cells. Cultures were washed twice with PBS, harvested in DMEM with 10% dimethyl sulphoxide and stored at −70° (approx. 1×10^7 cells/mL). Before use, cells were thawed, suspended in 50 mM Tris–HCl, pH 7.7 (30 mL/1 mL cell sample) and centrifuged for 10 min at 36,000 *g*. The cell pellet was homogenized in 20 mL 50 mM Tris–HCl, pH 7.7, with an Ultra Turrax homogenizer and centrifuged for 20 min at 36,000 *g*. The pellet was suspended in 25 mL incubation buffer (50 mM Tris–HCl, 4 mM $CaCl_2$ and 10 μM pargyline, pH 7.5) per mL of original cell suspension. The final cell membrane suspension corresponded to about 4×10^5 original cells/mL and contained 80–160 μg protein/mL. An incubation mixture was composed of 0.5 mL membrane suspension, 0.025 mL solvent (for total binding) or drug dissolved in 0.1% ethanol (for inhibition experiments) or spiroxatrine (1 μM final concentration in the assay, for non-specific binding) and 0.025 mL [^{3}H]8-OH-DPAT (final concentration 0.5 nM for inhibition experiments). The incubation mixture was incubated for 30 min at 37°; 5 mL ice-cold buffer (50 mM Tris–HCl, pH 7.5) was added and rapidly filtered under suction over Whatman GF/B glass fibre filters (diameter 2.5 cm). Filters were rinsed twice with 5 mL ice-cold buffer. A 40-well filtration manifold was used and incubation mixtures were poured manually over the filters. Filters were mixed with 5 mL Ultra Gold scintillant (Packard) and this mixture was counted in a Packard Tricarb liquid scintillation counter. Data were analysed graphically with inhibition curves and IC_{50} values (concentration of the compound producing 50% inhibition of specific binding) were derived. K_i values were calculated according to the equation: $K_i = IC_{50}/(1 + C/K_d)$ with C the concentration and K_d (1.44 ± 0.32 nM, N = 5) the equilibrium dissociation constant of the [^{3}H]ligand.

5-HT_{1A} receptor-mediated inhibition of cAMP formation in intact HA7 cells. Cultures were loaded with 2.0 μCi [^{3}H]adenine in 0.5 mL CSS buffer/well for 120 min at 37°. The cultures were washed with 1.0 mL CSS and incubated for 20 min with 1.0 mL CSS containing 0.5 mM isobutylmethylxanthine in the presence of 100 μM forskolin (or the indicated concentration) and test agent. Basal accumulation of cAMP was measured in the absence of forskolin and test agent. The reaction was stopped by the addition of 0.1 mL ice-cold $HClO_4$ to a final concentration of 0.1 N. The extract was neutralized with 0.5 M KH_2PO_4/K_2HPO_4 buffer (pH 7.4) for assay of the [^{3}H]cAMP content. The latter was assayed as described by Salomon *et al.* [19]. The neutralized extract was sequentially passed over Dowex 50W-X4 (200–400 mesh) and aluminum oxide columns, eluted with water and 0.1 M imidazol (pH 7.4) as described previously [20]. The [^{3}H]-cAMP eluate was mixed with 10 mL Pico-aqua scintillant (Packard) and this mixture was counted in a Packard Tricarb liquid scintillation counter. EC_{50} values (concentrations of test agent inhibiting 50% of forskolin-induced cAMP formation) were derived graphically. The antagonism of agonist-mediated inhibition of forskolin-induced cAMP formation was assayed with spiperone or the indicated compounds. Spiperone and the compounds were pre-incubated 15 min before 100 μM forskolin and 0.1 μM 5-HT were added for 20 min as described above.

Materials. The HeLa clonal HA7 cell line permanently expressing a human 5-HT_{1A} receptor gene [16] was commercially obtained from Tulco (Duke University, Durham, NC, U.S.A.). DMEM, foetal calf serum and 24-well tissue culture plates were obtained from Gibco Biocult Laboratories (Paisley, U.K.). Dowex 50W-X4 (200–400 mesh) was from Serva (Westbury, NY, U.S.A.). Aluminum oxide was from Merck (Darmstadt, Germany). [^{3}H]-Adenine (5 Ci/mmol) was from New England Nuclear (Dreieich, Germany). The compounds were

kindly provided by the companies of origin. The stock solutions of drugs were prepared in 100% ethanol. Dilutions were made in 0.1% of the solvent hydroxypropyl-β-cyclodextrin as previously described ([20], Janssen Biotech, Olen, Belgium).

RESULTS

The determination of the average number of 5-HT$_{1A}$ receptors in cultures of HA7 cells yielded 46,350 ± 8820 receptors per cells. Under these conditions, forskolin increased cAMP formation; a 25-, 60- and 104-fold increase was observed with 10, 30 and 100 μM forskolin, respectively. Figure 1a shows the inhibition of forskolin-induced cAMP formation by 5-HT. The inhibition by 5-HT was independent of the forskolin concentration, and the inhibition was maximal (between 90 and 92% of the stimulated cAMP formation) at 1 μM of 5-HT. Half-maximal inhibition by 5-HT was observed between 21 and 25 nM. The maximal inhibition of forskolin-induced cAMP formation by 5-HT was dependent on the subculture number of HeLa cells. In subcultures up to the 9th passage 0.1 μM 5-HT inhibited 81 ± 6% (N = 11) of forskolin-induced cAMP formation. Further subculturing attenuated the potency and maximal inhibitory effect of 5-HT (not shown). Hence, experiments were performed with cultures which showed at least 80% inhibition of forskolin-induced cAMP formation by 0.1 μM 5-HT. Spiperone reversed the 5-HT-mediated inhibition of forskolin-induced cAMP formation as is shown in Fig. 1b. Increasing forskolin concentrations slightly affected the IC_{50} value of spiperone [63–79 nM (N = 2), 25–50 nM (N = 2) and 20–45 nM (N = 3)] in the presence of 0.1 μM 5-HT and 10, 30 and 100 μM forskolin, respectively, whereas a steepening of the spiperone competition curve was apparent with increasing concentrations of forskolin. [^{3}H]8-OH-DPAT binding to HA7 cell membranes was not affected by these concentrations of forskolin. Further experiments on accumulation of cAMP were performed in the presence of 100 μM forskolin. Figure 2 shows that spiperone induced a parallel rightward shift of the 5-HT dose–response curve without affecting the maximal inhibitory effect of 5-HT.

Full inhibition of forskolin-induced cAMP for-

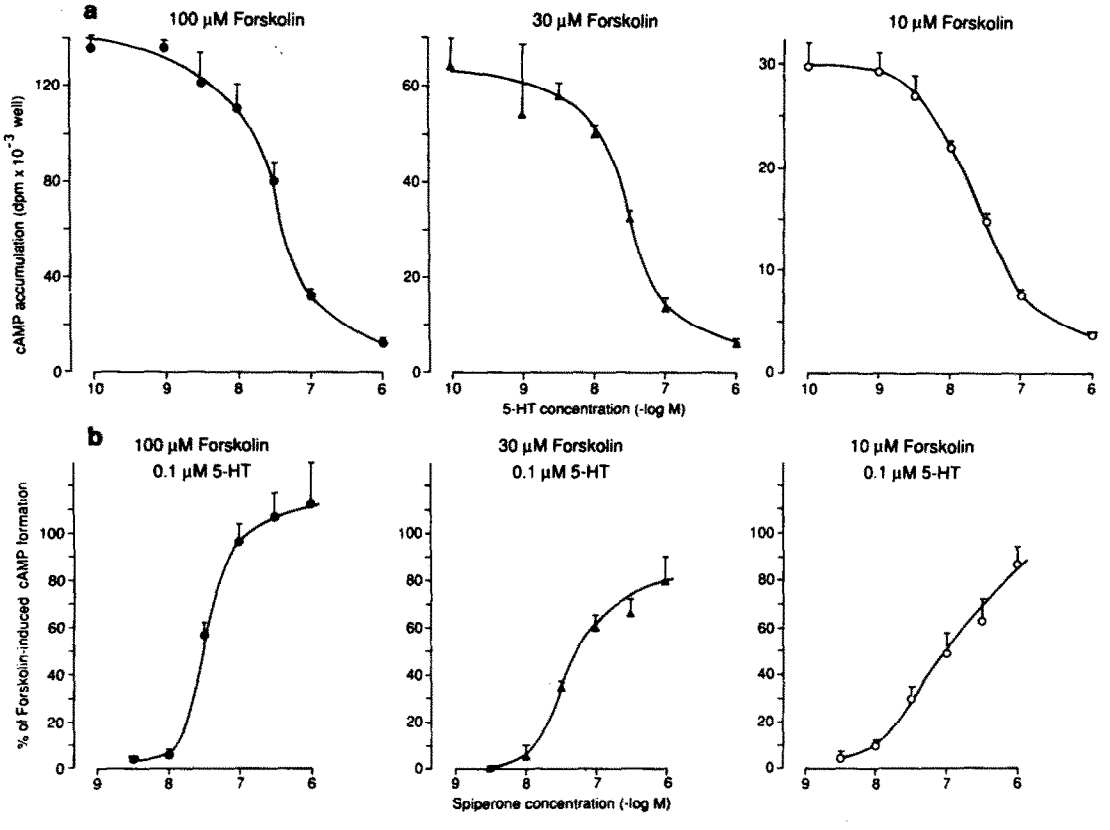

Fig. 1. Inhibition of forskolin-induced cAMP formation by 5-HT in the absence or the presence of spiperone in cultures of HA7 cells. (a) HA7 cells were cultured as described in Materials and Methods, and were exposed for 20 min to 10, 30 or 100 μM forskolin in the presence of increasing concentrations of 5-HT. Basal accumulation (in the absence of forskolin and 5-HT) of cAMP was 1133 ± 203 dpm/well. (b) HA7 cells were exposed to 0.1 μM 5-HT and 10, 30 or 100 μM forskolin in the absence or presence of increasing concentrations of spiperone. Results are expressed as the per cent of forskolin-induced cAMP formation inhibited by 0.1 μM 5-HT. Curves were constructed using mean values ± SD of one representative experiment out of two independent experiments, each performed in triplicate.

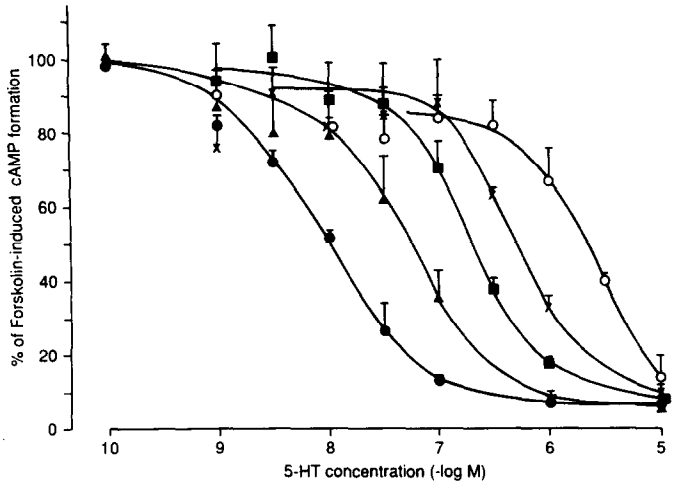

Fig. 2. Antagonism of 5-HT-mediated inhibition of forskolin-induced cAMP formation by spiperone in cultures of HA7 cells. HA7 cells were cultured as described in the legend to Fig. 1, and exposed to 100 μM forskolin and increasing concentrations of 5-HT in the absence or presence of increasing concentrations of spiperone. Results are expressed as the per cent of maximal forskolin-induced cAMP formation. Curves were constructed using mean values ± SD of one representative experiment out of two independent experiments, each performed in triplicate. (▲) 10 nM; (■) 100 nM; (×) 300 nM and (○) 1000 nM.

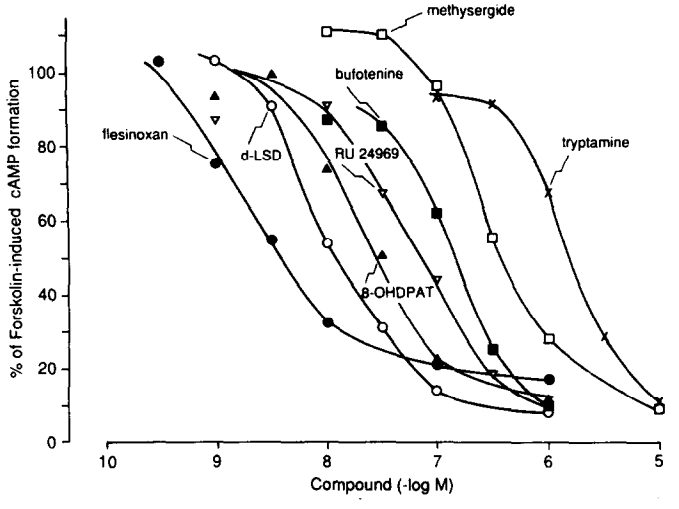

Fig. 3. Agonist-mediated inhibition of forskolin-induced stimulation of cAMP in cultures of HA7 cells. HA7 cells were cultured as described in the legend to Fig. 1, and exposed for 20 min to 100 μM forskolin and increasing concentrations of flesinoxan, d-LSD, 8-OH-DPAT, RU 24,969, bufotenine, methysergide and tryptamine. The inhibition is expressed as the per cent of maximal forskolin-induced cAMP formation. Curves were constructed using mean values of one representative experiment out of 3–8 independent experiments, each performed in triplicate. EC_{50} values are presented in Table 1.

mation was also observed with selective 5-HT_{1A} receptor agonists (8-OH-DPAT and flesinoxan) and non-selective 5-HT receptor agonists: *d*-lysergic acid diethylamide (d-LSD), RU 24,969, bufotenine, methysergide and tryptamine (Fig. 3). These compounds showed the following rank order of potency: flesinoxan > d-LSD > 8-OH-DPAT > RU 24,969 > bufotenine > methysergide > tryptamine. The concentrations for half-maximal effect varied between 3.3 nM and 1.9 μM and were between 6 and 25 times higher than the K_i values of the compounds for inhibition of [^{3}H]8-OH-DPAT binding to a membrane preparation of HA7 cells (Table 1). Spiperone (1 μM) fully prevented the inhibition of forskolin-induced stimulation of cAMP by these compounds (Fig. 4).

Partial inhibition of forskolin-induced cAMP formation was observed with submicromolar concentrations of buspirone, spiroxatrine and ipsapirone (Fig. 5). Inhibition at the highest dose tested was 65% for buspirone, whereas that due to 1 μM ipsapirone and spiroxatrine was between 42 and

Table 1. EC_{50} values of compounds for inhibition of forskolin-induced stimulation of cAMP in HA7 cells and apparent equilibrium inhibition constants (K_i values) of same compounds for inhibition of [^{3}H]8-OH-DPAT binding to a membrane preparation of HA7 cells

	Mean EC_{50} values (nM) ± SD for inhibition of 100 μM forskolin-induced stimulation of cAMP formation	Mean K_i values (nM) for inhibition of [^{3}H]8-OH-DPAT binding to HA7 membrane preparation
5-HT	21.3 ± 12.4	4.0
Flesinoxan	3.3 ± 0.6	0.6
d-LSD	16.2 ± 3.3	1.1
8-OH-DPAT	23.4 ± 12.0	1.9
RU-24969	90.0 ± 15.5	7.9
Bufotenine	132.0 ± 58.0	—
Methysergide	417.0 ± 62.0	17.0
Tryptamine	1886.0 ± 433.0	104.0
Buspirone	Partial inhibition (35%*, 90†)	15.0
Spiroxatrine	Partial inhibition (55%*, 30†)	1.0
Ipsapirone	Partial inhibition (58%*, 22†)	6.4

EC_{50} values and K_i values are the means ± SD of values obtained in 3–8 and 2–3 independent experiments, respectively.

* Per cent of maximal forskolin-induced cAMP formation at 1 μM.

† Concentration (nM) needed to obtain 50% of the maximal inhibition induced with this compound.

The Spearman rank-order correlation coefficient between the IC_{50} and K_i values was 0.96 ($P = 0.018$).

45%. Spiperone (1 μM) antagonized the buspirone-, spiroxatrine- and ipsapirone-mediated inhibition of forskolin-induced cAMP formation. Full inhibition of the forskolin-induced cAMP formation by the presence of 0.1 μM 5-HT was not affected by the simultaneous presence of 0.1 μM buspirone or spiroxatrine. Ipsapirone (1 μM) attenuated the 5-HT-mediated inhibition of cAMP formation by 23%.

Small effects (<35%) on forskolin-induced cAMP formation were observed with 1 μM of the 5-HT receptor agonist CP 93129, 0.1–1 μM of the 5-HT receptor antagonists mesulergine (5-HT_{1C} and 5-HT_2) and BW-501 (5-HT_{1C} and 5-HT_2) (Table 2). No inhibition of forskolin-induced cAMP formation was observed by the presence of 1 μM of the 5-HT_3 receptor agonist quipazine and non-selective 5-HT receptor agonists: 0.1–1 μM sumatriptan (5-HT_{1B} and 5-HT_{1D}) and 10 μM DOM (5-HT_{1C} and 5-HT_2). Neurotransmitter receptor antagonists reported to have 5-HT_{1A} receptor binding affinity, such as the

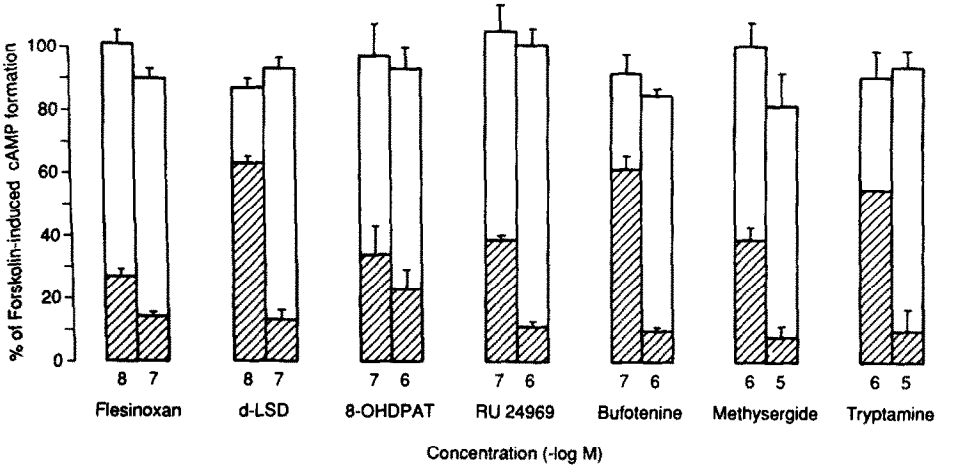

Fig. 4. Reversal by spiperone of the inhibitory effects of flesinoxan, d-LSD, 8-OH-DPAT, RU 24,969, bufotenine, methysergide and tryptamine on 100 μM forskolin-induced cAMP formation. HA7 cells were cultured as described in the legend to Fig. 1, and exposed for 20 min to 100 μM forskolin and the indicated concentrations of flesinoxan, d-LSD, 8-OH-DPAT, RU 24,969, bufotenine, methysergide and tryptamine in the absence or presence of 1 μM spiperone. Results are expressed as the per cent of the maximal forskolin-induced cAMP formation. Bars were constructed using mean values ± SD of one representative experiment out of two independent experiments, each performed in triplicate. Hatched bars, in the absence of spiperone; open bars, in the presence of 1 μM spiperone.

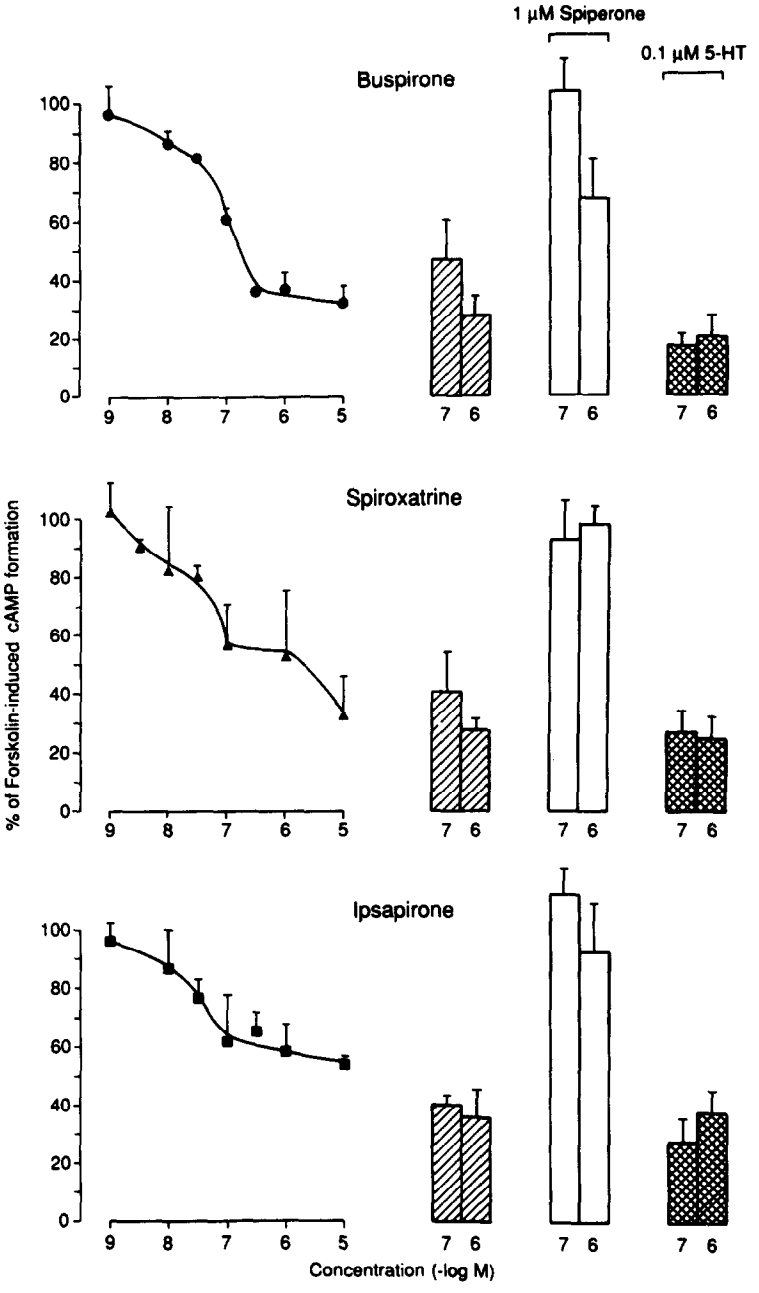

Fig. 5. Inhibition of forskolin-induced cAMP formation by buspirone, spiroxatrine and ipsapirone in the absence and presence of 5-HT or spiperone in cultures of HA7 cells. HA7 cells were cultured as described in the legend to Fig. 1, and exposed for 20 min to 100 μM forskolin and increasing concentrations of buspirone, spiroxatrine or ipsapirone (left side). The bars (right side) represent the cAMP accumulation in cultures that were exposed to 100 μM forskolin and the two indicated concentrations of buspirone, spiroxatrine or ipsapirone in the absence and presence of 1 μM spiperone or 0.1 μM 5-HT. Results are expressed as the per cent of maximal forskolin-induced cAMP formation; 0.1 μM 5-HT yielded 17 ± 2% of forskolin-induced cAMP formation. Curves and bars were constructed using mean values ± SD of three independent experiments performed in triplicate.

neuroleptic ocaperidone [21] and the β-blockers pindolol and nebivolol [22] show nanomolar binding affinity for 5-HT_{1A} sites in HA7 cells, whereas only 1 μM ocaperidone and 0.3 μM nebivolol slightly inhibited forskolin-induced cAMP formation. No effects were found with 0.1–1 μM of the neurotransmitter receptor antagonists ICS-205,930 (5-HT_3), CGP 20712-A (β1-adrenergic), prazosin (α_1-adrenergic), sulpiride (dopamine D_2) and pyrilamine (histamine H_1). With the exception of 1 μM pindolol

Table 2. Forskolin-induced stimulation of cAMP accumulation in the presence of various neurotransmitter receptor agonists and antagonists in the absence or simultaneous presence of 5-HT in cultures of HA7 cells and apparent equilibrium inhibition constants (K_i values) of same compounds for inhibition of [^{3}H]8-OH-DPAT binding to a membrane preparation of HA7 cells

		Per cent of 100 μM forskolin-induced stimulation of cAMP formation		Mean K_i values (nM) for inhibition of [^{3}H]8-OH-DPAT binding to HA7 membrane preparation
	μM	in the absence of 5-HT	in the presence of 0.1 μM 5-HT	
CP 93129	1	74 ± 10*	18 ± 11	3753
	0.1	108 ± 15	19 ± 3	
Quipazine	1	97 ± 7	16 ± 1	1697
Sumatriptan	1	87 ± 8	15 ± 3	255
	0.1	89 ± 7	17 ± 2	
DOM	10	95 ± 13	21 ± 1	7267
Mesulergine	1	73 ± 10*	18 ± 2	197
	0.1	83 ± 4*	13 ± 4	
BW-501	1	75 ± 15*	11 ± 2	2877
ICS 205-930	1	94 ± 12	11 ± 2	—
Ocaperidone	0.1	83 ± 17	34 ± 12	13.9
	1	66 ± 17*	57 ± 16	
Pindolol	1	95 ± 18	83 ± 15	28.0
Nebivolol	0.1	89 ± 18	27 ± 13	25.8
	0.3	70 ± 10*	25 ± 8	
CGP 20712-A	1	90 ± 3	11 ± 1	7364
Prazosin	1	86 ± 14	19 ± 2	12,881
Sulpiride	1	100 ± 6	16 ± 1	41,854
Pyrilamine	1	93 ± 4	12 ± 0	36,349

HA7 cells were cultured as described in the legend to Fig. 1, and exposed for 20 min to 100 μM forskolin and the indicated concentrations of the tested compound in the absence and presence of 0.1 μM 5-HT.

Results are expressed as the per cent of maximal forskolin-induced stimulation of cAMP obtained with 100 μM forskolin; 0.1 μM 5-HT yielded 17 ± 2% of forskolin-induced cAMP formation. Values are means ± SD of 2–3 independent experiments, each performed in triplicate are presented. K_i values are the means obtained in two independent experiments performed as described in Materials and Methods.

* $P < 0.05$.

and 1 μM ocaperidone, no attenuation of the 5-HT-mediated inhibition of forskolin-induced cAMP formation was observed (Table 2).

DISCUSSION

Membrane preparations from tissues used for receptor studies are seldom homogeneous. Most tissues contain several receptors or several subtypes of a particular receptor; the latter situation especially may pose serious problems for receptor studies. In contrast, cloned receptors expressed in cell lines have several major advantages: (1) one particular receptor subtype can be expressed in the absence of other receptor subtypes; (2) a human receptor can be studied; (3) a large amount of receptors can be obtained per cell, Fargin *et al.* [16] have shown that HA7 cells yield 0.5 pmol/mg protein of 5-HT_{1A} receptors; (4) signal transduction of receptors, in particular the negative coupling of 5-HT_{1A} receptors to adenylate cyclase, can be studied easily on intact cells. The cloned human 5-HT_{1A} receptor in HA7 cells is negatively coupled to adenylate cyclase and reduces stimulated cAMP accumulation [16]. This study shows 80% inhibition of forskolin-induced cAMP formation by 0.1 μM 5-HT in HA7 cells. Hence, we suggest HA7 cells over membrane preparations of brain tissue or primary neuronal cultures (see introduction) for measuring the negative coupling of 5-HT_{1A} receptors to adenylate cyclase.

The receptor mediating the inhibition of forskolin-induced stimulation of cAMP is likely to be the 5-HT_{1A} receptor because of its high affinity for 5-HT, 8-OH-DPAT and flesinoxan. The observed half-maximal effects for these agents and the non-selective 5-HT receptor agonists d-LSD, RU 24,969, bufotenine, methysergide and tryptamine are in good agreement with those reported for the inhibition of stimulated adenylate cyclase found in hippocampal membranes of guinea-pig [11, 23], calf [14] and in primary cultures of mouse hippocampal neurones [12]. The rank order of potency of the tested compounds correlated well with their binding affinity for 5-HT_{1A} receptors, measured by *in vitro* radioligand binding assays using membrane preparations of HA7 cells (Table 1), rat cortex, calf and rat hippocampus [12, 14]. Nevertheless, in HA7 cells the concentrations of the compounds for half-maximal inhibition of forskolin-induced cAMP formation were always higher than their K_i values for inhibition of [^{3}H]8-OH-DPAT binding (Table 1). This may be due to a poor coupling or a low receptor reserve of the human 5-HT_{1A} receptor in HA7 cells. 5-HT_{1B} and 5-HT_{1D} receptors which are also negatively coupled to adenylate cyclase [4] appeared not to be involved, since agents like

CP 93129 and sumatriptan did not inhibit forskolin-induced cAMP formation in HA7 cells.

Partial inhibition of stimulated cAMP formation was observed with submicromolar concentrations of buspirone, spiroxatrine and ipsapirone. Buspirone and spiroxatrine did not antagonize and ipsapirone only slightly antagonized the 5-HT-mediated inhibition of cAMP formation. These drugs have been described as agonists, partial agonists or even antagonists depending on the test model studied [12, 14, 24–27]. Hoyer *et al.* [27] discussed that agonists do not necessarily show the same intrinsic activity at different 5-HT_{1A} receptors, depending on the receptor reserve, coupling efficacy of the receptors and the possibility of coupling a receptor to different G-proteins. In the case of nebivolol and ocaperidone, there is apparently no correlation between the binding affinity for 5-HT_{1A} receptors and their effect on cAMP formation. Therefore, activity of a compound is difficult to predict and is most probably model-dependent as recently discussed by Boddeke *et al.* [26].

Within the series of tested neurotransmitter receptor antagonists, pindolol and spiperone were the only compounds that fully antagonized 5-HT-mediated inhibition of cAMP formation. The inhibition of spiperone was competitive and half-maximal at 30 nM, similar to the data reported by Fargin *et al.* [16]. In conclusion, HA7 cells with permanent and functional expression of a human 5-HT_{1A} receptor gene are a valid cellular system for studying the negative coupling of 5-HT_{1A} receptors to adenylate cyclase and their interaction with compounds.

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