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Stimulation of Growth Hormone Release by 5-Hydroxytryptamine (5-HT) in Cultured Rat Anterior Pituitary Cell Aggregates: Evidence for Mediation by 5-HT2B, 5-HT7, 5-HT1B, and Ketanserin-Sensitive Receptors

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5-Hydroxytryptamine (5-HT) promotes the release of GH by a hypothalamic site of action. The present study explores a putative pituitary action in a perifused rat anterior pituitary aggregate cell culture system. In aggregates cultured with 1 nM estradiol for expression of the 5-HT4, -5, and -6 receptor (R), 5-HT promptly stimulated GH secretion with a dose dependency between 1 and 10 nM. The effect of 5-HT was partially blocked by methiothepin and methysergide; by SB-206553, a 5-HTR2B/C antagonist; SB-269970, a 5-HTR7/5A antagonist; and SB-224289, a 5-HTR1B antagonist. The GH response was fully blocked by combined administration of SB-206553+SB-269970 and SB-206553+ketanserin but not by SB-206553+spiperone. Culturing the aggregates without estradiol diminished the magnitude of the GH response to 5-HT as well as the impact of 5-HTR7/5 blockade on the response. Basal GH release was stimulated by the 5-HTR2 agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, m-chlorophenyl piperazine, and α-methyl 5-HT; 5-carboxytryptamine (agonist at 5-HTR1, -5, and -7); tryptamine (preferential 5-HTR7 agonist); and the selective 5-HTR1B agonist CP93129 but not the 5-HTR1A agonists 7-(dipropylamino)tetralin-1-ol-8-hydroxy-2-(di-n-propylamino)tetralin and the 5-HTR1B/D agonist sumatriptan. The selective 5-HTR2B agonist BW 723C86 stimulated GH release, and the selective 5-HTR2B antagonist SB-204741 attenuated the GH response to 5-HT. The present data suggest that 5-HT may release GH through a pituitary site of action, and that the 5-HTR2B, 5-HTR7 and 5-HTR1B mediate this response, with possibly an inhibitory component of the 5-HTR1D. The relative contribution of these receptors may be modulated by estrogen. (*Endocrinology* 148: 4509–4522, 2007)

THE RAT ANTERIOR pituitary expresses virtually all known serotonin (5-HT) receptors (5-HTR) *in vivo* at the mRNA level, among which the 5-HTR4, 5-HTR5A, 5-HTR5B, and 5-HTR6 require estradiol (E_2) for expression *in vivo* (1). This diversity of expression is in search of a function. 5-HT is a biogenic monoamine involved in multiple functions within the central nervous system, peripheral organs, and the immune and coagulation systems (2). The actions of 5-HT are mediated by the 5-HTR family, which consists of 14 different subtypes (5-HTR1A/B/D/E/F, 2A-2C, -3, -4, -5A, -5B, -6, -7) of which all are G protein-coupled except for the 5-HTR3, which is a ligand-gated ion channel (3).

5-HT affects GH release through the serotoninergic projections from the medial and dorsal raphe nucleus of the brain stem to the hypothalamus (4). *In vivo*-manipulated 5-HT neurotransmission or treatment with serotonin agonists or antagonists suggests a stimulatory, inhibitory, no role, or controversial findings of 5-HT in GH release, depending on the physiological condition and species (5–19). The disparity in findings is probably related to the complexity of the serotoninergic system and the many receptor subtypes involved. Little is known concerning the receptors involved in 5-HT-modulated GH release because the above studies were conducted more than 2 decades ago, when selective 5-HTR agonists or antagonists were not available yet. At most, some data have shown the involvement of the 5-HTR1B/D, 5-HTR2A/C, and 5-HTR3 subtypes (20–25).

There is some evidence that 5-HT may also affect GH secretion at the level of the pituitary. One group of investigators showed that 5-HT is capable to stimulate GH release in intact rat pituitary *in vitro* (26). An *in vitro* effect was seen by another group but only when the posterior pituitary was coincubated (27). However, to our knowledge, the 5-HTR involved has not been studied. The 5-HTR6 is expressed at the mRNA level in the normal pituitary, but its function remains unknown (28). Although there may be an intrinsic hypothalamic serotoninergic system (29) and 5-HT has been detected in the portal blood, the concentration in the latter was not higher than in peripheral blood (30), suggesting no delivery of 5-HT from the median eminence to the anterior pituitary. Basal plasma levels of 5-HT in rats and humans have been reported to be 0.6–0.9 nM but can rise 50–100 times during treatment with amphetamines and selective serotonin reuptake inhibitors (31, 32) or under certain pathological

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Abbreviations: AUC, Area under the curve; 5-CT, 5-carboxytryptamine; Dex, dexamethasone; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; E_2, estradiol; 5-HT, 5-hydroxytryptamine; 5-HTR, 5-HT receptor; IBMX, isobutylmethylxantine; Ki, inhibitory constant; LSD, least significant differences; mCpp, m-chlorophenyl piperazine; 8-OHDPAT, 7-(dipropylamino)tetralin-1-ol-8-hydroxy-2-(di-n-propylamino)tetralin; PRL, prolactin; VIP, vasoactive intestinal peptide.

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conditions such as hypoxia (32, 33). There are some indications that 5-HT may be contained within the pituitary. Three decades ago, it was shown that the rat pituitary *ex vivo* is able to convert tryptophan into 5-HT (34), although it remains unknown whether the enzyme is located in glandular cells. Others demonstrated 5-HT in nerve fibers of the neurohypophysis (35) and the intermediate (36, 37) and anterior lobe (38). Interestingly, synapse-like structures between endocrine cells and nerve fibers have been demonstrated in the intermediate (36) and anterior lobes (39, 40). 5-HT has been identified in the secretory granules of gonadotrophs *in vivo* (35, 41, 42), and it is taken up by anterior pituitary cells via a fluoxetine-sensitive transport system, suggesting expression of the serotonin transporter (SERT) (41, 43). Because 5-HT may be present in the pituitary, the monoamine may influence hormone secretion by a local paracrine or neurocrine action.

The paucity of clear-cut data concerning the GH secretory response to 5-HT at the pituitary *vs.* hypothalamic level and the current availability of several selective 5-HTR antagonists and certain selective agonists prompted us to investigate whether 5-HT is capable of affecting basal GH secretion in reaggregated anterior pituitary cell cultures (1, 44) and to characterize the receptors behind any intrinsic activity. We report that 5-HT acutely stimulates GH release and present pharmacological evidence for the implication of the 5-HTR2B, 5-HTR7, 5-HTR1B, and possibly the 5-HTR1D. The magnitude of the response was increased by E_2, linking 5-HT to putative sexual dimorphic functions of the pituitary.

Materials and Methods

Chemicals and drugs

Serotonin (5-HT), SB-269970, ketanserin, SB-206553, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), WAY-100635, BRL-15572, GR113808, Ro 04-6790, isobutylmethylxantine (IBMX), SB-224289, SB-204741, clozapine, spiperone, and tryptamine were purchased from Sigma Chemical Co. (St. Louis, MO). Ondansetron and sumatriptan were purchased from GlaxoSmithKline (Middlesex, UK). Domperidone was obtained from Janssen Pharmaceutica (Beerse, Belgium). Methiothepin, methysergide, 5-carboxamidotryptamine, α-methyl 5-HT, m-chlorophenyl piperazine (mCpp), RS-102221, and 7-(dipropylamino)tetralin-1-ol-8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT), BW 723C86, and CP193129 were purchased from Tocris Biosciences (Avonmouth, UK). T_3, dexamethasone (Dex), and BSA (Cohn fraction V) was from Serva (Heidelberg, Germany). E_2 and vitamin C were from Merck (Darmstadt, Germany). 5-HT was dissolved in 1% vitamin C solution and made fresh. Domperidone, E_2, Dex, and ketanserin were dissolved in ethanol and stored at 4 C. IBMX was made fresh and dissolved in dimethylsulfoxide. GR113808 was dissolved in dimethylsulfoxide at a stock concentration of 10^{-2} M. All other chemicals (analytical grade) were dissolved in water and stored at −20 C. Stock concentrations were 10^{-3} M, except for Dex and E_2 (stock concentration 10^{-5} M) and T_3 (10^{-7} M stock concentration).

Animals

Adult male Wistar rats (3 months old) were obtained from Elevage Janvier (Schaijk, The Netherlands) and housed in an environment with constant temperature, humidity, and day-night cycles, with food and drink *ad libitum*. All experiments were conducted in accordance with the Guidelines for Care and Use of Experimental Animals and approved by the university ethical committee.

Anterior pituitary aggregate cell culture

Rats were decapitated after CO_2 anesthesia, and the anterior pituitary was dissected free from the neurointermediate lobe under sterile conditions and dispersed into single cells as described previously (1, 44). Dispersed cells were allowed to aggregate at 2×10^6 cells in nontreated 35-mm petri dishes (Iwaki, Japan) with 2 ml serum-free defined culture medium. For aggregation the dishes were placed on a gyratory shaker (AppliTek, Nazareth, Belgium) at 64 rpm in a 1.5% CO_2 humidified incubator at 37 C. Culture medium was serum-free defined medium as previously described (44, 45). It consists of HEPES/TES-buffered DMEM/F12 1:1 with selenite and ethanolamine (prepared as powder by Invitrogen, Grand Island, NY), supplemented with 0.5% BSA (endotoxin-poor; Serva), 5 mg/liter insulin-Zn, 5 mg/liter transferrin, 50 mg/liter streptomycin, 35 mg/liter penicillin, 10 mM ethanol, 1 mg/liter catalase, 8 mg/liter phenol red, and 1 g/liter $NaHCO_3$. Final concentration of iron (Fe^{2+} and Fe^{3+}) was 0.6 μM and glucose 1.4 g/liter (7.8 mM). Medium was replaced after 2 d and aggregates were tested on d 5 of culture. Depending on the experimental design, the medium was either supplemented or not with 1 nM E_2, 1 nM T_3, or 10 nM Dex during the entire culture period. In certain experiments, pertussis toxin (100 ng/ml; Sigma) was added in the 35-mm petri dishes containing the aggregates for a period of 24 h before the perifusion.

Perifusion of aggregates

The perifusion system was designed to study the continuous release of hormones from aggregates over a period of time, with the possibility of adding substances and observing their effect on hormone secretion at intervals of 1–2 min. A more elaborate description of its design and set-up can be found elsewhere (44). We have thoroughly validated the system and used it in numerous studies on secretory responses to GnRH and TRH, adrenergic agonists, GHRH and vasoactive intestinal peptide (VIP), cholinomimetics, dopamine, angiotensin II (44), bombesin (46), and prolactin (PRL)-releasing peptide (47). Briefly, aggregates (2×10^6 cells) are placed on a nylon mesh, in a chamber that is kept at 37 C, and immersed in medium [DMEM with 4.5 g/liter glucose, supplemented with 15 mM HEPES, 110 mg/liter Na-pyruvate, 1 g/liter $NaHCO_3$, and 0.5 g/liter NaCl (pH 7.5)], which is being pumped at a set rate through the chamber and collected in 2-min fractions. Aggregates are perifused for a period of 1.5 h to stabilize hormone release before any test substances are applied to the cells. A two-way valve system enables a smooth uninterrupted switch between a rest-stage, during which only medium (with vehicle if appropriate) is passed through the chamber, and an exposure stage, during which test substances (dissolved in the same perifusion medium) perifuse the aggregates. Fractions (0.5 ml per 2 min) are collected in 100 μl 2% BSA (Serva) in 0.01 M phosphosaline, vortexed, and stored at −20 C before analysis. To avoid any time delay in the onset of action of receptor antagonists, the antagonists were added to the perifusion medium 30 min before the start of fraction collections and were also present during the perifusion of the agonist to be blocked. In the figures exposure time to agonists is indicated (*horizontal bar*), taking into account the dead time in the system (4 min).

RIA

Hormone levels were measured from duplicate samples by specific competitive RIAs using the rat GH kit obtained from Dr. A. F. Parlow [National Hormone and Peptide Program, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Harbor-UCLA Medical Center, Torrance, CA] with monkey antirat GH (G-5) antiserum and rat GH-RP-2 as reference preparation. The reliability of the assays has been shown in previous work (44, 47). Both intra and interassay variations were less than 10%. Labeling of GH with I^{125} was done with the Iodogen method according to the manufacturer's instructions (Pierce, Perbio Sciences, Erembodegem, Belgium). Values were expressed in nanogram equivalents of NIDDK rat GH-releasing peptide-2 standard. The average basal GH release was 14 ng per 2 min in untreated and T_3-treated aggregates, 12 ng per 2 min in Dex-treated aggregates, and 11 ng per 2 min in E_2-treated aggregates.

cAMP measurements

Aggregates were cultured as mentioned above. Cells were subsequently placed in the same medium but with 0.5 mM IBMX and placed in nontreated 35-mm-diameter petri dishes at a density of roughly 1×10^6 cells/dish. The cells were then incubated in culture medium with IBMX and 5-HTR agonists or vehicle for 30 min at 37 C and in a CO_2-humidified incubator after which aggregates were collected and cell lysis was obtained through the addition of 0.1 M HCl and sonication. cAMP content was quantified through enzymatic immunoassay kit from Assay Designs (Ann Arbor, MI) (48).

Data expression and statistics

All data from the perifusion experiments are expressed as percentage of basal hormone secretion defined as the average secretion before each drug application. Values are expressed as mean ± SEM. Statistical analysis was performed using either a one-way ANOVA with Fisher's least significant differences (LSD) multiple comparison test on the cumulative secretion for the duration of drug application [area under the curve (AUC)] or two-way ANOVA with Fisher's LSD multiple comparison test using individual values of each 2-min fraction. All data from the cAMP experiments are expressed as percentage of control cAMP production and values are expressed as mean ± SEM. One-way ANOVA with Fisher's LSD multicomparison test was used for the statistical analysis after data were log transformed due to heterogeneity of variance.

Results

5-HT stimulates basal GH release in cultured anterior pituitary cell aggregates

Aggregates were routinely cultured in medium supplemented with 1 nM E_2 because we previously found that the expression of several 5-HTRs depended on estrogen (1). However, because E_2 is known to affect GH secretion in both positive and negative fashion, depending on the secretagogue involved (49), we wanted to examine the GH response to 5-HT also in steroid-free culture. In a first series of experiments, aggregates were perifused with 10 nM, 100 nM, and 1 μM doses of 5-HT, each for a period of 20 min, with rest periods of 40 min. As shown in Fig. 1A, 5-HT readily increased GH secretion ($P < 0.005$, $P < 0.02$, and $P < 0.001$ for 10 nM, 100 nM, and 1 μM, respectively), but there was no dose response at the doses used (AUC = 59, 52, and 57% above basal release, respectively). The GH responses to 5-HT (10 and 100 nM and 1 μM) in aggregates cultured in hormone-free medium was lower in magnitude than that seen in the E_2 (1 nM) condition ($P < 0.001$ at all doses), with significant increases at all concentrations ($P < 0.02$, $P < 0.005$, and $P < 0.01$) (Fig. 1B). The response appeared already maximal at the 10 nM dose.

In a next set of experiments (aggregates cultured with 1 nM E_2), doses of 1 nM and 3 nM 5-HT were tested and each time compared with a 10 nM dose given to aggregates in another perifusion chamber of the same experiment. Both the 1 and 3 nM dose gave significant increases in GH release (AUC 23 and 33% above basal release, compared with 32 and 36% for 10 nM 5-HT; $P < 0.04$ and $P < 0.02$, respectively) (Fig. 1C). The AUC at 1 nM 5-HT was not significantly different from that at 3 nM 5-HT, but there was a significant difference between the 1 and 10 nM dose ($P < 0.05$).

The presence of thyroid hormone and glucocorticoids during culture was also tested because these hormones also can influence GH release in response to various peptides and monoamines (49–51). The GH response was not markedly altered when aggregates were cultured in 10 nM Dex ($P < 0.01$, $P < 0.001$, and $P < 0.01$ for 10 and 100 nM and 1 μM 5-HT) or 1 nM T_3 ($P < 0.02$, $P < 0.001$, and $P < 0.001$ for 10 and 100 nM and 1 μM) (Fig. 1D).

For all further experiments presented below, aggregates were cultured with the 1 nM E_2 supplement unless otherwise stated, and the routine dose of 5-HT used was 10 nM.

Nonselective 5-HTR antagonists block the effect of 5-HT on GH release

To reach full blockade of the 5-HTRs, antagonists were used at a concentration at least 100 times larger than the inhibitory constant (Ki) value of their receptor binding at the respective 5-HTRs (estimated from competition with agonist tracer). The dose of 5-HT was fixed at 10 nM (Ki of 5-HT for agonist binding ranges between ~1 and 10 nM for most 5-HTRs; see http://pdsp.med.unc.edu/kidb.php).

Aggregates perifused prior and during exposure to 5-HT with 1 μM of the nonselective 5-HTR antagonists methysergide or methiothepin (blocking 5-HTR1, -2, -5, -6, and -7) (52–57) displayed a significantly reduced GH response, compared with 5-HT alone ($P < 0.001$ and $P < 0.001$, respectively) (Fig. 2, A and B). When the two antagonists were combined, the GH response to 5-HT was almost completely abolished (Fig. 2C). The application of methysergide (1 μM) on its own had no effect on basal GH release (Fig. 2D), excluding an agonistic activity by methysergide, although partial agonist activity has been previously described in some systems (58).

The GH response to 5-HT is depressed by different selective 5-HTR antagonists

The GH response to 10 nM 5-HT was tested in the presence of a battery of 5-HTR antagonists with different receptor selectivity. The 5-HTR1A antagonist WAY100635 (50 nM) (59) and the 5-HTR1D antagonist BRL 15572 (1 μM) (60) did not affect the GH response to 5-HT (data not shown, n = 5 and n = 3, respectively). The 5-HTR1B antagonist SB-224289 (1 μM) (61–63) slightly lowered the response (−20%) ($P < 0.05$) (Fig. 3A). The 5-HTR2A antagonist ketanserin (1 μM) (54, 64–66) caused the GH response to 5-HT to increase in magnitude ($P < 0.001$) (Fig. 3B). The 5-HTR2B/2C antagonist SB-206553 (1 μM) (61, 67, 68) significantly attenuated the GH response (64.5 ± 7% decrease) ($P < 0.005$), whereas the 5-HTR2C antagonist RS-102221 (1 μM) (52, 53, 61, 68) was without effect (Fig. 3, B and C). The 5-HTR3 antagonist ondansetron (1 μM, n = 3) (69, 70), the 5-HTR4 antagonist GR113808 (1 μM, n = 3) (71–73), and the 5-HTR6 antagonist Ro 04–6790 (1 μM, n = 3) (74) were inactive (data not shown). Finally, the 5-HTR7/5A antagonist SB-269970 (1 μM) (75) caused a 47.8 ± 17% decrease of the response (Fig. 3D) ($P < 0.05$).

A combination of selective 5-HTR antagonists can fully abolish the GH response to 5-HT

Because more than one selective antagonist weakened the GH response to 5-HT, the efficacy of these antagonists was tested in combination. When SB-206553 and SB-269970 were added together to the perifusion at the same concentration as when added solely, the GH response to 5-HT completely disappeared (Fig. 4A) ($P < 0.01$). Surprisingly, this was also

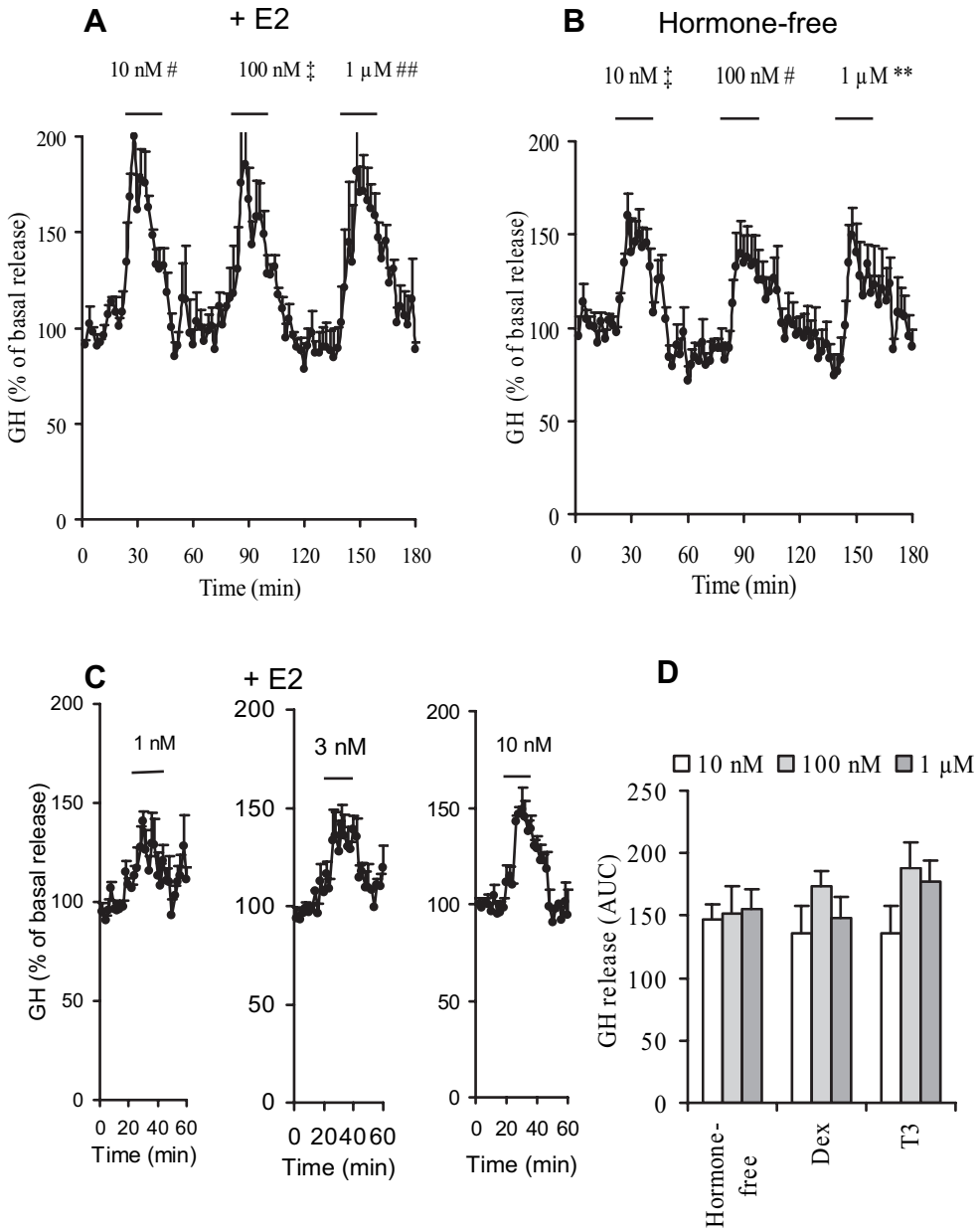

FIG. 1. Effect of 5-HT on GH release from pituitary cell aggregates cultured either with (A and C) or without (B) E_2 or with Dex or T_3 (D) in the culture medium. A, 10 nM, 100 nM, and 1 μM 5-HT (n = 5). B, 10 nM, 100 nM, and 1 μM 5-HT (n = 5). C, 1 nM 5-HT (n = 5) and 3 nM 5-HT (n = 3), compared with 10 nM. D, 10 nM, 100 nM, and 1 μM 5-HT (n = 3). Data are means ± SEM expressed as percentage of basal release (measured during the first 20 min). Statistics included one-way ANOVA performed with Fisher's LSD multiple comparison test on log-transformed AUC data comparing basal with stimulation values and between doses. ##, $P < 0.001$; #, $P < 0.005$; **, $P < 0.01$; ‡‡, $P < 0.04$; ‡, $P < 0.02$.

the case when the three selective 5-HTR2 antagonists (ketanserin, SB-206553, and RS-102221) (each 1 μM) were perifused together ($P = 0.000037$, compared with the release still detectable with SB-206553 alone), even though ketanserin and RS-102221 alone were ineffective in decreasing the response and the 5-HTR2B antagonist did not fully block the response (Fig. 4B). Complete annihilation of the GH response was also seen with a combination of ketanserin and SB-206553 (Fig. 4C) ($P < 0.05$, compared with the release still detectable with SB-206553 alone), but no change in GH response was seen with a combination of ketanserin and RS-102221 (data not shown, n = 3).

5-HTR agonists with affinity for the 5-HTR2 and the 5-HTR7 stimulate GH release

The above experiments suggest that the GH response to 5-HT is mediated through the 5-HTR2 and 5-HTR7 and for a minor part the 5-HTR1B. We therefore tested the effect on GH secretion of several 5-HTR agonists with different receptor selectivity at doses relevant according to their receptor binding affinity (Fig. 5). DOI, a partial agonist of the 5-HTR2A/2C and somewhat weaker than the 5-HTR2B (54, 76, 77) (Fig. 5A), caused an increase in GH release at both 100 nM and 1 μM ($P < 0.005$) as did mCpp, a partial agonist at the 5-HTR2B/2C (72, 76, 78) ($P < 0.01$ and $P < 0.05$ for 100 nM and 1 μM, respectively) (Fig. 5B). The magnitude of the response was markedly lower than that evoked by 5-HT tested concurrently ($P < 0.005$ at 100 nM). The 5-HTR2A/2B/2C agonist α-methyl 5-HT (100 nM and 1 μM) (54, 65, 66, 76, 79) caused a significant increase in GH release ($P < 0.03$ and $P < 0.04$ for 100 nM and 1 μM, respectively) (Fig. 5C), the maximal effect already seen at 100 nM but of lower magnitude than that elicited by 5-HT ($P < 0.002$ at 100 nM). Tryptamine, a medium-potent agonist of the 5-HTR7 and low potency agonist of 5-HTR2B (54, 57), significantly increased GH

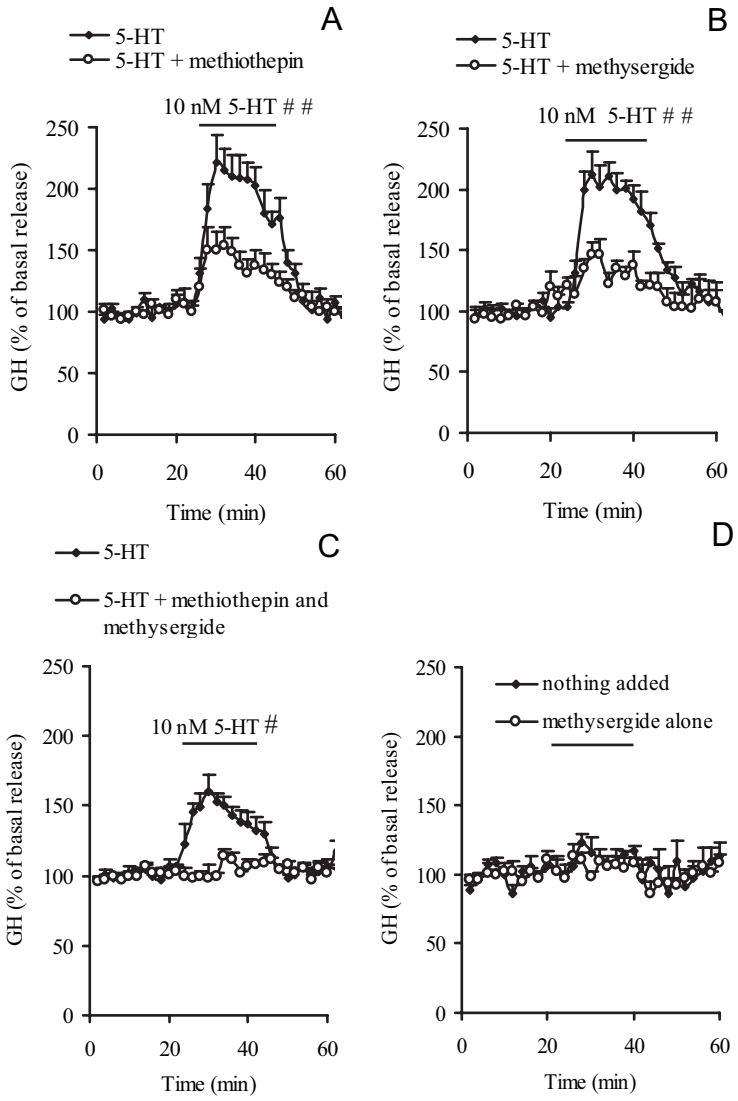

FIG. 2. Effect of nonselective 5-HT antagonists on the GH response to 10 nM 5-HT in E_2-treated anterior pituitary cell aggregates. 5-HT (10 nM) alone or in the presence of the nonselective 5-HTR antagonists methiothepin (1 μM) (A; n = 5), methysergide (1 μM) (B; n = 6), or both (C, n = 4). D, Effect of methysergide on basal GH release (n = 3). Data are means ± SEM expressed as percentage of basal release (measured during the first 20 min). Statistics included one-way ANOVA with Fisher's LSD multiple comparison test performed on AUC values from log-transformed data for 5-HT *vs.* 5-HT + antagonist. ##, $P < 0.001$, #, $P < 0.005$.

release at 10 and 100 nM ($P < 0.04$ and $P < 0.01$, respectively) (Fig. 5D), although no dose response was seen and efficacy remained below that of 5-HT ($P < 0.00001$ at 10 nM). A strong ($P < 0.01$ at 10 nM, $P < 0.001$ at 100 nM) and dose-dependent GH response ($P < 0.05$ between 10 and 100 nM) was seen with 5-carboxytryptamine (5-CT), an agonist of 5-HTR1A/B/D (62, 65, 80); 5-HTR5 (54, 55, 81); and 5-HTR7 (57, 82) (Fig. 5E). The magnitude of response remained below that to 5-HT ($P < 0.03$ at 10 nM; $P < 0.02$ at 100 nM). The 5-HTR1A agonist 8-OHDPAT (65, 83) (Fig. 5F) and sumatriptan (data not shown, n = 3), an agonist at the 5-HTR1B/D (54, 84), did not affect GH release. When comparing the efficacies (AUC found at 100 nM or 1 μM of each agonist divided by that of the 10 nM dose of 5-HT) of each agonist used, 5-CT had the highest efficacy (0.82), followed by α-methyl 5-HT (0.58) greater than tryptamine (0.48) equal to or greater than DOI (0.43) greater than mCPP (0.37).

Further characterization of the GH response with spiperone

The studies carried out with selective antagonists suggested that the 5-HTR2B, 5-HTR1B, and either the 5-HTR7 or 5-HTR5 receptors are mediating the GH response to 5-HT, with a possible negative contribution from the 5-HTR2A, particularly in the combined blockade experiments. However, the well-known 5-HTR2A antagonist ketanserin has also blocking activity at the 5-HTR1D in the rat (85, 86), and hence, the possibility should be envisaged that it is not the 5-HTR2A but the 5-HTR1D that is responsible for the full blockade of the GH response when ketanserin and the 5-HTR2B antagonist are coadministered. Furthermore, no distinction could be made between the implication of the 5-HTR7, 5-HTR5, or both because SB-269970 has affinity for both these receptors (75), and the only selective 5-HTR5A antagonist available is effective at the human (87) but not the rat 5-HTR5A (88), precluding the use of this antagonist to evaluate participation of the 5-HTR5A in the GH response. We wanted to further unravel these uncertainties with spiperone for the following reasons: 1) if the 5-HTR2A is involved, 1 μM spiperone would be capable of fully blocking the GH response when coadministered with the 5-HTR2B antagonist because it displays equally high affinity for blocking the 5-HTR2A as ketanserin (52, 54, 64, 89) and 2) spiperone displays in addition relatively potent 5-HTR7 antag-

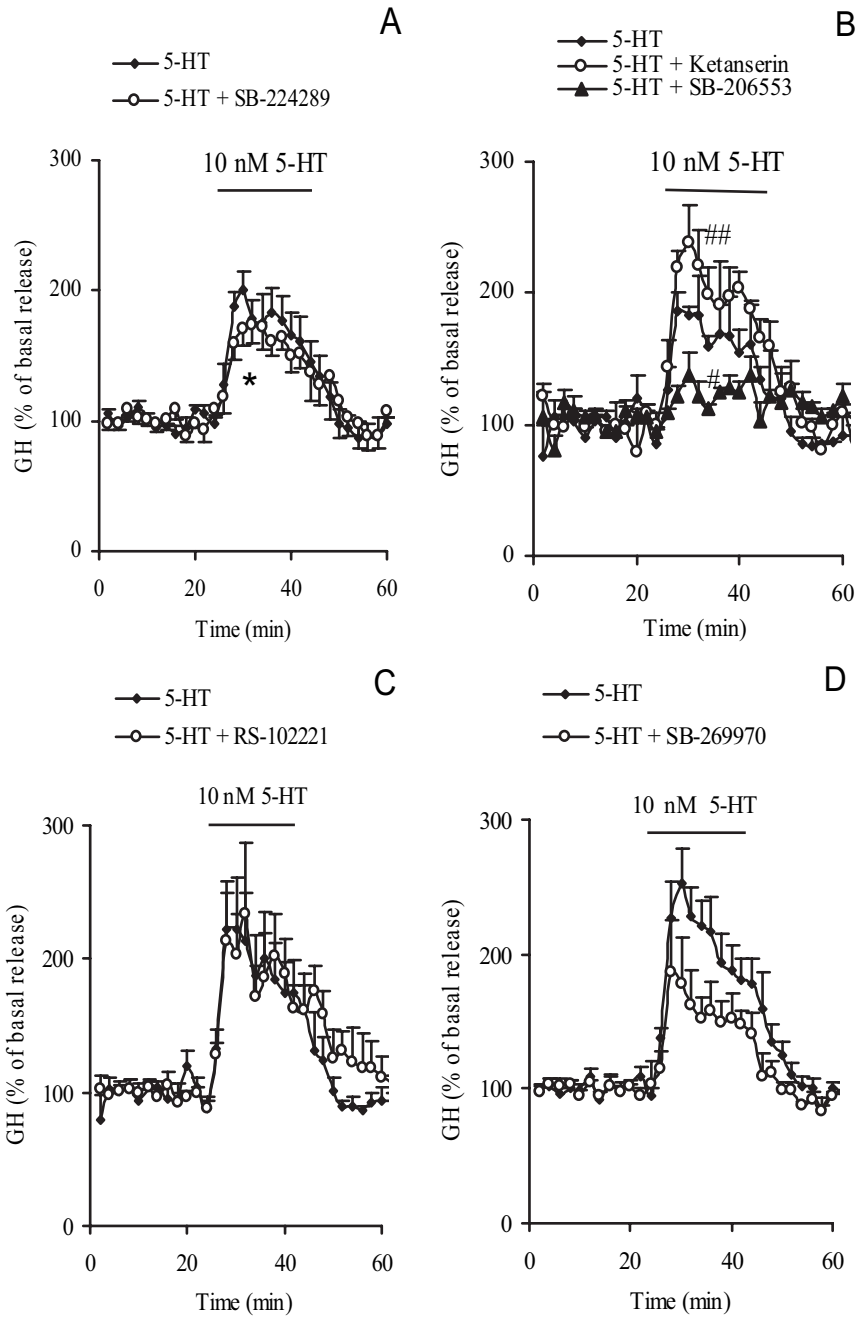

FIG. 3. Effect of selective antagonists on GH response to 10 nM 5-HT in E_2-treated anterior pituitary cell aggregates. 5-HT alone and in the presence of 1 μM of the 5-HTR1B antagonist SB-224289 (n = 4) (1 μM, n = 3) (A); 5-HTR2A antagonist ketanserin (1 μM, n = 3) and 5-HTR2B antagonist SB-206553 (1 μM, n = 3) (B); 5-HTR2C antagonist RS-102221 (1 μM, n = 4) (C); and 5-HTR7/5 antagonist SB-269970 (1 μM, n = 5) (D). Data are means ± SEM expressed as percentage of basal release (measured during the first 20 min). Statistics included one-way ANOVA with Fisher's LSD multiple comparison test performed on AUC values from log-transformed data for 5-HT *vs.* 5-HT + antagonist. ##, $P < 0.001$; #, $P < 0.005$; *, $P < 0.05$.

onism (Ki ~20 nM) (82) without affinity at the 5-HTR5A (81, 90), 5-HTR1D (91), and 5-HTR2B (52, 54) and can therefore be of further help in discriminating between the involvement of the 5-HTR2A or 5-HTR1D and between the 5-HTR5A and 5-HTR7.

At a dose of 1 μM, spiperone diminished the GH response to 5-HT only very slightly, although a 3 μM dose considerably attenuated the response ($P < 0.005$; $P < 0.01$ *vs.* basal release) (Fig. 6A). When combined with the 5-HTR2B/2C antagonist SB-206553 (1 μM), spiperone caused a partial inhibition of the GH response when used at 1 μM ($P < 0.01$ *vs.* 5-HT, $P < 0.01$ *vs.* basal release) (Fig. 6B), consistent with the above conclusion that it is not the 2A/2B blockade that is responsible for fully annihilating the GH response to 5-HT and the possible participation of the 5-HTR5A in establishing the GH response because the 5-HTR5A is not blocked by spiperone. Spiperone completely abolished the response when a 3 μM dose was used together with the 5-HTR2B antagonist SB-206553 (Fig. 6B). At this concentration spiperone fully blocks the 5-HTR7, explaining the full blockade when coadministered with the 5-HTR2B/2C antagonist SB-206553.

Further characterization of the GH response to 5-CT

5-CT at a dose of 10 nM is expected to occupy mainly the 5-HTR7 (54, 57, 82), 5-HTR5A (54), and 5-HTR5B (54, 81) (Ki 0.2, 13, and 1.3 nM, respectively) and the 5-HTR1B (54) (Ki ~0.3 nM) but little the 5-HTR2B (54, 92) (Ki ~150 nM). To

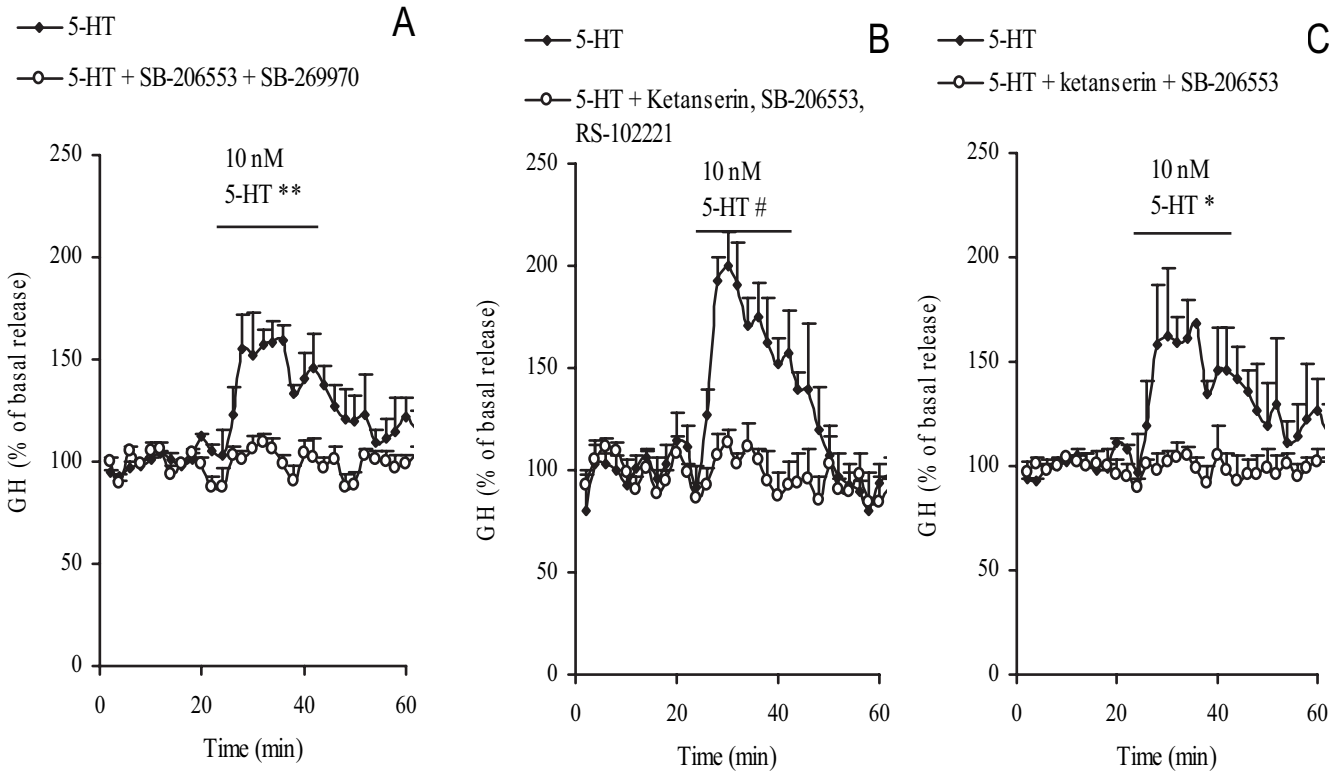

FIG. 4. Effect of combined administration of selective antagonists on the GH response to 10 nM 5-HT in E_2-treated anterior pituitary cell aggregates. A, Combination of 5-HTR2B antagonist SB-206553 and 5-HTR7 antagonist SB-269970 (1 μM, n = 4). B, Combination of 5-HTR2A/5-HTR1D antagonist ketanserin, 5-HTR2B antagonist SB-206553, and 5-HTR2C antagonist RS-102221 (1 μM, n = 4). C, Combination of ketanserin and SB-206553 (1 μM, n = 3). Data are means ± SEM expressed as percentage of basal release (measured during the first 20 min). Statistics included one-way ANOVA with Fisher's LSD multiple comparison test performed on AUC values for 5-HT *vs.* 5-HT + antagonist. #, $P < 0.005$; **, $P < 0.01$; *, $P < 0.05$.

further dissect out the putative participation of the former receptors, we tested the blocking behavior of the 5-HTR7/5 antagonist SB-269970 with 5-CT as agonist, with the expectation that SB-269970 would be more effective in reducing the GH response than with 5-HT as agonist. A 10 nM dose of 5-CT stimulated GH release as expected, but SB-269970 (1 μM) attenuated the GH response ($P < 0.04$) to a comparable extent that it did on the GH response to 10 nM 5-HT (39.6 ± 25% inhibition with 5-CT *vs.* 47.8 ± 17% with 5-HT) (data not shown, n = 3). These data are consistent with the contention that the GH response to 5-CT is not mediated solely by the 5-HTR7 but also indicate that at 10 nM 5-CT already acts at other 5-HTR(s) (such as the 5-HTR2B and/or 5-HTR1B).

Characterization of the GH response to 5-HT in aggregates cultured in hormone-free medium

For several reasons it looked important to also test the involvement of 5-HTRs in aggregates cultured in hormone-free medium. These reasons included: 1) the expression of different 5-HTRs is dependent or modulated by estrogen; 2) because the 5-HTR2C is repressed by estrogen, its participation in the GH response to 5-HT in the above experiments was considered negligible, and hence, the 5-HTR2B could be studied with the 5-HTR2B/2C antagonist SB-206553, but in the absence of estrogen a more selective 5-HTR2B antagonist and a selective 5-HTR2B agonist needed to be used to ascertain whether only the 5-HTR2B or also the 5-HTR2C mediates the GH response; and 3) in the above experiments, we studied the involvement of the 5-HTR1B only by using a 5-HTR1B/D antagonist. Because the expression of the 5-HTR1B is higher in the absence of estrogen than in its presence, it looked necessary to reexamine it again with a selective 5-HTR1B agonist and antagonist.

As shown in Fig. 7A, in aggregates cultured in hormone-free medium, the 5-HTR7/5 antagonist still diminished the GH response (−21.8%) ($P < 0.01$), whereas the 5-HTR2B/2C antagonist was strongly depressing it (86.1%), as expected ($P < 0.01$ *vs.* 5-HT; $P < 0.001$ *vs.* basal release). Interestingly, the impact of SB-269970 (−21.8%) was only half that in E_2-supplemented culture (−41%; see Fig. 3).

In the presence of the highly selective 5-HTR2B antagonist SB-204741 (1 μM) (61), the effect of 5-HT on GH release was significantly depressed ($P < 0.001$) (Fig. 7B), whereas the selective 5-HTR2B agonist BW 723C86 (100 nM) (93) significantly ($P < 0.001$) increased basal GH secretion (Fig. 7D). The GH response to 5-HT was slightly but not significantly ($P = 0.07$) affected by RS-102221 (Fig. 7B), suggesting that the 5-HTR2C does not significantly contribute to the response by 5-HT in hormone-free aggregates.

The selective 5-HTR1B antagonist SB-224289 (1 μM) slightly diminished the GH response to 5-HT ($P < 0.05$) (Fig. 7C), similarly to that seen in E_2-treated aggregates (see above), whereas the selective 5-HTR1B agonist CP 93129 (94)

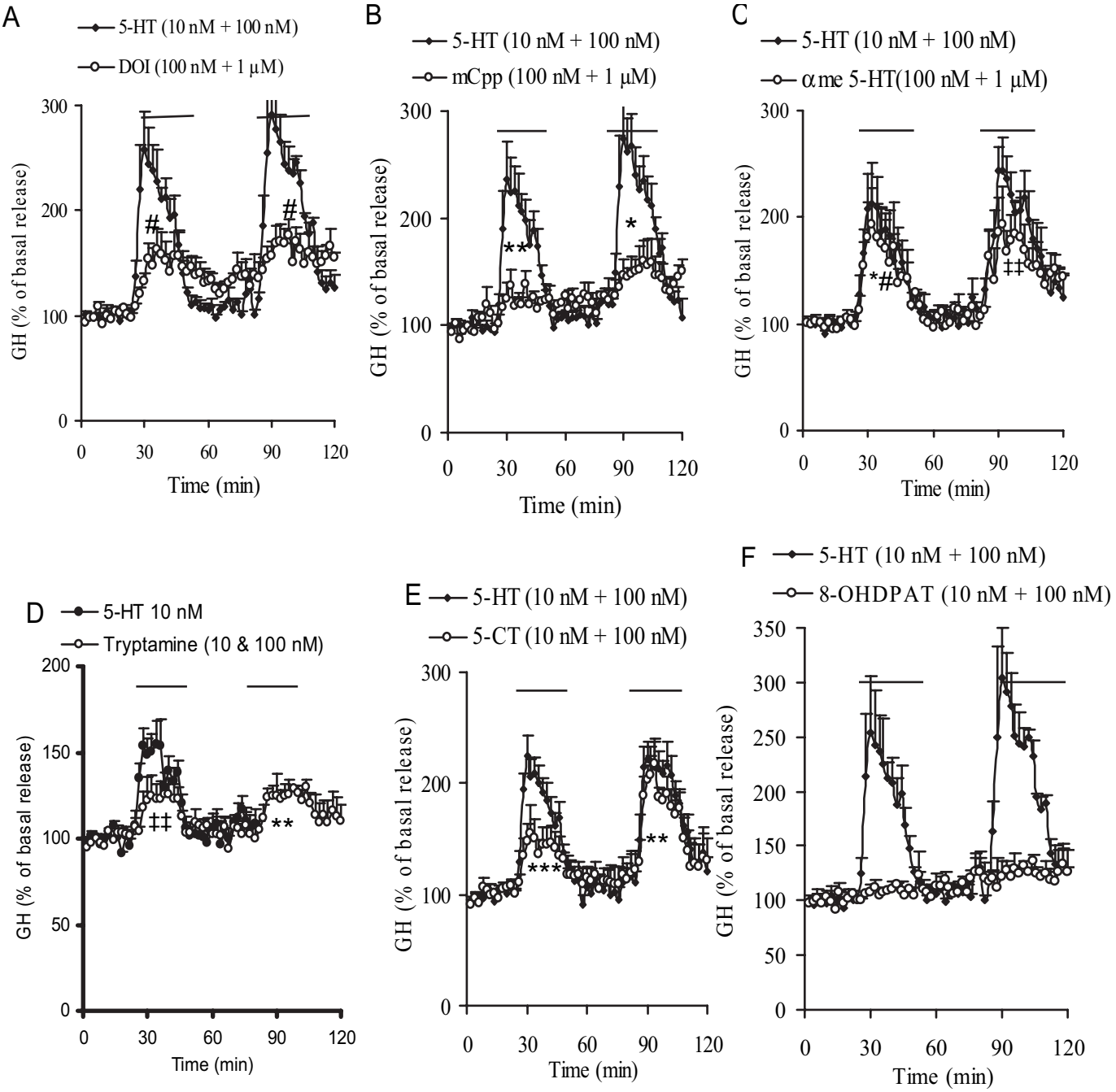

FIG. 5. Effect of 5-HTR agonists on basal GH release in E_2-treated anterior pituitary cell aggregates, compared with the effect of 10 and 100 nM 5-HT, tested simultaneously. A, 5-HTR2 agonist DOI (100 nM and 1 μM, n = 4). B, 5-HTR2 agonist mCpp (100 nM and 1 μM, n = 4). C, 5-HTR2 agonist α-methyl 5-HT (100 nM and 1 μM, n = 4). D, 5-HTR7 agonist tryptamine (10 nM and 100 nM, n = 4). E, 5-HTR1B/1D/5/7 agonist 5-CT (10 nM and 100 nM, n = 5). F, 5-HTR1A agonist 8-OHDPAT (10 and 100 nM, n = 3). Data are means ± SEM expressed as percentage of basal release (measured during the first 20 min). Statistics included one-way ANOVA performed with Fisher's LSD multiple comparison test on AUC data comparing basal with stimulation values and between doses. ##, $P < 0.001$; #, $P < 0.005$; **, $P < 0.01$; *, $P < 0.05$; ‡‡, $P < 0.04$; ‡, $P < 0.02$; *# , $P < 0.03$.

slightly yet significantly ($P < 0.001$) stimulated GH release (Fig. 7D).

5-CT stimulates cAMP production in aggregates cultured in E_2-free condition

In a previous paper reporting the PRL secretory response of the aggregates used in the present study, we showed that 5-HT increased intracellular cAMP levels (1). E_2 supplementation during culture significantly potentiated this response as a result of E_2 induction of 5-HTR4 expression. We here report that in E_2-free condition, 5-CT (100 nM), in which it cannot act via the 5-HTR4, 5-HTR5, and 5-HTR6 (not expressed), caused a 2.5-fold rise in cAMP accumulation ($P < 0.001$) (Fig. 8), consistent with an action at the 5-HTR7, 5-HTR2B, and 5-HTR1B.

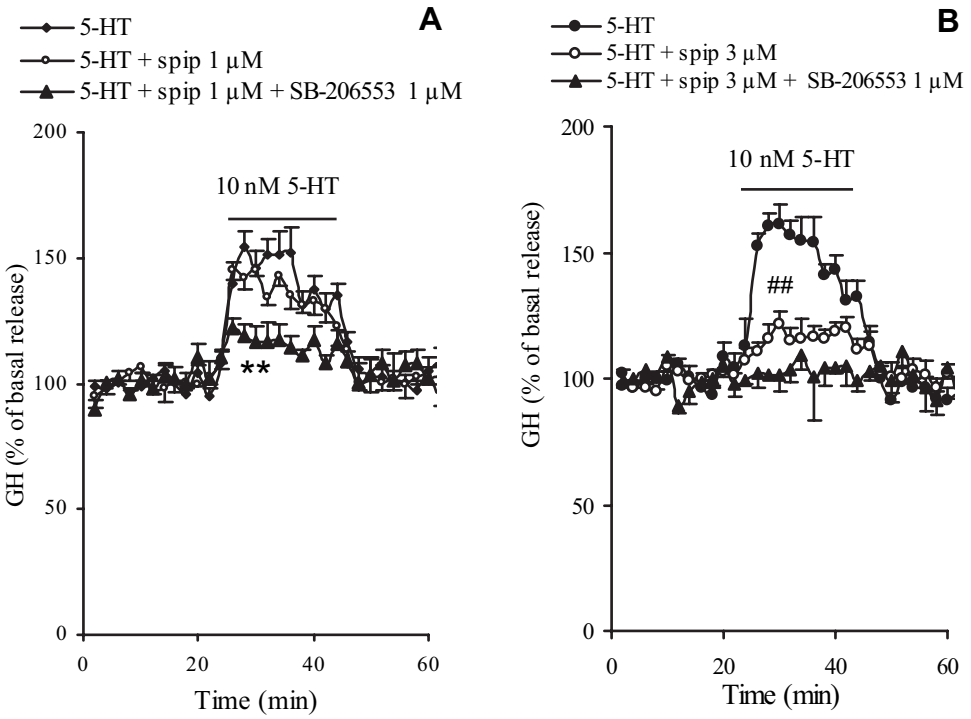

FIG. 6. Effect of spiperone (spip) alone and in combination with the 5-HTR2B antagonist SB-206553 on the GH response to 10 nM 5-HT from anterior pituitary cell aggregates cultured in E_2-supplemented medium. A, 10 nM 5-HT alone and in the presence of spiperone 1 μM (n = 5) or spiperone and 5-HTR2B antagonist SB-206553 1 μM (n = 4). B, 10 nM 5-HT alone and in the presence of spiperone 3 μM (n = 4) or spiperone 3 μM and 5-HTR2B antagonist SB-206553 1 μM (n = 3). Data are means ± SEM expressed as percentage of basal release (measured during the first 20 min). Statistics included one-way ANOVA with Fisher's LSD multiple comparison test performed on AUC values for 5-HT *vs.* 5-HT + antagonist. Basal secretion *vs.* 5-HT + spiperone 1 μM and SB-206553: two-way ANOVA with Fisher's LSD multiple comparison test performed on individual values of each 2-min fraction. 5-HT alone *vs.* 5-HT + 3 μM spiperone with one-way ANOVA performed on AUC values from log-transformed values. ##, $P < 0.001$; **, $P < 0.01$.

Discussion

The present investigation clearly demonstrates a direct stimulatory effect of 5-HT on GH release in anterior pituitary aggregate cell cultures, within minutes of exposure to this agonist in perifusion, and with a dose response between 1 and 10 nM. Although the magnitude of the GH response is much lower than that to the protagonist GH secretagogues, GHRH, and epinephrine, previously tested in the same system (95), it is comparable with the GH response to other peptides such as bombesin-like peptides (46), angiotensin II (51), VIP (96), and the cholinomimetic carbachol (50). It appears therefore that 5-HT is a putative fine-tuning agonist possibly involved in homeostasis or allostasis of basal GH release. The GH response to 5-HT was somewhat enhanced in magnitude by estradiol but not glucocorticoid and thyroid hormone. This is in clear contrast with the GH response to GHRH, VIP, epinephrine, and angiotensin II, which is strongly potentiated by glucocorticoid, with that to bombesin-like peptides being dependent on estradiol and depressed by glucocorticoids and with the effect of carbachol on GH release, which is inhibited in the presence of glucocorticoid but stimulated in the presence of thyroid hormone (49–51, 96). Thus, each peripheral hormone seems to establish adaptations of basal GH release through different signaling systems and sometimes in opposite direction. It is striking that estrogens augment the GH response to 5-HT because we (1) recently reported that 5-HT also increases PRL secretion in the present aggregates, an effect strongly dependent on estrogen. These observations extend growing evidence that estrogens may affect pituitary hormone secretion, gene expression, and differentiation in part via recruitment of growth factors, peptides, and, as found here, also monoamines (97).

An important issue of the present study was to determine which 5-HTR(s) mediate(s) the GH response to 5-HT. A rough indication for 5-HTR mediation is the finding that a 1 μM dose of the nonselective 5-HTR antagonists methiothepin and methysergide diminished the GH response to 10 nM 5-HT. Methiothepin is a high-affinity blocker (Ki ≤ 10 nM) of the 5-HTR2A/B/C, 5-HTR6, 5-HTR7, and 5-HTR1D, whereas methysergide is a high-affinity blocker of the 5-HTR2A/B/C and a medium-affinity antagonist of the 5-HTR7 and 5-HTR1D/F (52–55). Both antagonists display low affinity at other receptors and no blocking action at the 5-HTR3 and 5-HTR4 (98, 99). Because methiothepin and methysergide did fully block the GH response, when applied in combination, it is likely that the 5-HTR2A/B/C, 5-HTR5A/B, 5-HTR7, and some of the 5-HTR1s, but not the 5-HTR3 and 5-HTR4, are the candidate 5-HTRs that can mediate the GH response to 5-HT.

To discriminate between the candidate receptors, partially or fully selective antagonists and agonists were tested. The GH response to 10 nM 5-HT was not affected by the following antagonists of the following receptors: ondansetron (5-HTR3), GR-113808 (5-HTR4), Ro 046790 (5-HTR6), BRL 15572 (5-HTR1A/D), WAY100635 (5-HTR1A), and RS-102221 (5-HTR2C). The 5-HTR1B blocker SB-224289 displayed a small inhibitory effect on the GH response. The 5-HTR2B/2C antagonist SB-206553 behaved as an effective blocker. Participation of the 5-HTR2C was not expected in the presence of estrogen because the receptor is down-regulated. In the absence of estrogen, the 5-HTR2C is probably also not involved as the fully selective 5-HTR2B antagonist, SB-204741 (61), inhibited the GH response equally well as SB-206553, and the selective 5-HTR2C antagonist RS-102221 did not significantly reduce the GH response to 5-HT. Remarkably, ketanserin, the prototype 5-HTR2A antagonist but also a fairly potent 5-HTR1D antagonist [Ki ~10 nM in the rat (85, 86)], increased the response. This apparent disinhibition is not expected to be located at the 5-HTR2A because the canonical 5-HTR2A-

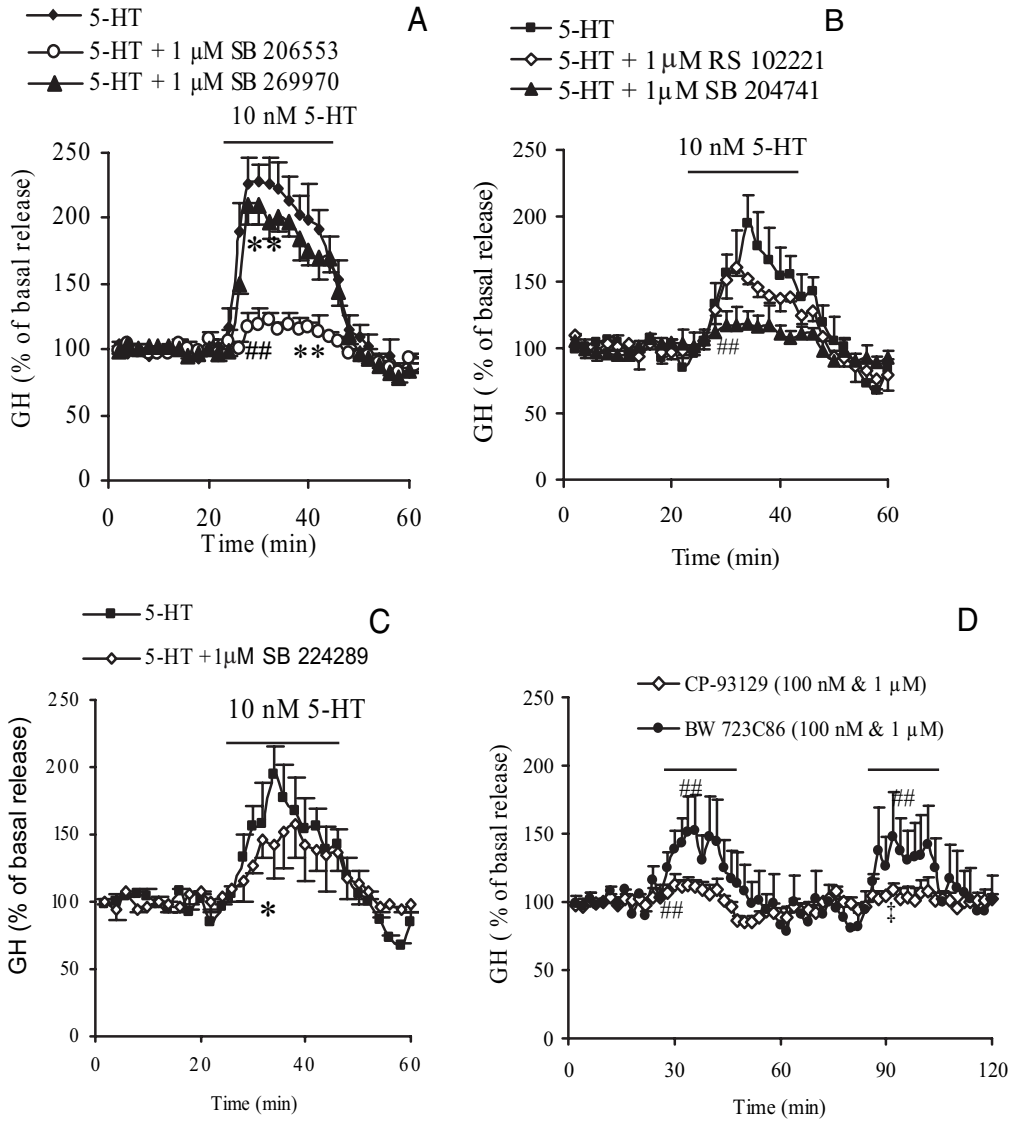

FIG. 7. Effect of 10 nM 5-HT in aggregates cultured in hormone-free medium alone and in the presence of either the 5-HTR2B antagonist SB-206553 (1 μM, n = 3) or the 5-HTR7/5 antagonist SB-269970 1 μM (n = 3) (A); the 5-HTR2B antagonist SB-204741 (1 μM, n = 3) or 5-HTR2C antagonist RS-102221 (1 μM, n = 4) (B); or the 5-HTR1B antagonist SB-224289 (1 μM, n = 3) (C). D, Effect of 100 nM and 1 μM of the selective 5-HTR2B agonist BW723C86 (n = 3) and 5-HTR1B agonist CP 93129, (n = 5) in aggregates cultured in hormone-free conditions. A 10 nM dose 5-HT was perifused as an internal control and consistently increased basal GH secretion (data not shown). Data are means ± SEM expressed as percentage of basal release (measured during the first 20 min). Statistics included ANOVA with Fisher's LSD multiple comparison test: 5-HT *vs.* 5-HT + 1 μM SB-206553, one-way ANOVA performed on AUC values, **, $P < 0.01$; 5-HT *vs.* 5-HT + 1 μM SB-269970, two-way ANOVA performed on individual values of each 2-min fraction, **, $P < 0.01$; basal secretion *vs.* 5-HT + 1 μM SB-206553, two-way ANOVA performed on individual values of each 2-min fraction; 5-HT *vs.* 5-HT + 1 μM SB-204741, two-way ANOVA performed on individual values of each 2-min fraction, $P < 0.001$; 5-HT *vs.* 5-HT + 1 μM RS 102221, two-way ANOVA performed on individual values of each 2-min fraction, $P < 0.08$; 5-HT *vs.* 5-HT + 1 μM SB-224289, two-way ANOVA with Fisher's LSD multiple comparison performed on individual values of each 2-min fraction, $P < 0.05$; basal secretion *vs.* BW723C86, two-way ANOVA performed on individual values of each 2-min fraction, $P < 0.001$; basal secretion *vs.* CP 93129, two-way ANOVA with Fisher's LSD multiple comparison performed on individual values of each 2-min fraction, $P < 0.001$ and $P < 0.02$ for 100 nM and 1 μM, respectively). ##, $P < 0.001$; *, $P < 0.05$; ‡, $P < 0.02$.

mediated pathway is stimulatory, although inhibitory responses have also been reported (100–102). An alternative explanation of the ketanserin effect is disinhibition of 5-HT through the 5-HTR1D, consistent with the negative coupling of 5-HTR1s to adenylyl cyclase (103, 104). SB-269970, a preferential 5-HTR7 antagonist but also displaying fairly good affinity for the 5-HTR5A (Ki ~15 nM), also partially antagonized the GH response to 5-HT. Taken together, these data suggest that the GH response to 5-HT is mediated by the 5-HTR2B, the 5-HTR7, and/or 5-HTR5A, with probably a contribution of the 5-HTR1B. In addition, the results obtained with ketanserin suggest that 5-HT may also have an inhibitory influence on GH secretion through either the 5-HTR2A or 5-HTR1D.

Further arguments for mediation by the 5-HTR2B, 5-HTR7, and 5-HTR1B was obtained by studies with agonists. The involvement of the 5-HTR2B was proved by the finding that the fully selective 5-HTR2B agonist BW723C86

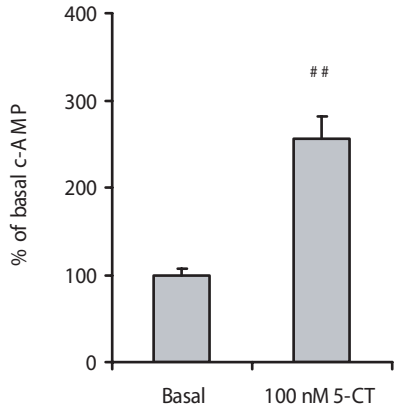

FIG. 8. Effect of 100 nM 5-CT (n = 3) and 100 nM 5-HT (n = 2) on cAMP levels in pituitary cell aggregates cultured without hormone supplement. Data are presented as percentage basal levels in control dishes. One-way ANOVA with Fisher's LSD multiple comparison test performed on log-transformed values for agonist data *vs.* basal release values. ##, $P < 0.001$.

(93) readily increased basal GH release. The stimulatory action of DOI, α-methyl-5-HT, and mCpp, which are agonists at all 5-HTR2 subtypes (Ki ~1–10 nM), without or with only low affinity for other 5-HTRs (54, 65, 66, 72, 76, 78), was consistent with a 5-HTR2 mediation. The lower efficacy of DOI and the even lower efficacy of mCPP are consistent with their reported partial agonism (105). The contribution of the 5-HTR1B was confirmed as the selective 5-HTR1B agonist CP 93129 (94) slightly stimulated GH release. Participation of the 5-HTR7 in GH secretion could not be tested with a fully selective agonist. However, GH release was stimulated by 5-CT, an agonist displaying highest affinity for the 5-HTR7 (Ki ~1 nM) (57, 82). Because 5-CT also has affinity for the 5-HTR5A and -B (Ki ~1 nM) (54, 81) and the 5-HTR1A, -B, and -D (Ki ~0.3–10 nM) (54, 84) but low affinity for the 5-HTR2B (Ki ~150 nM) (54, 76, 106), the effect of 5-CT at 10 nM is likely mediated mainly by the 5-HTR7 and 5-HTR1B/1D. At the 100 nM dose, 5-CT may also act through the 5-HTR2B. The GH response to 10 nM tryptamine is probably also through the 5-HTR7 because at that dose tryptamine is expected to occupy only the 5-HTR7 [Ki ~15 nM at 5-HTR7 (54, 57) and ~100 nM at 5-HTR2B (54, 76)]. Tryptamine, however, has also some affinity for the 5-HTR1D (Ki ~40–80 nM) (107), through which it may dampen the response, a hypothesis consistent with the absence of a dose response in the effect of tryptamine.

Our data are also intriguing because the different 5-HTRs that appear to be involved do not seem to simply add linearly to the GH response. Blocking both the 5-HTR2B and 5-HTR7 or -5A simultaneously are sufficient to completely abolish the GH response. The 5-HTR2B appears to contribute most. Moreover, the GH response was also completely abolished by combined application of ketanserin and SB-206553, even though ketanserin alone enhanced the magnitude of the GH response. Thus, there seems to be a cross talk among the 5-HTR subtypes or between the cells expressing these receptors. One possible model that may explain these observations is that to have a full GH response to 5-HT, both transduction via a Gs and a Gq-coupled mechanism must occur or that actions via Gs are gated by the Gq-induced intracellular messengers. The 5-HTR2 subtypes are known to signal through Gq (108), the 5-HTR7 through Gs (82), and the 5-HTR1 through Gi/o or Gs (109). According to this model, no or little GH response will then be seen in case there is either combined 5-HTR1D and 5-HTR2B blockade or combined 5-HTR2B and 5-HTR7 blockade.

Some further support for the involvement of the 5-HTR1D can be found in the spiperone experiments. Application of the 5-HTR2B antagonist SB-206553 together with spiperone, an antagonist of the 5-HTR2A with equal potency (~1 nM) and similar structure as ketanserin (54) but with no affinity for the 5-HTR1D (54, 84), did not fully block the GH response at a dose of 1 μM, whereas ketanserin + SB-206553 did. In fact, if spiperone and ketanserin would have acted through the 5-HTR2A, the spiperone + SB-206553 combination was expected to block the GH response more effectively than the ketanserin + SB-206553 combination because, unlike ketanserin, spiperone also displays blocking affinity for the 5-HTR7 (Ki ~20 nM) (82), and combined blockade of 5-HTR2B and 5-HTR7 fully abolishes the GH response. At a higher dose (3 μM), spiperone did fully block the GH response when combined with SB-206553, consistent with a blocking action of spiperone at the 5-HTR7. The requirement of a higher dose is consistent with the fact that spiperone has an affinity about 10 times lower than SB-269970 for the 5-HTR7.

With respect to the putative negative regulation by the 5-HTR1D, the puzzling finding has to be dealt with that sumatriptan, an agonist at the 5-HTR1B/D/F (54, 84), did not affect GH release, even at a 1 μM dose. It is possible that sumatriptan simultaneously exerts an inhibitory actions via the 5-HTR1D and a stimulatory one via the 5-HTR1B, resulting in no observable changes in GH secretion. However, at first look there is no argument for this because BRL15572, blocking the 5-HTR1A/D (60), also failed to affect the GH response to 5-HT. The ineffectiveness of BRL15572 may be the consequence of the fact that it is also a medium-potent blocker of the 5-HTR2B (60). Consequently, inhibition of GH secretion by 5-HT via the 5-HTR1D might be compensated for by blocking the stimulatory action via the 5-HTR2B. Future experiments exploring the separate effect of selective 5-HTR1D antagonists and agonists on basal and stimulated GH release are required to further evaluate 5-HTR1-mediated negative regulation.

We were not able to gather conclusive evidence as to the participation of the 5-HTR5 in the GH response because the only selective 5-HTR5 antagonist available is potently active at the human 5-HTR5A (87) but not at the rat 5-HTR5A (88) (no data available for 5-HTR5B). Therefore we tested the participation of the 5-HTR5A by indirect approaches. First, it was found that spiperone, known to block the 5-HTR7 (Ki ~20 nM) (82) but not the 5-HTR5A (Ki >1 μM) (81, 90), displayed a small inhibitory action on the GH response at a dose of 1 μM and a pronounced depression at 3 μM, indicating that the fraction of the GH response blockable by SB-269970 is not mediated by the 5-HTR5A alone but at least in addition by the 5-HTR7. The effect at 1 μM was less than that of the 5-HTR7 antagonist SB-269970, possibly because spiperone is about 10 times less potent at the 5-HTR7 than SB-269970 (Ki ~1 nM). In a second approach, the 5-HTR7

blocking capacity of SB-269970 was tested in aggregates cultured in E_2-free medium. Because in the latter condition, the 5-HTR5A and -B is not expressed to a detectable level (1), it was expected that SB-269970 would be ineffective on the GH response if the 5-HTR5A (or -B) was the only mediating receptor. However, we found that SB-269970 still inhibited the GH response, suggesting that the 5-HTR7 is indeed involved. Nevertheless, the GH response was less affected by SB-269970 in E_2-free condition than in the E_2-supplemented condition, consistent with at least a possibility for participation of the 5-HTR5A or -B in the latter. We cannot, however, exclude the possibility that treatment with E_2 does in fact increase the functional output of the 5-HTR7, in addition to rendering the 5-HTR5 functional.

No studies have examined the role of 5-HTRs other than 5-HTR1B/D, 5-HTR2A/C, and 5-HTR3 subtypes in GH regulation *in vivo*. The present study shows for the first time that the effect of 5-HT at the pituitary level is not mediated by the 5-HTR2A, 5-HTR2C, or 5-HTR3 but by the 5-HTR2B, 5-HTR7, and 5-HTR1B. Partial effect by the estrogen-induced 5-HTR5 remains a possibility, extending our previous observation of estrogen-induced 5-HTR4 in PRL release (1). The present findings open a new avenue in understanding the complexity of 5-HT in GH regulation. It is conceivable that 5-HT participates in holding basal GH release to a certain level beyond the contribution of the GHRH/somatostatin balance. It is interesting in this context to consider a role of estrogen induction of 5-HTR4, 5-HTR5, and 5-HTR6 (1) because estrogens have been shown to increase not only basal PRL release but also basal GH release by a pituitary site of action (110). We previously reported that the 5-HTR4 is upregulated by E_2 treatment and is responsible in mediating the effect of 5-HT on the PRL secretion of anterior pituitary cells (1). What is of particular interest is that the 5-HTRs mediating the GH response are different from the one involved in mediating the PRL response, suggesting that there is a necessity to keep these two systems separate during adaptive functional changes in the pituitary. The present studies demonstrate a unique and complicated role for 5-HT at the level of the pituitary in the regulation of hormone secretion that had not previously been demonstrated.

In conclusion, the present data show a stimulatory action at the pituitary level of 5-HT on GH release and that this effect is mediated by several 5-HTRs. The largest part of the response is mediated by the 5-HTR2B with a contribution of the 5-HTR7 and the 5-HTR1B. The 5-HTR1D may also be involved in a negative fashion. These receptors appear to cross talk, which may be important for stabilizing basal GH release at the pituitary level.

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The authors have nothing to disclose.

References

1. **Papageorgiou A, Denef C** 2007 Estradiol induces expression of 5-hydroxytryptamine (5-HT) 4, 5-HT5 and 5-HT6 receptor mRNA in rat anterior pituitary cell aggregates and allows prolactin release via the 5-HT4 receptor. Endocrinology 148:1384–1395
2. **Hardman JG, Limbird LE** 2001 Goodman and Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill
3. **Hoyer D, Hannon JP, Martin GR** 2002 Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 71:533–554
4. **Raap D, Van de Kar LD** 1999 Selective serotonin reuptake inhibitors and neuroendocrine function. Life Sci 65:1217–1235
5. **Collu R, Fraschini F, Visconti P, Martini L** 1972 Adrenergic and serotoninergic control of growth hormone secretion in adult male rats. Endocrinology 90:1231–1237
6. **Imura H, Nakai Y, Yoshimi T** 1973 Effect of 5-hydroxytryptophan (5-HTP) on growth hormone and ACTH release in man. J Clin Endocrinol Metab 36:204–206
7. **Feldman JM, Plonk JW, Bivens CH, Lebovitz HE, Handwerger S** 1975 Growth hormone and prolactin secretion in the carcinoid syndrome. Am J Med Sci 269:333–347
8. **Smythe GA, Brandstater JF, Lazarus L** 1975 Serotoninergic control of rat growth hormone secretion. Neuroendocrinology 17:245–257
9. **Stuart M, Lazarus L, Smythe GA, Moore S, Sara V** 1976 Biogenic amine control of growth hormone secretion in the fetal and neonatal rat. Neuroendocrinology 22:337–342
10. **Delitala G, Devilla L, Bionda S, Franca V** 1977 Suppression of human growth hormone secretion by cyproheptadine. Metabolism 26:931–936
11. **Arnold MA, Fernstrom JD** 1978 Serotonin receptor antagonists block a natural, short term surge in serum growth hormone levels. Endocrinology 103: 1159–1163
12. **Martin JB, Durand D, Gurd W, Faille G, Audet J, Brazeau P** 1978 Neuropharmacological regulation of episodic growth hormone and prolactin secretion in the rat. Endocrinology 102:106–113
13. **Collu R, Du RP, Tache Y** 1979 Role of putative neurotransmitters in prolactin, GH and LH response to acute immobilization stress in male rats. Neuroendocrinology 28:178–186
14. **Kletzky OA, Marrs RP, Nicoloff JT** 1980 Effects of cyproheptadine on insulin-induced hypoglycaemia secretion of PRL, GH and cortisol. Clin Endocrinol (Oxf) 13:231–234
15. **Parati EA, Zanardi P, Cocchi D, Caraceni T, Muller EE** 1980 Neuroendocrine effects of quipazine in man in health state or with neurological disorders. J Neural Transm 47:273–297
16. **Rabii J, Buonomo F, Scanes CG** 1981 Role of serotonin in the regulation of growth hormone and prolactin secretion in the domestic fowl. J Endocrinol 90:355–358
17. **Casanueva FF, Villanueva L, Penalva A, Cabezas-Cerrato J** 1984 Depending on the stimulus, central serotoninergic activation by fenfluramine blocks or does not alter growth hormone secretion in man. Neuroendocrinology 38:302–308
18. **Hall TR, Harvey S, Chadwick A** 1984 Serotonin and acetylcholine affect the release of prolactin and growth hormone from pituitary glands of domestic fowl *in vitro* in the presence of hypothalamic tissue. Acta Endocrinol (Copenh) 105:455–462
19. **Argenio GF, Bernini GP, Sgro M, Vivaldi MS, Del Corso C, Santoni R, Franchi F** 1991 Blunted growth hormone (GH) responsiveness to GH-releasing hormone in obese patients: influence of prolonged administration of the serotoninergic drug fenfluramine. Metabolism 40:724–727
20. **Franceschini R, Cataldi A, Garibaldi A, Cianciosi P, Scordamaglia A, Barreca T, Rolandi E** 1994 The effects of sumatriptan on pituitary secretion in man. Neuropharmacology 33:235–239
21. **Herdman JR, Delva NJ, Hockney RE, Campling GM, Cowen PJ** 1994 Neuroendocrine effects of sumatriptan. Psychopharmacology (Berl) 113:561–564
22. **Katz LM, Nathan L, Kuhn CM, Schanberg SM** 1996 Inhibition of GH in maternal separation may be mediated through altered serotonergic activity at 5-HT2A and 5-HT2C receptors. Psychoneuroendocrinology 21:219–235
23. **Whale R, Bhagwagar Z, Cowen PJ** 1999 Zolmitriptan-induced growth hormone release in humans: mediation by 5-HT1D receptors? Psychopharmacology 145:223–226
24. **Valverde I, Penalva A, Dieguez C** 2000 Influence of different serotonin receptor subtypes on growth hormone secretion. Neuroendocrinology 71: 145–153
25. **Pinilla L, Gonzalez LC, Tena-Sempere M, Aguilar E** 2001 5-HT1 and 5-HT2 receptor agonists blunt ± α-amino-3-hydroxy-5-methylisoxazole-4-propionic

acid (AMPA)-stimulated GH secretion in prepubertal male rats. Eur J Endocrinol 144:535–541
26. **Lopez F, Gonzalez D, Aguilar E** 1986 Serotonin stimulates GH secretion through a direct pituitary action: studies in hypophysectomized autografted animals and in perifused pituitaries. Acta Endocrinol (Copenh) 113:317–322
27. **Balsa JA, Sanchez-Franco F, Pazos F, Lara JI, Lorenzo MJ, Maldonado G, Cacicedo L** 1998 Direct action of serotonin on prolactin, growth hormone, corticotropin and luteinizing hormone release in cocultures of anterior and posterior pituitary lobes: autocrine and/or paracrine action of vasoactive intestinal peptide. Neuroendocrinology 68:326–333
28. **Gerard C, el Mestikawy S, Lebrand C, Adrien J, Ruat M, Traiffort E, Hamon M, Martres MP** 1996 Quantitative RT-PCR distribution of serotonin 5-HT6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. Synapse 23:164–173
29. **Lowry CA** 2002 Functional subsets of serotonergic neurones: implications for control of the hypothalamic-pituitary-adrenal axis. J Neuroendocrinol 14: 911–923
30. **Johnston CA, Gibbs DM, Negro-Vilar A** 1983 High concentrations of epinephrine derived from a central source and of 5-hydroxyindole-3-acetic acid in hypophysial portal plasma. Endocrinology 113:819–821
31. **Zolkowska D, Rothman RB, Baumann MH** 2006 Amphetamine analogs increase plasma serotonin: implications for cardiac and pulmonary disease. J Pharmacol Exp Ther 318:604–610
32. **Herve P, Launay JM, Scrobohaci ML, Brenot F, Simonneau G, Petitpretz P, Poubeau P, Cerrina J, Duroux P, Drouet L** 1995 Increased plasma serotonin in primary pulmonary hypertension. Am J Med 99:249–254
33. **Callebert J, Esteve JM, Herve P, Peoc'h K, Tournois C, Drouet L, Launay JM, Maroteaux L** 2006 Evidence for a control of plasma serotonin levels by 5-hydroxytryptamine-2B receptors in mice. J Pharmacol Exp Ther 317:724–731
34. **Saavedra JM, Palkovits M, Kizer JS, Brownstein M, Zivin JA** 1975 Distribution of biogenic amines and related enzymes in the rat pituitary gland. J Neurochem 25:257–260
35. **Payette RF, Gershon MD, Nunez EA** 1985 Serotonergic elements of the mammalian pituitary. Endocrinology 116:1933–1942
36. **Leranth C, Palkovits M, Krieger DT** 1983 Serotonin immunoreactive nerve fibers and terminals in the rat pituitary-light and electron-microscopic studies. Neuroscience 9:289–296
37. **Saland LC** 2001 The mammalian pituitary intermediate lobe: an update on innervation and regulation. Brain Res Bull 54:587–593
38. **Westlund KN, Childs GV** 1982 Localization of serotonin fibers in the rat adenohypophysis. Endocrinology 111:1761–1763
39. **Ju G** 1999 Evidence for direct neural regulation of the mammalian anterior pituitary. Clin Exp Pharmacol Physiol 26:757–759
40. **Liu Y, Morris JF, Ju G** 1996 Synaptic relationship of substance P-like-immunoreactive nerve fibers with gland cells of the anterior pituitary in the rat. Cell Tissue Res 285:227–234
41. **Nunez EA, Gershon MD, Silverman AJ** 1981 Uptake of 5-hydroxytryptamine by gonadotrophs of the bat's pituitary: A combined immunocytochemical radioautographic analysis. J Histochem Cytochem 29:1336–1346
42. **Payette RF, Gershon MD, Nunez EA** 1986 Colocalization of luteinizing hormone and serotonin in secretory granules of mammalian gonadotrophs. Anat Rec 215:51–58
43. **Johns MA, Azmitia EC, Krieger DT** 1982 Specific *in vitro* uptake of serotonin by cells in the anterior pituitary of the rat. Endocrinology 110:754–760
44. **Denef C, Maertens P, Allaerts W, Mignon A, Robberecht W, Swennen L, Carmeliet P** 1989 Cell-to-cell communication in peptide target cells of anterior pituitary. Methods Enzymol 168:47–71
45. **Hauspie A, Seuntjens E, Vankelecom H, Denef C** 2003 Stimulation of combinatorial expression of prolactin and glycoprotein hormone α-subunit genes by gonadotropin-releasing hormone and estradiol-17β in single rat pituitary cells during aggregate cell culture. Endocrinology 144:388–399
46. **Houben H, Denef C** 1990 Stimulation of growth hormone and prolactin release from rat pituitary cell aggregates by bombesin and ranatensin-like peptides is potentiated by estradiol, 5α-dihydrotestosterone, and dexamethasone. Endocrinology 126:2257–2266
47. **Swinnen E, Boussemaere M, Denef C** 2005 Stimulation and inhibition of prolactin release by prolactin-releasing peptide in rat anterior pituitary cell aggregates. J Neuroendocrinol 17:379–386
48. **Langouche L, Pals K, Denef C** 2002 Structure-activity relationship and signal transduction of gamma-MSH peptides in GH3 cells: further evidence for a new melanocortin receptor. Peptides 23:1077–1086
49. **Houben H, Denef C** 1992 Negative regulation by dexamethasone of the potentiation of neuromedin C-induced growth hormone and prolactin release by estradiol in anterior pituitary cell aggregates. Life Sci 50:775–780
50. **Carmeliet P, Baes M, Denef C** 1989 The glucocorticoid hormone dexamethasone reverses the growth hormone-releasing properties of the cholinomimetic carbachol. Endocrinology 124:2625–2634
51. **Robberecht W, Denef C** 1988 Stimulation and inhibition of pituitary growth hormone release by angiotensin II *in vitro*. Endocrinology 122:1496–1504
52. **Bonhaus DW, Bach C, DeSouza A, Salazar FH, Matsuoka BD, Zuppan P, Chan HW, Eglen RM** 1995 The pharmacology and distribution of human 5-hydroxytryptamine-2B (5-HT2B) receptor gene products: comparison with 5-HT2A and 5-HT2C receptors. Br J Pharmacol 115:622–628
53. **Bonhaus DW, Weinhardt KK, Taylor M, DeSouza A, McNeeley PM, Szczepanski K, Fontana DJ, Trinh J, Rocha CL, Dawson MW, Flippin LA, Eglen RM** 1997 RS-102221: a novel high affinity and selective, 5-HT2C receptor antagonist. Neuropharmacology 36:621–629
54. **Boess FG, Martin IL** 1994 Molecular biology of 5-HT receptors. Neuropharmacology 33:275–317
55. **Erlander MG, Lovenberg TW, Baron BM, de Lecea L, Danielson PE, Racke M, Slone AL, Siegel BW, Foye PE, Cannon K, Burns JE, Sutcliffe JG** 1993 Two members of a distinct subfamily of 5-hydroxytryptamine receptors (5htr5) differentially expressed in rat brain. Proc Natl Acad Sci USA 90:3452–3456
56. **Lovenberg TW, Baron BM, de Lecea L, Miller JD, Prosser RA, Rea MA, Foye PE, Racke M, Slone AL, Siegel BW** 1993 A novel adenylyl cyclase-activating serotonin receptor (5-HT7) implicated in the regulation of mammalian circadian rhythms. Neuron 11:449–458
57. **Shen Y, Monsma Jr FJ, Metcalf MA, Jose PA, Hamblin MW, Sibley DR** 1993 Molecular cloning and expression of a 5-hydroxytryptamine7 serotonin receptor subtype. J Biol Chem 268:18200–18204
58. **Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ, Roth BL** 2000 evidence for possible involvement of 5-HT2B receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. Circulation 102:2836–2841
59. **Johansson L, Sohn D, Thorberg SO, Jackson DM, Kelder D, Larsson LG, Renyi L, Ross SB, Wallsten C, Eriksson H, Hu PS, Jerning E, Mohell N, Westlind-Danielsson A** 1997 The pharmacological characterization of a novel selective 5-hydroxytryptamine1A receptor antagonist, NAD-299. J Pharmacol Exp Ther 283:216–225
60. **Price GW, Burton MJ, Collin LJ, Duckworth M, Gaster L, Gothert M, Jones BJ, Roberts C, Watson JM, Middlemiss DN** 1997 SB-216641 and BRL-15572—compounds to pharmacologically discriminate h5-HT1B and h5-HT1D receptors. Naunyn Schmiedebergs Arch Pharmacol 356:312–320
61. **Knight AR, Misra A, Quirk K, Benwell K, Revell D, Kennett G, Bickerdike M** 2004 Pharmacological characterisation of the agonist radioligand binding site of 5-HT2A, 5-HT2B and 5-HT2C receptors. Naunyn Schmiedebergs Arch Pharmacol 370:114–123
62. **Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A** 2002 Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J Pharmacol Exp Ther 303:791–804
63. **Selkirk JV, Scott C, Ho M, Burton MJ, Watson J, Gaster LM, Collin L, Jones BJ, Middlemiss DN, Price GW** 1998 SB-224289—a novel selective (human) 5-HT1B receptor antagonist with negative intrinsic activity. Br J Pharmacol 125:202–208
64. **Leysen JE, Eens A, Gommeren W, van Gompel P, Wynants J, Janssen PA** 1988 Identification of nonserotonergic [3H]ketanserin binding sites associated with nerve terminals in rat brain and with platelets; relation with release of biogenic amine metabolites induced by ketanserin and tetrabenazine-like drugs. J Pharmacol Exp Ther 244:310–321
65. **Hoyer D, Engel G, Kalkman HO** 1985 Molecular pharmacology of 5-HT1 and 5-HT2 recognition sites in rat and pig brain membranes: radioligand binding studies with [3H]5-HT, [3H]8-OH-DPAT, (-)[125I]iodocyanopindolol, [3H]mesulergine and [3H]ketanserin. Eur J Pharmacol 118:13–23
66. **Hoyer D** 1988 Molecular pharmacology and biology of 5-HT1C receptors. Trends Pharmacol Sci 9:89–94
67. **Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T, Thomas D, Baxter GS, Forbes IT, Ham P, Blackburn TP** 1996 *In vitro* and *in vivo* profile of SB 206553, a potent 5-HT2C/5-HT2B receptor antagonist with anxiolytic-like properties. Br J Pharmacol 117:427–434
68. **Cussac D, Newman-Tancredi A, Quentric Y, Carpentier N, Poissonnet G, Parmentier JG, Goldstein S, Millan MJ** 2002 Characterization of phospholipase C activity at h5-HT2C compared with h5-HT2B receptors: influence of novel ligands upon membrane-bound levels of [3H]phosphatidylinositols. Naunyn Schmiedebergs Arch Pharmacol 365:242–252
69. **Bonhaus DW, Loury DN, Jakeman LB, To Z, DeSouza A, Eglen RM, Wong EH** 1993 [3H]BIMU-1, a 5-hydroxytryptamine3 receptor ligand in NG-108 cells, selectively labels σ-2 binding sites in guinea pig hippocampus. J Pharmacol Exp Ther 267:961–970
70. **Barnes NM, Sharp T** 1999 A review of central 5-HT receptors and their function. Neuropharmacology 38:1083–1152
71. **Van den Wyngaert I, Gommeren W, Verhasselt P, Jurzak M, Leysen J, Luyten W, Bender E** 1997 Cloning and expression of a human serotonin 5-HT4 receptor cDNA. J Neurochem 69:1810–1819
72. **Bonhaus DW, Berger J, Adham N, Branchek TA, Hsu SAO, Loury DN, Leung E, Wong EHF, Clark RD, Eglen RM** 1997 [3H]RS 57639, a high affinity, selective 5-HT4 receptor partial agonist, specifically labels guinea-pig striatal and rat cloned (5-HT4S and 5-HT4L) receptors. Neuropharmacology 36:671–679.
73. **Ansanay H, Sebben M, Bockaert J, Dumuis A** 1996 Pharmacological comparison between [3H]GR 113808 binding sites and functional 5-HT4 receptors in neurons. Eur J Pharmacol 298:165–174
74. **Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, Med-**

hurst AD 2003 Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. Mol Pharmacol 64:1295–1308

75. **Lovell PJ, Bromidge SM, Dabbs S, Duckworth DM, Forbes IT, Jennings AJ, King FD, Middlemiss DN, Rahman SK, Saunders DV, Collin LL, Hagan JJ, Riley GJ, Thomas DR** 2000 A novel, potent, and selective 5-HT(7) antagonist: (R)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl) phenol (SB-269970). J Med Chem 43:342–345
76. **Wainscott DB, Lucaites VL, Kursar JD, Baez M, Nelson DL** 1996 Pharmacologic characterization of the human 5-hydroxytryptamine-2B receptor: evidence for species differences. J Pharmacol Exp Ther 276:720–727
77. **Egan C, Grinde E, Dupre A, Roth BL, Hake M, Teitler M, Herrick-Davis K** 2000 Agonist high and low affinity state ratios predict drug intrinsic activity and a revised ternary complex mechanism at serotonin 5-HT(2A) and 5-HT(2C) receptors. Synapse 35:144–150
78. **Herrick-Davis K, Grinde E, Gauthier C, Teitler M** 1998 Pharmacological characterization of the constitutively activated state of the serotonin 5-HT2C receptor. Ann NY Acad Sci 861:140–145
79. **May JA, Chen HH, Rusinko A, Lynch VM, Sharif NA, McLaughlin MA** 2003 A novel and selective 5-HT2 receptor agonist with ocular hypotensive activity: (S)-(+)-1-(2-aminopropyl)-8,9-dihydropyrano[3,2-e]indole. J Med Chem 46:4188–4195
80. **Lovenberg TW, Erlander MG, Baron BM, Racke M, Slone AL, Siegel BW, Craft CM, Burns JE, Danielson PE, Sutcliffe JG** 1993 Molecular cloning and functional expression of 5-HT1E-like rat and human 5-hydroxytryptamine receptor genes. Proc Natl Acad Sci USA 90:2184–2188
81. **Wisden W, Parker EM, Mahle CD, Grisel DA, Nowak HP, Yocca FD, Felder CC, Seeburg PH, Voigt MM** 1993 Cloning and characterization of the rat 5-HT5B receptor: evidence that the 5-HT5B receptor couples to a G protein in mammalian cell membranes. FEBS Lett 333:25–31
82. **Ruat M, Traiffort E, Leurs R, Tardivel-Lacombe J, Diaz J, Arrang JM, Schwartz JC** 1993 Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT7) activating cAMP formation. Proc Natl Acad Sci USA 90:8547–8551
83. **Peroutka SJ** 1986 Pharmacological differentiation and characterization of 5-HT1A, 5-HT1B, and 5-HT1C binding sites in rat frontal cortex. J Neurochem 47:529–540
84. **Hamblin MW, Metcalf MA, McGuffin RW, Karpells S** 1992 Molecular cloning and functional characterization of a human 5-HT1B serotonin receptor: a homologue of the rat 5-HT1B receptor with 5-HT1D-like pharmacological specificity. Biochem Biophys Res Commun 184:752–759
85. **Pauwels PJ, Tardif S, Palmier C, Wurch T, Colpaert FC** 1997 How efficacious are 5-HT1B/D receptor ligands: an answer from gtp[γ]S binding studies with stably transfected C6-glial cell lines. Neuropharmacology 36:499–512
86. **Wurch T, Palmier C, Colpaert FC, Pauwels PJ** 1997 Sequence and functional analysis of cloned guinea pig and rat serotonin 5-HT1D receptors: common pharmacological features within the 5-HT1D receptor subfamily. J Neurochem 68:410–418
87. **Corbett DF, Heightman TD, Moss SF, Bromidge SM, Coggon SA, Longley MJ, Roa AM, Williams JA, Thomas DR** 2005 Discovery of a potent and selective 5-HT5A receptor antagonist by high-throughput chemistry. Bioorg Med Chem Lett 15:4014–4018
88. **Thomas DR, Soffin EM, Roberts C, Kew JNC, de la Flor RM, Dawson LA, Fry VA, Coggon SA, Faedo S, Hayes PD** 2006 SB-699551-A (3-cyclopentyl-*N*-[2-(dimethylamino)ethyl]-*N*-[(4′-{[(2-phenylethyl)amino]methyl}-4-biphenylyl)methyl]propanamide dihydrochloride), a novel 5-ht5A receptor-selective antagonist, enhances 5-HT neuronal function: evidence for an autoreceptor role for the 5-ht5A receptor in guinea pig brain. Neuropharmacology 51:566–577
89. **Roth BL, Ciaranello RD, Meltzer HY** 1992 Binding of typical and atypical antipsychotic agents to transiently expressed 5-HT1C receptors. J Pharmacol Exp Ther 260:1361–1365
90. **Rees S, den Daas I, Foord S, Goodson S, Bull D, Kilpatrick G, Lee M** 1994 Cloning and characterisation of the human 5-HT5A serotonin receptor. FEBS Lett 355:242–246
91. **Hamblin MW, McGuffin RW, Metcalf MA, Dorsa DM, Merchant KM** 1992 Distinct 5-HT1B and 5-HT1D serotonin receptors in rat: Structural and pharmacological comparison of the two cloned receptors. Mol Cell Neurosci 3:578–587
92. **Kursar JD, Nelson DL, Wainscott DB, Baez M** 1994 Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine2B receptor. Mol Pharmacol 46:227–234
93. **Kennett GA, Bright F, Trail B, Baxter GS, Blackburn TP** 1996 Effects of the 5-HT2B receptor agonist, BW 723C86, on three rat models of anxiety. Br J Pharmacol 117:1443–1448
94. **Millan MJ, Newman-Tancredi A, Lochon S, Touzard M, Aubry S, Audinot V** 2002 Specific labelling of serotonin 5-HT1B receptors in rat frontal cortex with the novel, phenylpiperazine derivative, [3H]GR125,743: a pharmacological characterization. Pharmacol Biochem Behav 71:589–598
95. **Baes M, Denef C** 1987 Evidence that stimulation of growth hormone release by epinephrine and vasoactive intestinal peptide is based on cell-to-cell communication in the pituitary. Endocrinology 120:280–290
96. **Denef C, Schramme C, Baes M** 1984 Influence of corticosteroids on prolactin release from anterior pituitary cell aggregates cultured in serum-free medium. Differential effects on dopamine-induced inhibition, post-dopamine rebound and stimulation by TRH, vasoactive intestinal peptide (VIP), angiotensin II and isoproterenol. J Steroid Biochem 20:197–202
97. **Denef C** 2003 Paracrine control of lactotrope proliferation and differentiation. Trends Endocrinol Metab 14:188–195
98. **Grossman CJ, Kilpatrick GJ, Bunce KT** 1993 Development of a radioligand binding assay for 5-HT4 receptors in guinea-pig and rat brain. Br J Pharmacol 109:618–624
99. **Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP** 1994 International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 46:157–203
100. **Edagawa Y, Saito H, Abe K** 2000 The serotonin 5-HT2 receptor-phospholipase C system inhibits the induction of long-term potentiation in the rat visual cortex. Eur J Neurosci 12:1391–1396
101. **Marcoli M, Rosu C, Bonfanti A, Raiteri M, Maura G** 2001 Inhibitory presynaptic 5-hydroxytryptamine2A receptors regulate evoked glutamate release from rat cerebellar mossy fibers. J Pharmacol Exp Ther 299:1106–1111
102. **Wang SJ, Wang KY, Wang WC, Sihra TS** 2006 Unexpected inhibitory regulation of glutamate release from rat cerebrocortical nerve terminals by presynaptic 5-hydroxytryptamine-2A receptors. J Neurosci Res 84:1528–1542
103. **Lin SL, Setya S, Johnson-Farley NN, Cowen DS** 2002 Differential coupling of 5-HT(1) receptors to G proteins of the G (i) family. Br J Pharmacol 136: 1072–1078
104. **Francken BJ, Jurzak M, Vanhauwe JF, Luyten WH, Leysen JE** 1998 The human 5-ht5A receptor couples to Gi/Go proteins and inhibits adenylate cyclase in HEK 293 cells. Eur J Pharmacol 361:299–309
105. **Porter RH, Benwell KR, Lamb H, Malcolm CS, Allen NH, Revell DF, Adams DR, Sheardown MJ** 1999 Functional characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C receptors in CHO-K1 cells. Br J Pharmacol 128:13–20
106. **Kursar JD, Nelson DL, Wainscott DB, Cohen ML, Baez M** 1992 Molecular cloning, functional expression, and pharmacological characterization of a novel serotonin receptor (5-hydroxytryptamine-2F) from rat stomach fundus. Mol Pharmacol 42:549–557
107. **Weinshank RL, Zgombick JM, Macchi MJ, Branchek TA, Hartig PR** 1992 Human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-HT1Dα and 5-HT1Dβ. Proc Natl Acad Sci USA 89:3630–3634
108. **Roth BL, Willins DL, Kristiansen K, Kroeze WK** 1998 5-Hydroxytryptamine2-family receptors (5-hydroxytryptamine2A, 5-hydroxytryptamine2B, 5-hydroxytryptamine-2C): where structure meets function. Pharmacol Ther 79:231–257
109. **Lanfumey L, Hamon M** 2004 5-HT1 receptors. Curr Drug Targets CNS Neurol Disord 3:1–10
110. **Carlsson L, Eriksson E, Seeman H, Jansson JO** 1987 Oestradiol increases baseline growth hormone levels in the male rat: possible direct action on the pituitary. Acta Physiol Scand 129:393–399

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