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Human 5-HT_{1B} receptor stimulated inositol phospholipid hydrolysis in CHO cells: synergy with G_q-coupled receptors

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Abstract

We have previously reported that the transfected G_i/G_o protein-coupled human adenosine A_1 receptor (expressed at 200 fmol/mg of protein) and the endogenous 5-HT_{1B} receptor (not detectable using radioligand binding) suppress forskolin-stimulated cyclic AMP accumulation and stimulate increases in $[Ca^{2+}]_i$ in Chinese hamster ovary cells (CHO). In addition, co-activation of the adenosine A_1 receptor (but not the 5-HT_{1B} receptor) potentiates the hydrolysis of inositol phospholipids elicited by receptors coupled to G_q-proteins (Dickenson and Hill, 1996. Eur. J. Pharmacol. 320, 141–151). In order to establish whether this difference in ability to modulate G_q-coupled receptor responses is a consequence of low 5-HT_{1B} receptor density, we have stably transfected CHO-K1 cells with the human 5-$HT_{1D\beta}$ cDNA (the human homologue of the rodent 5-HT_{1B} receptor). We initially isolated a clonal cell line (designated CHO5-HT_{1B} cells) displaying moderate specific [^{3}H]5-HT binding (pK_d of 8.17 ± 0.07 and a B_{max} of 140 fmol/mg protein). In CHO5-HT_{1B} cells, the selective human 5-$HT_{1B/1D}$ receptor agonist sumatriptan produced a concentration-dependent inhibition of forskolin-stimulated cyclic AMP accumulation ($pEC_{50} = 7.92 \pm 0.04$). Sumatriptan also elicited a moderate and pertussis toxin-sensitive increase in [^3H]inositol phosphate formation in CHO-5HT_{1B} cells ($pEC_{50} = 6.51 \pm 0.05$). Finally, sumatriptan synergistically enhanced P_{2U} purinoceptor stimulated [^{3}H]inositol phosphate accumulation through a pertussis toxin-sensitive mechanism. These findings clearly show the significance of 5-HT_{1B} receptor expression level in determining whether 5-HT_{1B} receptor activation can modulate the accumulation of [^{3}H]inositol phosphates elicited by a G_q-protein coupled receptor. The observation that 5-HT_{1B} receptor activation can potentiate G_q-coupled receptor stimulated second messenger responses may have an important physiological role in the regulation of vascular smooth muscle contraction.

Keywords: 5-HT_{1B} receptor; P_{2U} purinoceptor; Inositol phosphate; CHO cell

1. Introduction

Members of the 5-HT_1 receptor subtype family (5-HT_{1A}, 5-HT_{1B}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E} and 5-HT_{1F}) are predominantly coupled to the pertussis toxin-sensitive family of G-proteins (G_{i1}, G_{i2}, G_{i3} and G_o). The signal transduction pathways associated with these 'inhibitory' receptors include: inhibition of adenylyl cyclase activity, closing of voltage-sensitive Ca^{2+} channels, and opening of potassium channels (Boess and Martin, 1994).

Pharmacological evidence suggests that 5-$HT_{1B/1D}$-like receptors are partly responsible for the vasoconstrictor effects of 5-HT observed in coronary and cerebral vascular smooth muscle (Maclean et al., 1996; Nishimura, 1996; Terron, 1996). Molecular biology studies have shown that 5-$HT_{1D\beta}$ receptor mRNA (now designated the human 5-HT_{1B} receptor; Hartig et al., 1996) is expressed in cerebral and peripheral blood vessels of several species including humans (Hamel et al., 1993; Ullmer et al., 1995; Bouchelet et al., 1996). These findings suggest that 5-HT_{1B} receptors mediate vasoconstriction in brain and pulmonary blood vessels. However, at present, little is known about the mechanism(s) involved in 5-HT_{1B} receptor-mediated contraction of vascular smooth muscle. Interestingly, recombinant human 5-HT_{1B} and 5-HT_{1D} receptors stimulate inositol phospholipid hydrolysis, and increase intracellular free calcium ($[Ca^{2+}]_i$) in transfected murine fibroblasts (Zgombick et al., 1993). Furthermore, endogenous 5-HT_{1B}-like receptors have been shown to stimulate in-

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creases in $[Ca^{2+}]_i$ in cells derived from bovine basilar artery and Chinese hamster ovary cells (Ebersole et al., 1993; Dickenson and Hill, 1995). Clearly, 5-HT_{1B} receptor-mediated increases in $[Ca^{2+}]_i$ may underlie the vasoconstrictor effects of 5-HT in vascular smooth muscle.

5-HT_1-like receptor activation can also amplify the vasoconstrictor responses elicited by vascular smooth muscle spasmogens such as histamine, angiotensin and thromboxane A_2 (MacLennan et al., 1993; Choppin and O'Connor, 1995, 1996; Smith et al., 1996). The second messenger pathway(s) underlying the 5-HT_1-like receptor-mediated amplification of vascular smooth muscle contraction remain unclear. Vascular smooth muscle contraction can also be amplified by other G_i-protein coupled receptors e.g., neuropeptide Y_1 and α_2-adrenoceptors (MacLennan et al., 1993). In vascular smooth muscle activation of Ca^{2+}-mobilising receptors such as histamine H_1 and angiotensin II leads to the breakdown of phosphatidylinositol 4,5-biphosphate into inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (Berridge, 1993). $InsP_3$-induced Ca^{2+} release is involved in the initial phase of smooth muscle contraction, whereas diacylglycerol-activation of protein kinase C is thought to play a role in the maintenance of contraction (Berridge, 1993). Interestingly, recent studies have shown that co-activation of G_i-protein coupled receptors can enhance $InsP_3$ formation, Ca^{2+} release and protein kinase C activation elicited by G_q-protein coupled receptors (Gerwins and Fredholm, 1995; Selbie et al., 1995; Dickenson and Hill, 1996). Hence, co-activation of G_i- and G_q protein-coupled receptors in vascular smooth muscle may lead to the increased formation of $InsP_3$ and activation of protein kinase C resulting in enhanced cellular contraction.

We recently reported that co-activation of the endogenous 5-HT_{1B} receptor in Chinese hamster ovary (CHO) cells had no significant effect on CCK_A receptor stimulated inositol phospholipid hydrolysis (Dickenson and Hill, 1996). In contrast, the human transfected adenosine A_1 receptor in CHO cells synergistically enhances G_q-coupled receptor (CCK_A receptor and P_{2U} purinoceptors) stimulated inositol phospholipid hydrolysis (Megson et al., 1995; Dickenson and Hill, 1996). Interestingly, the endogenous 5-HT_{1B} receptor is expressed at levels that are not detectable using specific [^{3}H]5-HT or [^{125}I]cyanopindolol binding (Giles et al., 1996) whereas, the human transfected adenosine A_1 receptor is expressed at 200 fmol/mg of protein (Iredale et al., 1994). The low level of 5-HT_{1B} receptor expression may explain why 5-HT_{1B} receptor activation fails to augment CCK_A receptor-stimulated inositol phospholipid hydrolysis in CHO cells. To investigate this intriguing possibility, we have increased the level of 5-HT_{1B} receptor expression by stably transfecting CHO-K1 cells with the human 5-HT_{1B} receptor cDNA. In this study, we report for the first time that 5-HT_{1B} receptor activation can augment the hydrolysis of inositol phospholipids elicited by a G_q-protein coupled receptor.

2. Materials and methods

2.1. Expression of recombinant human 5-HT_{1B} receptors in Chinese hamster ovary cells

The Bluescript expression vector containing the human 5-HT_{1B} receptor cDNA was obtained from the American Type Culture Collection. The Bluescript plasmid was initially digested with *Hin*dIII and *Bam*HI restriction enzymes and the resulting 1.79-kb fragment (containing the 5-HT_{1B} receptor cDNA) subcloned into the *Hin*dIII/*Bam*HI site of the eukaryotic expression vector pcDNA3. The resulting plasmid (pcDNA35-HT_{1B}) was used to transfect CHO-K1 cells (European Collection of Animal Cell Cultures, Porton Down, Salisbury, UK) by the calcium phosphate precipitation technique. Stably transfected CHO-K1 cells were selected using 500 μg/ml geneticin (G418) for two weeks. CHO-K1 cells resistant to G418 were subsequently cloned by dilution cloning method. Clone five (termed CHO-5HT_{1B} cells) was found to display moderate specific [^{3}H]5-HT binding, and was used throughout the study. CHO-5HT_{1B} cells were cultured in 75 cm^2 flasks (Costar) in Dulbeco's modified Eagle's Medium/Nutrient F12 (1:1) supplemented with 2 mM L-glutamine, 10% (v/v) foetal calf serum and 500 μg/ml G418. Cells were maintained at 37°C in a humidified 5% CO_2 atmosphere until confluency and were subcultured (1:5 split ratio) using trypsin (0.05% w/v)/EDTA (0.02% w/v) solution. Cells for [^{3}H]inositol phosphate and [^{3}H]cyclic AMP determinations were grown in 24-well cluster dishes (Costar).

2.2. Inositol phospholipid hydrolysis

[^{3}H]inositol phosphate accumulation was measured by pre-labelling cell monolayers with [^{3}H]*myo*-inositol as described previously (Dickenson and Hill, 1996).

2.3. Cyclic AMP accumulation

[^{3}H]cyclic AMP accumulation was measured by pre-labelling cell monolayers with [^{3}H]adenine as described previously (Megson et al., 1995).

2.4. [^{3}H]5-HT binding

CHO-5HT_{1B} cells were grown to confluence in 162 cm^2 flasks. When confluent, cells from two flasks were detached using Dulbeco's phosphate buffered saline solution (Sigma) containing 5 mM EDTA at 37°C for 5 min. After centrifugation ($150 \times g$ for 5 min) membranes were prepared by re-suspending the cells in 10 ml of ice-cold Tris buffer (50 mM, pH 7.4), followed by homogenisation using a glass-teflon homogeniser (approximately 10

strokes) and centrifugation at 20 000 × *g* for 15 min. The resulting pellet was re-suspended in 600 μl of Tris buffer and kept on ice until required.

Saturation binding experiments were performed in 50 mM Tris buffer, pH 7.4, containing 4 mM $CaCl_2$, 100 μM ascorbic acid, 10 μM pargyline and 10 μM paroxetine with increasing concentrations of [^{3}H]5-HT (0.5 to 32 nM). CHO-$5HT_{1B}$ cell membranes (10 μl; approximately 250 μg protein) were incubated in the presence (non-specific binding) or absence (total binding) of GR 127935 (1 μM) in a total volume of 200 μl. After 45 min at 37°C, the incubation was stopped by rapid filtration using a Brandel MR24 cell harvester and washing with ice-cold Tris buffer (3 times, approximate volume 10 ml) over Whatman GF/B filters (pre-soaked for 1 h in 0.3% polyethylenimine to reduce non-specific binding). Filters were transferred to scintillation vial inserts, and 4 ml of Emulsifier-Safe scintillator (Packard) added. The filters were left at room temperature for at least 4 h before liquid-scintilllation spectrometry.

Protein determinations were made using the method of Bradford (1976) using bovine serum albumin as the standard.

2.5. Data analysis

pEC_{50} ($-\log EC_{50}$; concentration of drug producing 50% of the maximal response) values were obtained by computer-assisted curve fitting by use of the computer programme InPlot (GraphPAD, San Diego, CA, USA). Statistical significance was determined by Student's unpaired *t*-test ($P < 0.05$ was considered statistically significant). All data are presented as mean ± S.E.M. The *n* in the text refers to the number of separate experiments.

GraphPAD was also used to perform nonlinear regression analysis for fitting data from saturation binding experiments as previously described (Dickenson and Hill, 1994).

2.6. Chemicals

[2-^3H]*myo*-inositol and [2,8-^3H]adenine were supplied by New England Nuclear (Stevenage, UK). [^3H]-5-hydroxytryptamine trifluoroacetate (97 Ci mmol^{-1}) was from Amersham International. Pertussis toxin was obtained from Porton Products. Uridine 5′ triphosphate, forskolin, pargyline and ascorbic acid were purchased from Sigma (Poole, Dorset). The kind gifts of sumatriptan and GR 127935 (from Glaxo Wellcome Research Group (Stevenage, UK) and CP 93,127 (3-(1,2,5,6-tetrahydropyrid-4-yl) pyrrolo[3,2-b]pyrid-5-one) from Pfizer (Groton, CT, USA) are gratefully acknowledged. Dulbeco's modified Eagles

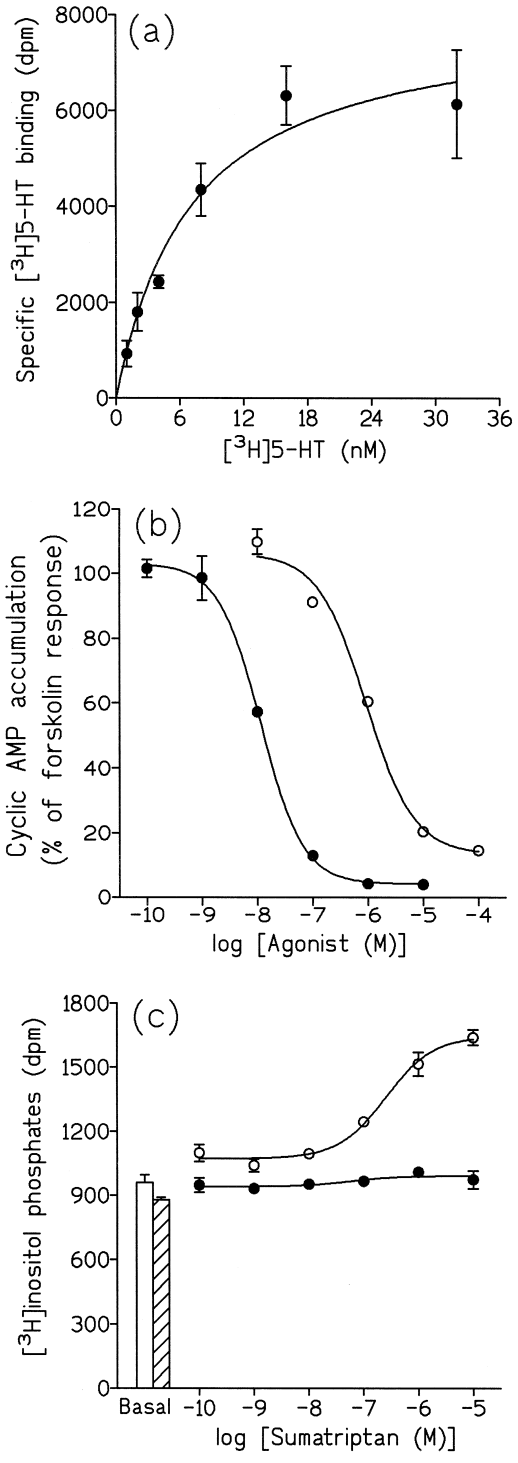

Fig. 1. Functional expression of human $5\text{-}HT_{1B}$ receptors in CHO-K1 cells. (a) Saturation binding of [^{3}H]5-HT to CHO-$5HT_{1B}$ cell membranes. Results are the mean ± S.E.M. from three independent experiments each performed in triplicate. (b) Sumatriptan (●) and CP 93,127 (○) mediated inhibition of forskolin (3 μM) stimulated cAMP formation. Results are expressed as the % of the forskolin response (in the absence of agonist = 100%) and represent the mean ± S.E.M. from three independent experiments each performed in triplicate. (c) Sumatriptan-stimulated [^{3}H]inositol phosphate accumulation in control cells (○) and cells pre-treated for 24 h with 100 ng/ml pertussis toxin (●). The histograms represent basal [^{3}H]inositol phosphate accumulation in control (open) and pertussis toxin-treated cells (shaded). Values represent the mean ± S.E.M. of triplicate determinations measured in a single experiment. Similar results were obtained in at least two further experiments.

Medium/Nutrient Mix F-12 (1:1) and foetal calf serum were from Sigma (Poole, Dorset, UK). All other chemicals were of analytical grade.

3. Results

3.1. Stable expression of the human 5-HT_{1B} receptor in CHO-K1 cells

Fig. 1a shows the specific binding of [^{3}H]5-HT to cell membranes prepared from CHO-5HT_{1B} cells. Saturation binding experiments using [^{3}H]5-HT produced a dissociation constant (pK_d) of 8.17 ± 0.07 ($n = 3$) and a B_{max} of 140 ± 26 ($n = 3$) fmol/mg of protein. Previous studies have shown that CHO-K1 cells express an endogenous 5-HT_{1B} receptor, which cannot be detected using specific binding of [^{3}H]5-HT or [^{125}I]cyanopindolol (Giles et al., 1996). In CHO-5HT_{1B} cells, the selective human 5-$HT_{1B/1D}$ receptor agonist sumatriptan, suppressed forskolin-stimulated cyclic AMP accumulation with a pEC_{50} of 7.92 ± 0.04 ($n = 3$; Fig. 1b). Similarly, the selective rodent 5-HT_{1B} receptor agonist CP 93,127 (Macor et al., 1990) also inhibited forskolin-stimulated cyclic AMP accumulation with an pEC_{50} of 5.99 ± 0.07 ($n = 3$; Fig. 1b). Maximally effective concentrations of sumatriptan (1 μM) and CP 93,127 (100 μM) inhibited the forskolin (3 μM) response by $95.9 \pm 0.3\%$ ($n = 3$) and $85.4 \pm 1.8\%$ ($n = 3$), respectively. The EC_{50} value obtained for CP 93,127 in CHO5-HT_{1B} cells (983 nM) is indicative of the human 5-HT_{1B} receptor (Parker et al., 1993). CP 93,127 has a 100-fold higher potency for the rodent 5-HT_{1B} receptor and the endogenous 5-HT_{1B}-like receptor in CHO cells (EC_{50} ca. 10 nM; Schoeffter et al., 1995; Giles et al., 1996).

Sumatriptan (1 μM) also stimulated a modest increase in the accumulation of total [^{3}H]inositol phosphates ($1.8 \pm$ 0.4-fold increase relative to basal levels; $n = 7$; $P < 0.05$). The response to sumatriptan was concentration-dependent with an pEC_{50} of 6.51 ± 0.05 ($n = 7$; Fig. 1c). Finally, pre-treatment of CHO5-HT_{1B} cells with pertussis toxin (100 ng/ml for 24 h) completely abolished sumatriptan-stimulated [^{3}H]inositol phosphate accumulation (Fig. 1c).

3.2. Interactions between 5-HT_{1B} receptors and P_{2U} purinoceptors in CHO-5HT_{1B} cells

We have previously reported that co-activation of the endogenous 5-HT_{1B} receptor in CHO cells had no effect on CCK_A receptor stimulated inositol phospholipid hydrolysis (Dickenson and Hill, 1996). In CHO-5HT_{1B} cells, we

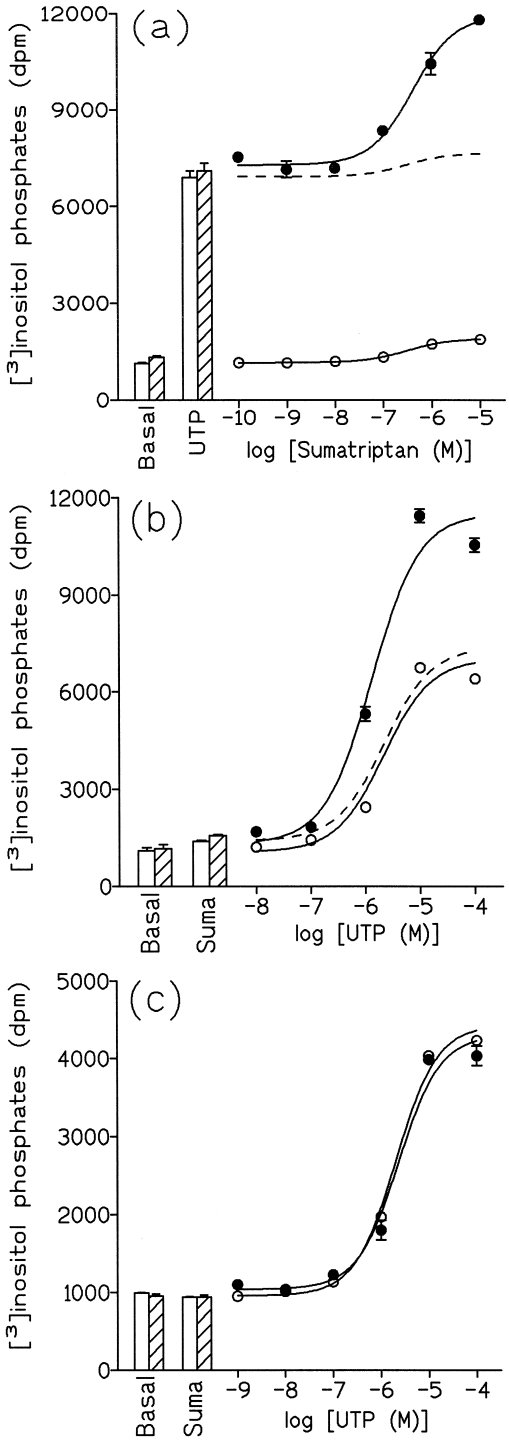

Fig. 2. Effect of sumatriptan on P_{2U} purinoceptor-stimulated [^{3}H]inositol phosphate accumulation in CHO-5HT_{1B} cells. (a) Concentration–response curve to sumatriptan in the absence (○) and presence of 100 μM UTP (●). (b) Concentration–response curve to UTP in the absence (○) and presence of 1 μM sumatriptan (●). (c) As in (b), but cells were pretreated for 24 h with 100 ng/ml pertussis toxin. The histograms represent basal and responses to UTP (100 μM) and sumatriptan (1 μM) alone, measured in each experiment. The dotted lines represent the predicted additive responses to UTP and sumatriptan. These were calculated by adding the response obtained with the fixed concentration of agonist (sumatriptan or UTP and after subtracting basal [^{3}H]inositol phosphate accumulation) to that obtained with each concentration of agonist in the dose–response curve (sumatriptan or UTP). Values represent mean ± S.E.M. of triplicate determinations measured in a single experiment. Similar data were obtained in at least two other experiments.

did not observe any measurable increase in [^{3}H]inositol phosphate accumulation following stimulation of the CCK_A receptor (data not shown), and therefore in this study, we examined the effect of 5-HT_{1B} recepter activation on P_{2U}-purinoceptor-stimulated [^{3}H]inositol phosphate accumulation. Fig. 2a shows the effect of increasing concentrations of sumatriptan on P_{2U}-purinoceptor stimulated [^{3}H]inositol phosphate accumulation in CHO5-HT_{1B} cells. Co-activation with sumatriptan and UTP produced a synergistic increase in [^{3}H]inositol phosphate accumulation. For example, maximally effective concentrations of UTP (100 μM) and sumatriptan (10 μM) produced increases in [^{3}H]inositol phosphates of 5.4 ± 0.4 ($n = 4$) and 1.7 ± 0.1 ($n = 4$) fold relative to basal, respectively, whereas a combination of UTP (100 μM) and sumatriptan (10 μM) elicited a 8.7 ± 0.2 ($n = 4$) fold relative to basal increase in [^{3}H]inositol phosphate accumulation. This response is significantly greater than the predictive additive fold relative to basal response of 6.1 ± 0.5 ($n = 4$; $P < 0.05$). The pEC_{50} value for sumatriptan in the presence of UTP (100 μM) was 6.55 ± 0.12 ($n = 4$). Fig. 2b shows the effect of 1 μM sumatriptan on the concentration–response curve for UTP-stimulated [^{3}H]inositol phosphate accumulation (pEC_{50} for UTP $= 5.62 \pm 0.01$; $n = 3$). Sumatriptan augmented the responses elicited by 1, 10 and 100 μM UTP. The pEC_{50} for UTP in the presence of 1 μM sumatriptan was 5.88 ± 0.02 ($n = 3$). Finally, the synergistic interactions between sumatriptan and UTP were blocked in cells pre-treated for 24 h with 100 ng/ml pertussis toxin (Fig. 2c).

4. Discussion

Chinese hamster ovary (CHO) cells express endogenous 5-HT_{1B}-like receptors functionally coupled to the inhibition of adenylyl cyclase through pertussis toxin-sensitive G_i/G_o proteins (Berg et al., 1994; Dickenson and Hill, 1995). Furthermore, we have recently shown that 5-HT_{1B} receptor activation in CHO-K1 and CHO-A1 cells (a CHO cell line stably expressing the human adenosine A_1 receptor) also triggers a pertussis toxin-sensitive increase in $[Ca^{2+}]_i$ (Dickenson and Hill, 1995). Interestingly, these functional 5-HT_{1B} receptors are expressed at levels that are not detectable using specific [^{3}H]5-HT or [^{125}I]cyanopindolol binding (Giles et al., 1996).

In CHO-K1 cells, transfected G_i-protein coupled receptors (adenosine A_1 and neuropeptide Y_1 receptors) have been shown to potentiate G_q-coupled receptor (P_{2U}, CCK_A and α_{1B}-adrenoceptors) stimulated inositol phospholpid hydrolysis (Selbie et al., 1995; Megson et al., 1995; Dickenson and Hill, 1996). However, activation of the endogenous G_i-coupled 5-HT_{1B} receptor in CHO cells had no effect on the [^{3}H]inositol phosphate response elicited by the G_q-protein coupled receptor, CCK_A (Dickenson and Hill, 1996). We spectulated that the inability of the endogenous 5-HT_{1B} receptor to augment G_q-protein coupled receptor responses (compared to the transfected G_i-coupled receptors) may be a consequence of the low level of 5-HT_{1B} receptor expression and/or the coupling of the endogenous hamster 5-HT_{1B} receptor to different pertussis toxin-sensitive G_i/G_o protein.

In this study, we have investigated the second messenger interactions between the 5-HT_{1B} receptor and the endogenous P_{2U} purinoceptor, in CHO cells expressing increased levels of 5-HT_{1B} receptor (140 ± 26 fmol/mg of protein). Increased levels of 5-HT_{1B} receptor expression were achieved by stably transfecting CHO-K1 cells with the cDNA for the human 5-$HT_{1D\beta}$ receptor. The 5-$HT_{1D\beta}$ receptor is considered to be the human homologue of the 5-HT_{1B} receptor found in rats, mice, hamster and opossum (Hoyer et al., 1994; Hartig et al., 1996). Functional responses in CHO5-HT_{1B} cells were evaluated using the selective human 5-$HT_{1B/1D}$ receptor agonist sumatriptan. The EC_{50} value (12 nM) for sumatriptan-mediated inhibition of forskolin-induced cAMP accumulation obtained in this study is comparable to the values reported for transfected human 5-HT_{1B} receptors in murine fibroblasts ($EC_{50} = 2$ nM; Zgombick et al., 1993) and adrenocortical Y-1 cells ($EC_{50} = 7.6$ nM; Zgombick et al., 1996). In contrast, sumatriptan has a 10–100-fold lower potency for the rat 5-HT_{1B} receptor (Hoyer et al., 1994). For example, in rat substantia nigra and cultured renal mesangial cells, sumatriptan inhibits forskolin-stimulated cAMP accumulation with EC_{50} values of 1 μM and 512 nM, respectively (Hoyer et al., 1994; Schoeffter et al., 1995). These values are similar to the potency of sumatriptan (EC_{50} ca. 1 μM) at the endogenous 5-HT_{1B}-like receptor in CHO cells (Dickenson and Hill, 1995; Giles et al., 1996). In addition to inhibiting forskolin-stimulated cAMP formation, sumatriptan elicited a pertussis toxin-sensitive increase in the accumulation of [^{3}H]inositol phosphates in CHO5-HT_{1B} cells. These data are in agreement with Zgombick et al. (1993), who reported that the human 5-HT_{1B}-receptor stimulated a pertussis toxin-sensitive increase in inositol phospholipid hydrolysis in transfected murine fibroblasts. These observations suggest that 5-HT_{1B} receptors are capable of stimulating phospholipase C through the activation of G_i and/or G_o proteins.

However, the main finding of the present study is that 5-HT_{1B} receptor activation can markedly potentiate P_{2U}-purinoceptor stimulated [^{3}H]inositol phosphate accumulation in CHO5-HT_{1B} cells. The level of 5-HT_{1B} receptor expression in CHO5-HT_{1B} cells is comparable to the expression level of the human transfected adenosine A_1 receptor in CHO-A1 cells (200 fmol/mg of protein; Iredale et al., 1994). In these cells, co-activation of the adenosine A_1-receptor synergistically enhances CCK_A, P_{2U} and thrombin receptor stimulated accumulation of [^{3}H]inositol phosphates (Dickenson and Hill, 1996, 1997; Megson et al., 1995). These observations suggest that synergistic interactions between G_q- and G_i-coupled receptors (in terms

of inositol phospholipid hydrolysis) may be dependent upon the expression level of the G_i-coupled receptor involved. Physiologically, the level of 5-HT_{1B} receptor expression in a given vascular smooth muscle preparation may determine whether 5-HT_{1B} receptor activation results in the synergistic amplification of contractions elicited by other spasmogens. The ability of transfected human 5-HT_{1B} receptor (compared to the endogenous hamster 5-HT_{1B} receptor) to augment G_q-coupled receptor responses in CHO cells may reflect species differences in receptor-coupling to different members of the G_i/G_o protein family. It is possible that receptor-coupling to a specific G_i/G_o protein (i.e., $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$ or $G\alpha_o$) is required in order to observe synergy between G_i and G_q coupled receptors.

The ability of the 5-HT_{1B} receptor to directly stimulate inositol phosholipid hydrolysis and to potentiate G_q-coupled receptor responses may be important in certain clinical and pathological conditions. For example, 5-HT has been implicated in pulmonary hypertension (Hervé et al., 1990) and recently Maclean et al. (1996) reported 5-HT_1-like receptor stimulated vasoconstriction in isolated human pulmonary arteries. Furthermore, recent studies have shown that basilar arteries from stroke-prone spontaneously hypertensive rats display increased 5-HT_1-like mediated contractions (Nishimura, 1996). Interestingly, increased plasma levels of 5-HT are associated with pulmonary hypertension (Hervé et al., 1995). It is conceivable that in the presence of elevated 5-HT levels, vascular smooth muscle contraction stimulated by G_q-coupled receptors such as histamine H_1 and angiotensin II may be enhanced.

In summary, we have shown for the first time that 5-HT_{1B} receptor activation can markedly potentiate the accumulation of inositol phosphates elicited by a G_q-protein coupled receptor (in this case the P_{2U} purinoceptor). The ability of 5-HT_{1B} receptors to augment agonist-stimulated inositol phospholipid hydrolysis may underlie the synergistic interactions between 5-HT_1-like receptors and G_q-coupled receptors observed in vascular smooth muscle preparations.

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References

Berg, K.A., Clarke, W.P., Sailstad, C., Saltzman, A., Maayani, S., 1994. Signal transduction differences between 5-hydroxytrptamine type 2A and type 2C receptor systems. Mol. Pharmacol. 46, 477–484.

Berridge, M.J., 1993. Inositol trisphosphate and calcium signalling. Nature 361, 315–325.

Boess, F.G., Martin, I.L., 1994. Molecular biology of 5-HT receptors. Neuropharmacology 33, 275–317.

Bouchelet, I., Cohen, Z., Case, B., Seguela, P., Hamel, E., 1996. Differential expression of sumatriptan-sensitive 5-hydroxytryptamine receptors in human trigeminal ganglia and cerebral blood vessels. Mol. Pharmacol. 50, 219–223.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Choppin, A., O'Connor, S.E., 1995. Presence of vasoconstrictor 5-HT_1-like receptors revealed by precontraction of rabbit isolated mesenteric artery. Br. J. Pharmacol. 114, 309–314.

Choppin, A., O'Connor, S.E., 1996. Influence of vascular tone on vasoconstrictor responses to the 5-HT_1-like receptor agonist sumatriptan in anaesthetised rats. Eur. J. Pharmacol. 304, 87–92.

Dickenson, J.M., Hill, S.J., 1994. Characteristics of [^{3}H]mepyramine binding in DDT_1MF-2 cells: evidence for high affinity binding to a functional histamine H_1 receptor. Eur. J. Pharmacol. 268, 257–262.

Dickenson, J.M., Hill, S.J., 1995. Coupling of an endogenous 5-HT_{1B}-like receptor to increases in intracellular calcium through a pertussis toxin-sensitive mechanism in CHO-K1 cells. Br. J. Pharmacol. 116, 2889–2896.

Dickenson, J.M., Hill, S.J., 1996. Synergistic interactions between human transfected adenosine A_1 receptors and endogenous cholecystokinin receptors in CHO cells. Eur. J. Pharmacol. 302, 141–151.

Dickenson, J.M., Hill, S.J., 1997. Transfected adenosine A_1 receptor-mediated modulation of thrombin-stimulated phospholipase C and phospholipase A_2 activity in CHO cells. Eur. J. Pharmacol. 321, 77–86.

Ebersole, B.J., Diglio, C.A., Kaufman, D.W., Berg, K.A., 1993. 5-hydroxytryptamine$_1$-like receptors linked to increases in intracellular calcium concentration and inhibition of cyclic AMP accumulation in cultured vascular smooth muscle cells derived from bovine basilar artery. J. Pharmacol. Exp. Ther. 266, 692–699.

Gerwins, P., Fredholm, B.B., 1995. Activation of adenosine A_1 and bradykinin receptors increases protein kinase C and phospholipase D activity in smooth muscle cells. Naunyn-Schmiedebergs Arch. Pharmacol. 351, 186–193.

Giles, H., Lansdell, S.J., Bolofo, M-L., Wilson, H.L., Martin, G.R., 1996. Characterization of a 5-HT_{1B} receptor in CHO cells: functional responses in the absence of radioligand binding. Br. J. Pharmacol. 117, 1119–1126.

Hamel, E., Fan, E., Linville, D., Ting, V., Villemure, J.G., Chia, L.S., 1993. Expression of mRNA for the serotonin 5-hydroxytryptamine$_{1D\beta}$ receptor subtype in human and bovine cerebral arteries. Mol. Pharmacol. 44, 242–249.

Hartig, P.R., Hoyer, D., Humphrey, P.P.A., Martin, G.R., 1996. Alignment of receptor nomenclature with the human genome: classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes. Trends Pharmacol. Sci. 17, 103–105.

Hervé, P., Drouet, L., Dosquet, C., Launay, J-M., Rain, B., Simonneau, G., Caen, J., Duroux, P., 1990. Primary pulmonary hypertension in a patient with familial storage pool disease: role of serotonin. Am. J. Med. 89, 117–120.

Hervé, P., Launay, J-M., Scrobohaci, M-L., 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.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P.A., 1994. VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46, 157–203.

Iredale, P.A., Alexander, S.P.H., Hill, S.J., 1994. Coupling of a transfected human brain adenosine A_1 receptor in CHO-K1 cells to calcium mobilisation via a pertussis toxin-sensitive mechanism. Br. J. Pharmacol. 111, 1252–1256.

Maclean, M.R., Clayton, R.A., Templeton, A.G.B., Morecroft, J., 1996. Evidence for 5-HT_1-like receptor-mediated vasoconstriction in human pulmonary artery. Br. J. Pharmacol. 119, 277–282.

MacLennan, S.J., Bolofo, M.L., Martin, G.R., 1993. Amplifying interactions between spasmogens in vascular smooth muscle. Biochem. Soc. Trans. 21, 1145–1150.

Macor, J.W., Burkhart, C.A., Heym, J.H., Ives, J.L., Lebel, L.A., Newman, M.E., Nielson, J.A., Ryan, K., Schultz, D.W., Torgerson, L.K., Koe, K., 1990. 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one: a potent and selective serotonin (5-HT_{1B}) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. J. Med. Chem. 33, 2087–2093.

Megson, A.C., Dickenson, J.M., Townsend-Nicholson, A., Hill, S.J., 1995. Synergy between the inositol phosphate responses to transfected human adenosine A_1-receptors and constitutive P_2-purinoceptors in CHO-K1 cells. Br. J. Pharmacol. 115, 1415–1424.

Nishimura, Y., 1996. Characterization of 5-hydroxytryptamine receptors mediating contractions in basilar arteries from stroke-prone spontaneously hypertensive rats. Br. J. Pharmacol. 117, 1325–1333.

Parker, E.M., Grisel, D.A., Iben, L.G., Shapiro, R.A., 1993. A single amino acid difference accounts for the pharmacological distinctions between the rat and human5-hydroxytryptamine$_{1B}$ receptors. J. Neurochem. 60, 380–383.

Schoeffter, P., Pfeilschifter, J., Bobirnac, I., 1995. 5-hydroxytryptamine 5-HT_{1B} receptors inhibiting cyclic AMP accumulation in rat renal mesangial cells. Naunyn-Schmiedebergs Arch. Pharmacol. 351, 35–39.

Selbie, L.A., Darby, K., Schmitz-Peiffer, C., Browne, C.L., Herzog, H., Shine, J., Biden, T.J., 1995. Synergistic interaction of Y1-neuropeptide Y and α_{1b}-adrenergic receptors in the regulation of phospholipase C, protein kinase C and arachidonic acid production. J. Biol. Chem. 270, 11789–11796.

Smith, R.R., Maxwell, M.P., Martin, G.R., 1996. Further investigations into amplifying interactions between 5-HT_{1D} and angiotensin AT_1 receptors in vascular smooth muscle. Br. J. Pharmacol. 117, 250P.

Terron, J.A., 1996. GR127935 is a potent antagonist of the 5-HT_1-like receptor mediating contraction in the canine coronary artery. Eur. J. Pharmacol. 300, 109–112.

Ullmer, C., Schmuck, K., Kalkman, H.O., Lubbert, H., 1995. Expression of serotonin receptor mRNAs in blood vessels. FEBS Lett. 370, 215–221.

Zgombick, J.M., Borden, L.A., Cochran, T.L., Kucharewicz, S.A., Weinshank, R.L., Branchek, T.A., 1993. Dual coupling of cloned human 5-hydroxytryptamine$_{1D\alpha}$ and 5-hydroxtryptamine$_{1D\beta}$ receptors stably expressed in murine fibroblasts: inhibition of adenylate cyclase and elevation of intracellular calcium concentrations via pertussis toxin-sensitive G protein(s). Mol. Pharmacol. 44, 575–582.

Zgombick, J.M., Schechter, L.E., Adham, N., Kucharewicz, S.A., Weinshank, R.L., Branchek, T.A., 1996. Pharmacological characterizations of recombinant human $5\text{-HT}_{1D\alpha}$ and $5\text{-HT}_{1D\beta}$ receptor subtypes coupled to adenylate cyclase inhibition in clonal cell lines: apparent differences in drug intrinsic efficacies between human 5-HT_{1D} subtypes. Naunyn-Schmiedebergs Arch. Pharmacol. 354, 226–236.