0026-895X/96/041017-07$3.00/0

MOLECULAR PHARMACOLOGY, 50:1017–1023 (1996).

5-Hydroxytryptamine$_{2C}$ Receptor Activation Inhibits 5-Hydroxytryptamine$_{1B}$-like Receptor Function via Arachidonic Acid Metabolism

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Received May 6, 1996; Accepted July 2, 1996

SUMMARY

We previously reported that in Chinese hamster ovary (CHO) cells, 5-hydroxytryptamine (5-HT)$_{1B}$-like (CHO/5-HT$_{1B}$) receptor-mediated inhibition of forskolin-stimulated cAMP accumulation is inhibited by activation of transfected human 5-HT$_{2C}$ receptors but not 5-HT$_{2A}$ receptors. In the current study, we investigated the mechanism involved in the regulation of receptor-mediated inhibition of adenylyl cyclase as a means to further elucidate differences between the signal transduction cascades of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes. Activation of 5-HT$_{2C}$ receptors with 5-HT or (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane increased release of arachidonic acid via a phospholipase A$_2$ (PLA$_2$)-dependent mechanism. Incubation with (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (1 μM) abolished 5-carboxamidotryptamine (5 nM)-mediated inhibition of forskolin-stimulated cAMP accumulation, which was blocked by the PLA$_2$ inhibitor mepacrine (100 μM) and the cyclooxygenase inhibitor indomethacin (2 μM). Furthermore, purinergic receptor-mediated PLA$_2$ activation as well as direct activation of PLA$_2$ with melittin reduced CHO/5-HT$_{1B}$ responsiveness. These data indicate that activation of the PLA$_2$/arachidonic acid signaling cascade mediates 5-HT$_{2C}$ receptor regulation of the CHO/5-HT$_{1B}$ receptor pathway. Consistent with our previous report and in contrast to activation of 5-HT$_{2C}$ or purinergic receptors, activation of 5-HT$_{2A}$ receptors had no effect on CHO/5-HT$_{1B}$ receptor function, although 5-HT$_{2A}$ receptor-mediated activation of PLA$_2$ was measured. Interestingly, purinergic receptor-mediated inhibition of CHO/5-HT$_{1B}$ receptor function was blocked when 5-HT$_{2A}$ receptors were activated simultaneously. These data suggest that the lack of 5-HT$_{2A}$-mediated regulation of CHO/5-HT$_{1B}$ receptors may be due to activation of a third pathway (in addition to PLC and PLA$_2$ pathways), which results in the inhibition of the production or the actions of a cyclooxygenase-dependent arachidonic acid metabolite.

5-HT$_{2A}$ and 5-HT$_{2C}$ receptors are members of the 5-HT$_2$ receptor subfamily of G protein-coupled receptors. The 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors have a high degree of amino acid sequence homology and share similar pharmacological profiles (1–3). In addition, both of these receptors are known to activate PLC-mediated PI hydrolysis (4, 5), which leads to increases in diacylglycerol and InsP$_3$. Diacylglycerol activates PKC, whereas InsP$_3$ increases [Ca^{2+}]$_i$ (6, 7). Increases in PKC activity and [Ca^{2+}]$_i$ have been shown to occur on activation of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (8–10).

Although the signal transduction elicited by activation of these receptors has generally been thought to be similar, we have shown previously that there are differences in signal transduction between these two receptor subtypes. Receptor expression levels and the magnitude (E_{max}) of 5-HT-mediated PI hydrolysis were similar in CHO cell lines expressing the human 5-HT$_{2A}$ receptor (CHO-FA3) or the human 5-HT$_{2C}$ receptor (CHO-1C19). However, activation of 5-HT$_{2C}$, but not 5-HT$_{2A}$, receptors reduced the inhibition of cAMP formation in response to activation of an endogenously expressed 5-HT$_{1B}$-like (CHO/5-HT$_{1B}$) receptor (11). Furthermore, the 5-HT$_{2C}$-mediated reduction in CHO/5-HT$_{1B}$ responsiveness was independent of PKC activation and increases in [Ca^{2+}]$_i$.

We have continued to investigate the signal transduction differences between the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor systems in CHO cells. In this report, we show that 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and an endogenously expressed purinergic receptor are coupled to PLA$_2$-mediated release of AA. Furthermore,

This work was supported by United States Public Health Service Grants DA09094 (K.A.B., S.M.), HD26437 (W.P.C.), and MH48125 (W.P.C., S.M.).

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; AA, arachidonic acid; 5-CT, 5-carboxamidotryptamine; [Ca^{2+}]$_i$, intracellular calcium concentration; CHO, Chinese hamster ovary; DMSO, dimethylsulfoxide; DOI, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; FScA, forskolin-stimulated cAMP accumulation; HBSS, Hanks' balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IP, inositol phosphates; InsP$_3$, inositol trisphosphate; PI, phosphatidylinositol; PKC, protein kinase C; PLA$_2$, phospholipase A$_2$; PLC, phospholipase C.

we demonstrate that 5-HT_{2C}- and purinergic receptor-mediated inhibition of CHO/5-HT_{1B} receptor function is due to an indomethacin-sensitive metabolite of AA. Although activation of 5-HT_{2A} receptors also increases AA release, 5-HT_{2A} receptor activation did not alter CHO/5-HT_{1B} receptor function as we have shown previously (11). Furthermore, 5-HT_{2A} receptor activation blocked the effect of the purinergic receptor on CHO/5-HT_{1B} receptor function, suggesting that the 5-HT_{2A} receptor may couple to a third pathway (in addition to PLC and PLA_2 pathways), which results in the inhibition of the production or the actions of a cyclooxygenase-dependent AA metabolite.

Experimental Procedures

Materials. Forskolin was purchased from Calbiochem (San Diego, CA); *myo*-[^{3}H]inositol, ^{125}I-cAMP tracer, and [^{14}C]AA were from New England Nuclear Research Products (Boston, MA); anti-cAMP antibody was from ICN Biochemicals (Lisle, IL); 5-CT, 5-HT HCl, and DOI were from Research Biochemicals (Natick, MA); indomethacin was from BIOMOL Research Laboratories (Plymouth Meeting, PA); and mepacrine, melittin, and hygromycin were from Sigma Chemical (St. Louis, MO). Rolipram was a generous gift from Berlex Laboratories (Cedar Knolls, NJ). All tissue culture reagents and HBSS were purchased from GIBCO (Grand Island, NY). All other drugs and chemicals (reagent grade) were purchased from Sigma Chemical.

Stable transfection and cell culture. The human 5-HT_{2C} and 5-HT_{2A} genes were isolated, and stable transfections of CHO-K1 cells were done as described previously (11, 12). The cell lines CHO-1C19 (5-HT_{2C}) and CHO-FA3 (5-HT_{2A}) were used for this study and are the same cell lines that we used previously (11). These lines have comparable expression levels of receptor protein (~200 fmol/mg of protein) and comparable maximal levels of IP accumulation in response to 5-HT. Cells were maintained in α-minimal essential medium supplemented with 5% fetal bovine serum, and 300 μg/ml hygromycin. For all experiments, cells were seeded onto 12- or 24-well tissue culture vessels at a density of 4×10^4 cells/cm^2. After a 24-hr plating period, cells were washed with HBSS and placed into Dulbecco's modified Eagle's medium/F-12 (1:1) with 5 μg/ml insulin, 5 μg/ml transferrin, 30 nM selenium, 20 nM progesterone, and 100 μM putrescine (serum-free media). Cells were grown in serum-free media for 24 hr before all experiments.

Measurement of CHO/5-HT_{1B} receptor responsiveness. Cells that had been washed twice with HBSS containing calcium and magnesium supplemented with 10 mM HEPES (wash buffer, pH 7.4) were preincubated in 500 μl of wash buffer/well for 15 min in a CO_2 incubator (5% at 37°). Where indicated, inhibitors were present during this preincubation period. CHO/5-HT_{1B} receptor-mediated responsiveness was determined by measuring the 5-CT-induced inhibition of cAMP accumulated in the presence of 1 μM forskolin (15 min at 37°) and the phosphodiesterase inhibitor rolipram (0.1 mM). Cellular cAMP content was measured by radioimmunoassay as described previously (11) and normalized to protein content, which was measured according to the method of Lowry (13).

AA release. Cells were labeled with 0.1 μCi/ml [^{14}C]AA (57 mCi/mmol) for 4 hr at 37° (5% CO_2). Under these conditions, >90% of label was taken up by the cells during this 4-hr period. After labeling, cells were washed three times with HBSS containing calcium and magnesium supplemented with 20 mM HEPES and 0.1% bovine serum albumin (experimental medium). Between washes, the cells were incubated for 5 min in a 37° water bath (for a total preincubation period of 15 min). For calcium-free experiments, cells were washed three times with experimental medium, with or without 1 mM calcium, after the third wash interval (i.e., cells were exposed to an extracellular calcium ion-free environment for ≤5 min before agonist challenge). After the wash paradigm, cells were incubated in 1 ml of experimental medium containing vehicle (H_2O or 0.01% DMSO as necessary) or the indicated drug concentrations. Aliquots (100 μl) were taken after 10 and 30 min of incubation and added directly to scintillation vials. The ^{14}C radioactivity was determined with liquid scintillation counting in a Beckman 5000 or Beckman 7500 scintillation counter (Beckman Instruments, Palo Alto, CA).

IP accumulation measurements. Cells were labeled with 1 μCi/ml *myo*-[^{3}H]inositol in serum-free medium for 24 hr. Total IP accumulation in response to agonist stimulation in the presence of 20 mM LiCl for 10 min at 37° was determined as described previously (9, 11). The amount of [^{3}H]IPs (inositol monophosphate, inositol bisphosphate, and $InsP_3$ are collectively referred to as IP) formed was separated according to the ion exchange method of Berridge *et al.* (14).

Data analysis. For cAMP determinations, data were normalized for each experiment by defining the response to 1 μM forskolin as 100%. Concentration-response data were fit by nonlinear regression to the model $E = (E_{max})/[1 + (EC_{50}/A)^n]$, where E is the measured response at the given agonist concentration A, E_{max} is maximal response, EC_{50} is the concentration of agonist producing half-maximal response, and n is the slope index. The Student's t test was used for statistical comparisons.

Result

5-HT agonists increase AA release via PLA_2 activation in CHO cells stably expressing the 5-HT_{2C} receptor. In nontransfected (parent) CHO-K1 cells, no release of AA was detected after a 10- or 30-min incubation with either 5-HT or the 5-$HT_{2A/2C}$ receptor agonist DOI (data not shown). However, incubation of CHO-1C19 (5-HT_{2C}) cells with 5-HT or DOI increased the amount of ^{14}C radioactivity released into the medium in a concentration-dependent manner (Fig. 1A). The maximal response to 5-HT was 170 ± 11% above basal release, and the EC_{50} value for 5-HT was 28 nM (pEC_{50} = 7.56 ± 0.18; mean ± standard error values from five experiments). DOI was a partial agonist eliciting a maximal response of 127 ± 11% above basal release with an EC_{50} value of 121 nM (pEC_{50} = 6.92 ± 0.13; mean ± standard error values from five experiments). These EC_{50} values are similar to those obtained previously for 5-HT- and DOI-mediated IP accumulation in CHO-1C19 cells (11).

After pretreatment with the PLA_2 inhibitor mepacrine (100 μM, Fig. 1B), the amount of ^{14}C radioactivity released in response to a maximal concentration of 5-HT was completely inhibited. The selectivity of mepacrine for inhibiting 5-HT_{2C} receptor-mediated responses was determined by measuring [^{14}C]AA release and IP accumulation in response to DOI (1 μM) in CHO-1C19 cells. Pretreatment with mepacrine (100 μM for 15 min) completely inhibited DOI-mediated AA release (168 ± 52% above basal release; mean ± standard error values from three experiments) to basal levels. In contrast, mepacrine had no effect on DOI-mediated PI hydrolysis. IP accumulation was 270 ± 35% above basal levels in the presence of 1 μM DOI alone versus 245 ± 26% above basal levels in the presence of DOI and 100 μM mepacrine (mean ± standard error values from four experiments, $p \leq 0.375$). Mepacrine had no effect on basal AA release or IP accumulation.

When calcium was removed from the incubation medium, basal release levels were unchanged after 10 min of incubation (1878 ± 176 versus 2168 ± 292 cpm in the presence and absence of calcium, respectively); however, 5-HT-mediated release was reduced from 227 ± 25% above basal levels under normal calcium-containing conditions to 12 ± 8% above basal

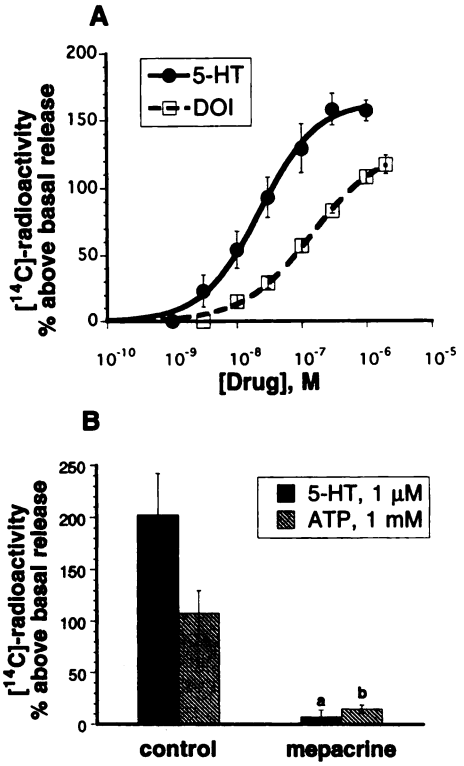

Fig. 1. Activation of transfected 5-HT$_{2C}$ receptors and endogenous purinergic receptors increase the release of AA via a PLA$_2$-dependent mechanism in CHO-1C19 cells. CHO-K1 cells, stably transfected with human 5-HT$_{2C}$ receptor cDNA, were labeled with [^{14}C]AA as described in Experimental Procedures. A, Concentration-response curves to 5-HT and DOI after 30 min of agonist treatment. Data are normalized to percentage above basal release and represent the mean ± standard error of five experiments. Individual concentration-response data were fit to a logistic equation (see Experimental Procedures) to determine the mean E_{max} and EC$_{50}$ parameters, which are provided in the text. B, 5-HT- and ATP-mediated AA release in the presence and absence of the PLA$_2$ inhibitor mepacrine. After treatment with or without 100 μM mepacrine for 15 min, aliquots of media were taken after a 10-min incubation with 1 μM 5-HT or 1 mM ATP. Data represent the mean ± standard error values from two (ATP) or four (5-HT) experiments measured in triplicate. Basal AA release was 1719 ± 133 and 2047 ± 228 cpm for vehicle and mepacrine, respectively (mean ± standard error values from four experiments; p ≤ 0.375). *a*, $p < 0.005$; *b*, p ≤ 0.05, versus corresponding control.

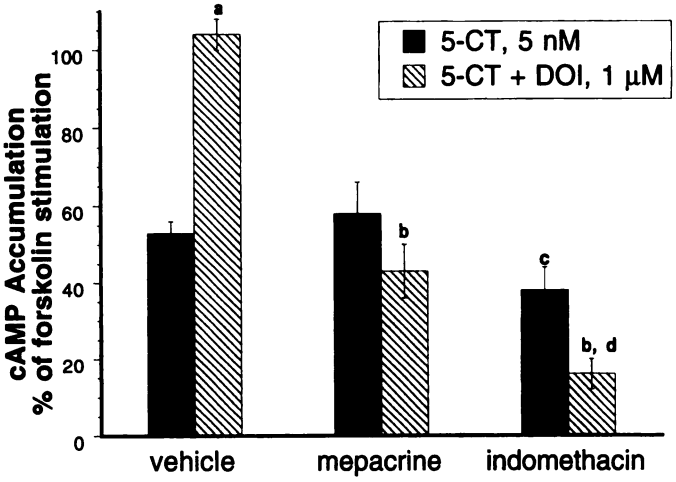

Fig. 2. 5-HT$_{2C}$ receptor-mediated inhibition of CHO/5-HT$_{1B}$ receptor responsiveness is abolished in the presence of the PLA$_2$ inhibitor, mepacrine, and the cyclooxygenase inhibitor indomethacin. After preincubation with vehicle (0.01% DMSO), 100 μM mepacrine, or 2 μM indomethacin (37° for 15 min), cells were incubated with 5 nM (EC$_{50}$ concentration) 5-CT with and without 1 μM DOI. The inhibition of 1 μM FScA was measured as described in Experimental Procedures. Data represent the mean ± standard error of five experiments. FScA was 199 ± 24 for forskolin alone, 159 ± 9 in the presence of mepacrine, and 212 ± 32 pmol/mg of protein in the presence of indomethacin (mean ± standard error values from five experiments). Similar to our previous findings, DOI did not alter FScA (11). *a*, p ≤ 0.0005 versus vehicle without 5-CT; *b*, p ≤ 0.0005 versus vehicle without 5-CT plus DOI; *c*, p ≤ 0.025 versus vehicle without 5-CT; *d*, $p \leq 0.025$ versus indomethacin plus 5-CT.

levels in nominally calcium-free conditions (mean ± standard error values from four experiments). Taken together, these data indicate that transfected 5-HT$_{2C}$ receptors couple to PLA$_2$ in a calcium-sensitive manner in CHO cells.

Consistent with reports of purinergic receptor-mediated activation of PLA$_2$ in CHO-K1 cells (15, 16), incubation with ATP (1 mM) also increased the release of ^{14}C radioactivity in a mepacrine-sensitive manner (see Figs. 1 and 5). Furthermore, incubation with melittin (2.5 μg/ml for 10 min), a direct activator of PLA$_2$ (17), increased the release of ^{14}C radioactivity 352 ± 18% above basal release (mean ± standard error values from five experiments).

Activation of the PLA$_2$-AA signal transduction pathway inhibits CHO/5-HT$_{1B}$ receptor function. As previously reported in CHO-1C19 cells (11), activation of transfected 5-HT$_{2C}$ receptors with a maximal concentration of DOI (1 μM) abolished the 5-CT-mediated inhibition of FScA (Fig. 2) with no effect on FScA itself. However, in the presence of the PLA$_2$ inhibitor mepacrine (100 μM), the effect of DOI on 5-CT-mediated inhibition of FScA was completely blocked, suggesting that the PLA$_2$/AA pathway mediates the 5-HT$_{2C}$ receptor regulation of CHO/5-HT$_{1B}$ receptor function. Because AA can be metabolized by a variety of pathways [i.e., cyclooxygenase, lipoxygenase, and cytochrome P450 and by auto-oxidation (18)], we first tested the effect of a cyclooxygenase inhibitor on 5-HT$_{2C}$ receptor-mediated inhibition of CHO/5-HT$_{1B}$ responsiveness. Inhibition of the cyclooxygenase pathway with indomethacin (2 μM) blocked the effect of DOI on 5-CT-mediated inhibition of FScA. Interestingly, in the presence of both indomethacin and DOI, 5-HT$_{1B}$ responsiveness was enhanced (Fig. 2).

The reduction in CHO/5-HT$_{1B}$ responsiveness by 5-HT$_{2C}$ receptor activation was mimicked in CHO-1C19 cells by melittin (2.5 μg/ml, Fig. 3), as well as by activation of endogenously expressed purinergic receptors with ATP (1 mM, Fig. 4). Furthermore, the reduction of 5-CT-mediated inhibition of FScA in the presence of melittin or ATP was blocked by prior treatment with either mepacrine (100 μM) or indomethacin (2 μM), again suggesting that a cyclooxygenase metabolite of AA inhibits CHO/5-HT$_{1B}$ responsiveness.

Activation of 5-HT$_{2A}$ receptors blocks AA-mediated inhibition of CHO/5-HT$_{1B}$ receptor function. In CHO cells transfected with the human 5-HT$_{2A}$ receptor cDNA (CHO-FA3), incubation with 5-HT for 10 min increased the release of [^{14}C]AA by 68 ± 9% above basal release (mean ± standard error values from six experiments; Fig. 5), which was antagonized after pretreatment with mepacrine (11 ± 5% above basal release; mean ± standard error values from four experiments). Similarly, activation of endogenously expressed purinergic receptors with ATP increased the release of [^{14}C]AA by 73 ± 9% (mean ± standard error values from

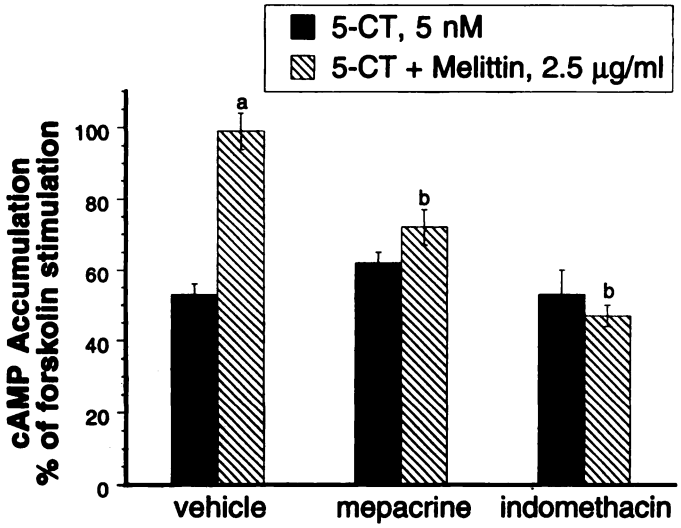

Fig. 3. Direct activation of PLA_2 with melittin abolishes CHO/5-HT_{1B} responsiveness. After preincubation (37° for 15 min) with vehicle (0.01% DMSO), 100 µM mepacrine, or 2 µM indomethacin, cells were incubated with 5 nM 5-CT with and without 2.5 µg/ml melittin. The inhibition of 1 µM FScA was measured as described in Experimental Procedures. Data represent the mean ± standard error values of five experiments. FScA was 199 ± 24 for forskolin alone and 185 ± 12 pmol/mg of protein in the presence of melittin. *a*, $p < 0.005$ versus corresponding vehicle control; *b*, $p < 0.005$ versus vehicle without 5-CT.

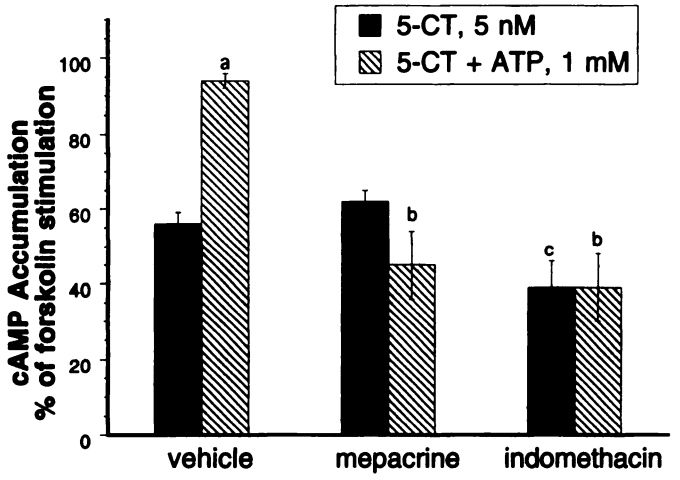

Fig. 4. Purinergic receptor-mediated activation of PLA_2 inhibits CHO/5-HT_{1B} responsiveness. After preincubation (37° for 15 min) with vehicle (0.01% DMSO), 100 µM mepacrine, or 2 µM indomethacin, cells were incubated with 5 nM 5-CT with and without 1 mM ATP. The inhibition of 1 µM FScA was measured as described in Experimental Procedures. Data represent the mean ± standard error of five experiments. FScA was 205 ± 36 for forskolin alone and 248 ± 44 pmol/mg of protein in the presence of ATP (six experiments). *a*, $p \leq 0.0005$ versus corresponding vehicle control; *b*, $p \leq 0.0005$ versus vehicle without 5-CT; *c*, $p \leq 0.025$ versus vehicle without 5-CT.

five experiments; Fig. 5), an amount comparable to that produced by 5-HT_{2A} receptor activation. As indicated in Fig. 5, ATP-mediated AA release was sensitive to prior treatment with mepacrine. These results are consistent with published reports of 5-HT_{2A} receptor (19) and ATP receptor (15, 16) coupling to PLA_2.

Consistent with our previous report (11), activation of the 5-HT_{2A} receptor had no effect on CHO/5-HT_{1B} receptor function (Fig. 6). In contrast, activation of purinergic receptors

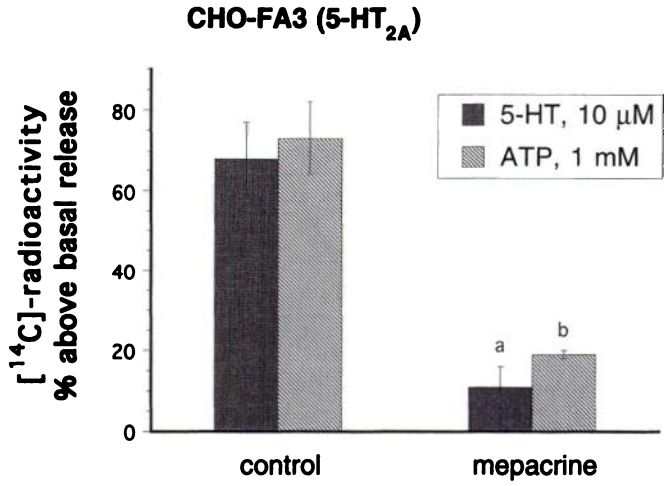

Fig. 5. Activation of transfected 5-HT_{2A} receptors and endogenous purinergic receptors increase the release of AA via a PLA_2-dependent mechanism in CHO-FA3 cells. CHO-K1 cells, stably transfected with human 5-HT_{2A} receptor cDNA, were labeled with [^{14}C]AA as described in Experimental Procedures. Cells were treated with 100 µM mepacrine or vehicle for 15 min as indicated in Experimental Procedures. Aliquots of media were taken after 10 min of incubation at 37° with 10 µM 5-HT or 1 mM ATP. Data shown represent the mean ± standard error values from four experiments measured in triplicate. Basal AA release was not significantly altered by treatment with mepacrine ($p \leq 0.375$) and was 1984 ± 195 and 2537 ± 286 cpm for vehicle and mepacrine, respectively. *a*, $p \leq 0.0005$ versus 5-HT control; *b*, $p \leq 0.005$ versus ATP control.

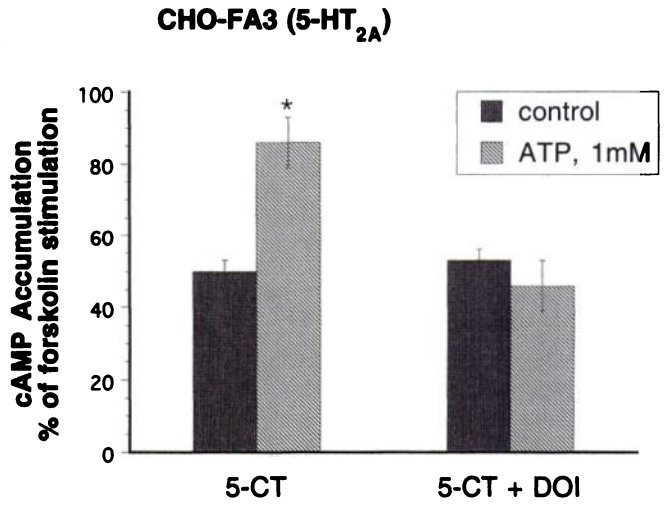

Fig. 6. Purinergic receptor-mediated inhibition of 5-HT_{1B}-like responsiveness is abolished by 5-HT_{2A} receptor activation. CHO cells, stably transfected with human 5-HT_{2A} receptor cDNA, were incubated with vehicle, 5-CT (5 nM), or 5-CT plus DOI (1 µM) in the presence and absence of 1 mM ATP. The inhibition of 1 µM FScA was measured as described in Experimental Procedures. Data represent the mean ± standard error of four experiments. FScA was 104 ± 11 for forskolin alone and 94 ± 11 pmol/mg of protein in the presence of 1 mM ATP. *, $p \leq 0.0005$ versus 5-CT control.

with ATP in CHO-FA3 cells reduced CHO/5-HT_{1B} receptor function. 5-CT (5 nM) inhibited FScA by 50 ± 3% in vehicle-treated cells compared with 14%± 7% in cells incubated with ATP (mean ± standard error values from four experiments). These data are similar to those for ATP-mediated inhibition of the CHO/5-HT_{1B} receptor responsiveness in the CHO-1C19 (5-HT_{2C}) cell line (Fig. 4) and in parent CHO-K1 cells (not shown). Interestingly, ATP-mediated reduction of CHO/

5-HT_{1B} receptor responsiveness was blocked when 5-HT_{2A} receptors were activated. 5-CT-mediated inhibition of FScA in the presence of both ATP and DOI was not different from control (Fig. 6). Although DOI is known to be a very selective drug for 5-HT_2 receptor subtypes, the hypothesis that DOI acted as an antagonist at the purinergic receptor was tested by measuring ATP-mediated IP accumulation in parent CHO cells in the presence and absence of 1 μM DOI. IP accumulation in response to ATP (1 mM) was 123 ± 16% above basal versus 127 ± 16% above basal in the presence of DOI (mean ± standard error values from three experiments). These data indicate that the DOI-mediated blockade of ATP receptor regulation of the CHO/5-HT_{1B} receptor was not due to antagonism of the purinergic receptor by DOI. In addition to blocking ATP-mediated effects on CHO/5-HT_{1B} receptor responsiveness, activation of 5-HT_{2A} receptors with DOI blocked the effects of melittin in CHO-FA3 cells. In these experiments, 5-CT-mediated inhibition of FScA was 39 ± 5% in the presence of vehicle and 45 ± 7%, 16 ± 3%, and 44 ± 8% in the presence of 1 μM DOI, 2.5 μg/ml melittin, and melittin plus DOI, respectively (mean ± standard error values from three experiments).

Discussion

Previously, we reported that 5-HT_{2C}, but not 5-HT_{2A}, receptor activation inhibits the responsiveness of an endogenously expressed 5-HT_{1B}-like receptor in CHO cells (11). Although the classic signal transduction pathway coupled to the 5-HT_{2C} receptor is the PLC/PI pathway (4), we showed that neither activation of PKC nor increases in $[Ca^{2+}]_i$ were involved in the 5-HT_{2C} effect. Also, independence of the 5-HT_{2C} effect from the PLC/PI pathway was further supported because 5-HT_{2A} receptors activated PLC-mediated IP accumulation to a similar degree as 5-HT_{2C} receptors but did not inhibit CHO/5-HT_{1B} responsiveness (11).

Because many PLC-coupled receptors are also known to activate the PLA_2/AA pathway (19, 20), we investigated the PLA_2-AA pathway as a possible mediator of the 5-HT_{2C} receptor-mediated effect on CHO/5-HT_{1B} receptor function. Our data show that activation of stably transfected 5-HT_{2C} receptors increases AA release (Fig. 1, A and B). This 5-HT_{2C}-mediated AA release was completely blocked by the PLA_2 inhibitor mepacrine at a concentration that did not affect 5-HT_{2C}-mediated PI hydrolysis. Mepacrine also blocked AA release induced by activation of PLA_2 directly with melittin (data not shown) or by activation of endogenously expressed purinergic receptors with ATP (Figs. 1B and 5). These data suggest that 5-HT_{2C} receptors in CHO cells increase AA release by activating PLA_2. Recently, activation of 5-HT_{2C} receptors has been reported to release AA as well as to increase cGMP levels in rat choroid plexus, effects that were sensitive to PLA_2 and cyclooxygenase/lipoxygenase inhibitors (21). Thus, in addition to activation of PLC, 5-HT_{2C} receptor-mediated activation of PLA_2 may also occur in brain.

Our results also suggest that the PLA_2/AA signaling cascade mediates the inhibition of CHO/5-HT_{1B} receptor responsiveness by 5-HT_{2C} receptor activation. Direct activation of PLA_2 with melittin as well as activation of PLA_2 by endogenously expressed purinergic receptors mimicked the 5-HT_{2C} receptor effect (Figs. 2–4). Furthermore, mepacrine completely blocked the 5-HT_{2C}-mediated inhibition of CHO/5-HT_{1B} responsiveness. Mepacrine also blocked the effect of melittin and ATP on CHO/5-HT_{1B} responsiveness.

In the presence of the cyclooxygenase inhibitor indomethacin, receptor- and non-receptor-mediated inhibition of CHO/5-HT_{1B} receptor function was blocked (Figs. 2–4), indicating that a metabolite of AA mediates the interaction between the PLA_2/AA and adenylyl cyclase signaling cascades. Furthermore, in the presence of indomethacin and DOI, 5-CT-mediated inhibition of FScA was actually enhanced (Fig. 2). These data suggest that a cyclooxygenase-dependent metabolite of AA (e.g., prostaglandins or thromboxanes) is involved in the negative regulation of the CHO/5-HT_{1B} receptor system. This enhancement of 5-HT_{1B}-like receptor responsiveness by indomethacin in the presence of 5-HT_{2C} activation is interest-

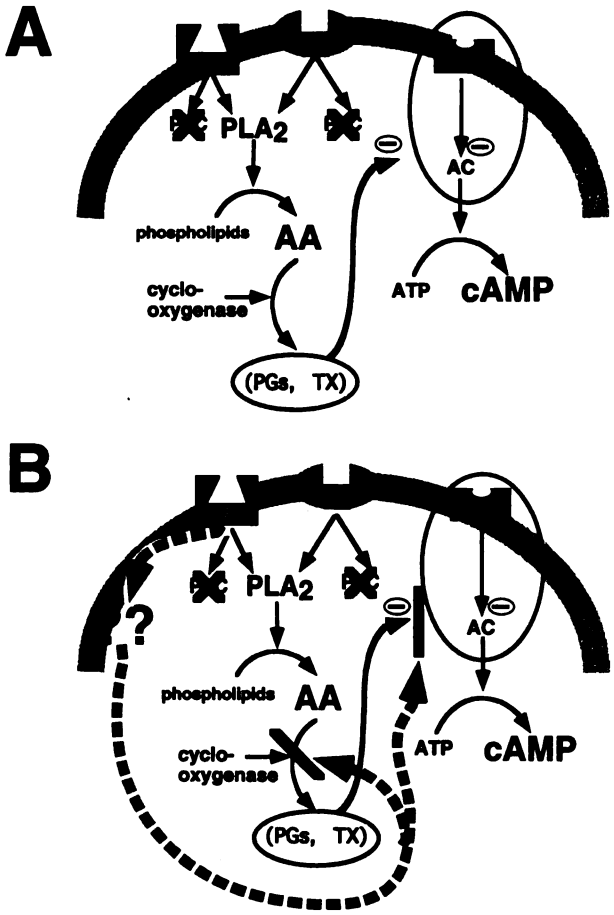

Fig. 7. Illustration of 5-HT_{2C}- (A) and 5-HT_{2A}- (B) mediated effects on CHO/5-HT_{1B} receptor function. A, In CHO-1C19 cells, transfected 5-HT_{2C} receptors and endogenous purinergic (likely P2 purinergic) receptors couple to both PLC and PLA_2. Previous work (11) has demonstrated that the consequences of PLC activation (i.e., increased $[Ca^{2+}]_i$ and activation of PKC) are not involved in regulation of CHO/5-HT_{1B} receptor function. Activation of 5-HT_{2C} or purinergic receptors inhibits CHO/5-HT_{1B} receptor function via a PLA_2/AA cyclooxygenase-dependent pathway. B, In CHO-FA3 cells, transfected 5-HT_{2A} receptors and endogenous purinergic receptors couple to both PLC and PLA_2. Although activation of purinergic receptors inhibits CHO/5-HT_{1B} receptor function, 5-HT_{2A} receptor activation does not. In addition, 5-HT_{2A} receptor activation inhibits the purinergic receptor action on CHO/5-HT_{1B} receptor function. We propose that the 5-HT_{2A} receptor couples to an additional pathway that can either block the effect or inhibit the production of a cyclooxygenase product. *AC*, adenylyl cyclase; *PGs*, prostaglandins; *TX*, thromboxane.

ing. Perhaps by inhibiting cyclooxygenase activity, AA released in response to 5-HT_{2C} receptor activation is shunted to another metabolic pathway (e.g., lipoxygenase, cytochrome P450), which produces an eicosanoid that enhances receptor-mediated inhibition of adenylyl cyclase. However, if this were the case, one would expect enhancement of CHO/5-HT_{1B} function with indomethacin in the presence of ATP or melittin as well.

A number of reports have indicated that considerable interaction occurs between the adenylyl cyclase/cAMP and the PLA_2/AA signal transduction pathways. Elevation of cellular cAMP levels has been shown to inhibit AA release (22), and activation of receptors coupled to the inhibition of adenylyl cyclase activity has been shown to amplify (23–25) as well as inhibit (26, 27) the release of AA in response to activation of PLA_2. In CHO cells stably expressing the 5-HT_{2C} receptor, we found that receptor-mediated inhibition of adenylyl cyclase activity is blocked by activation of the PLA_2/AA pathway. To our knowledge, this is the first report of regulation of a G_i-coupled receptor system by activation of the PLA_2/AA signaling cascade. In addition to its role as an important intracellular messenger (28, 29), AA may function as an intercellular messenger (28). As such, AA has been implicated as a retrograde messenger that may be involved in the control of the efficacy of synaptic transmission (30). Regulation of drug efficacy at G protein-coupled receptors may be an additional mechanism by which AA influences synaptic events. Although it is not yet known whether 5-HT_{2C} and 5-HT_{1B} receptors colocalize to the same cells in brain, there is evidence that these receptors colocalize to the same brain region (e.g., substantia nigra and hippocampus) (31, 32). Because AA and many of its metabolites are membrane permeable, it is possible that the mechanisms for 5-HT_{1B} regulation described here may occur even if the receptors are not found on the same cell.

Although 5-HT_{2A} receptor-mediated activation of the PLA_2/AA pathway has been reported to occur in hippocampal neurons (19), we were somewhat surprised to find 5-HT_{2A}-mediated AA release in our CHO-FA3 cell line (Fig. 5) because activation of 5-HT_{2A} receptors does not inhibit 5-HT_{1B} receptor function (11) (Fig. 6). However, the finding that 5-HT_{2A} receptor activation blocked the ATP-mediated reduction in CHO/5-HT_{1B} receptor function suggests that a third pathway (in addition to the PLC/PI and the PLA_2/AA pathways) is activated by the 5-HT_{2A} receptor in CHO-FA3 cells. Similar results were found in a second clonal cell line stably expressing 5-HT_{2A} receptors (CHO-FA4). A consequence of activation of this third pathway is the inhibition of either the production, or the actions of the cyclooxygenase-dependent AA metabolite. The specific eicosanoids generated (e.g., prostaglandins and thromboxanes) in response to 5-HT_{2A}, as well as 5-HT_{2C}, receptor activation in CHO cells are currently being investigated. A third signal transduction pathway coupled to 5-HT_{2A} receptors adds an additional mode of signaling and opens further avenues for 5-HT_{2A} receptor regulation of cellular functions.

As illustrated in Fig. 7, we conclude that a cyclooxygenase-dependent AA metabolite inhibits the function of endogenously expressed 5-HT_{1B}-like receptors in CHO cells. Although both 5-HT_{2C} and 5-HT_{2A} receptors seem to couple to PLA_2-mediated AA release, the 5-HT_{2A} receptor subtype may also couple to (or release) a second messenger or messengers, which can either block the effect or inhibit the production of the cyclooxygenase product.

Acknowledgments

We gratefully acknowledge the expert technical assistance of Jodie D. Cropper and Lynn Cordner. We also thank Drs. Stuart Sealfon and Barbara Ebersole for critical review of the manuscript.

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