Cross-talk between 5-Hydroxytryptamine Receptors in a Serotonergic Cell Line

IN Volvement OF ARACHIDONIC ACID METABOLISM*

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The study of signaling cascades and of functional interactions between 5-hydroxytryptamine (5-HT) receptor pathways with heterogeneous brain cell populations remains an arduous task. We took advantage of a serotonergic cell line to elucidate cross-talks between 5-HT receptors and to demonstrate the involvement of two 5-HT2 receptor subtypes in the regulation of 5-HT1B/1D function. The inducible 1C11 cell line has the unique property of acquiring within 4 days a complete serotonergic phenotype (1C11* cells), including three 5-HT receptors. 5-HT1B/1D and 5-HT2B receptors are expressed since day 2 of the serotonergic differentiation while 5-HT2A receptors are induced at day 4. We first established that 5-HT2B receptors are coupled with the phospholipase A2 (PLA2)-mediated release of arachidonic acid (AA) and that the activation of 5-HT2B receptors in 1C11*d4 cells inhibits the 5-HT1B/1D receptor function via a cyclooxygenase-dependent AA metabolite. At day 4, this 5-HT2B-mediated inhibition of the 5-HT1B/1D function can be blocked upon concomitant 5-HT2A activation although a 5-HT2A/PLA2 positive coupling was evidenced. This suggests the existence in 1C11*d4 cells of pathway(s) for 5-HT2A receptors, distinct from PLC and PLA2. Finally, this study reveals the antagonistic roles of 5-HT2A and 5-HT2B receptors in regulating the function of 5-HT1B/1D, a receptor involved in neuropsychiatric disorders and migraine pathogenesis.

In view of the pivotal role of serotonin (5-hydroxytryptamine (5-HT))1 in neurologic and neuropsychiatric disorders, to identify the mechanisms that mediate the cellular functions controlled by 5-HT is a major challenge. To achieve this goal, the study of signaling cascades and of functional interactions between 5-HT receptor pathways with heterogeneous brain cell populations still appears impractical. An alternative is to use a clonal cell line, such as 1C11, expressing a definite set of 5-HT receptors (1).

The 1C11 clone was derived from F9 multipotential embryonal carcinoma cells through immortalization and differentiation (2, 3). The 1C11 clone has the properties of a neuroectodermal progenitor able to differentiate into 5-hydroxytryptaminergic cells (1C11*) by binding dibutyryl cyclic AMP (Bt2cAMP) and cyclohexane carboxylic acid (CCA). The switch from the undifferentiated committed 1C11 cell type to the 1C11* serotonergic cells occurs within 4 days in more than 90% of the cell population.

We recently demonstrated that, in addition to the onset of 5-HT metabolism, storage, and transport, 1C11* cells also acquire 5-HT1B/1D, 5-HT2A, and 5-HT2B receptors (1). Binding and transductional experiments excluded the functional presence of any other 5-HT receptor subtypes. On day 2 of the serotonergic differentiation, 5-HT1B/1D and 5-HT2B receptors became expressed and remained functional until at least day 4, at which time the 5-HT2A receptor was induced. The appearance of the latter receptor coincided with the onset of an active 5-HT transport system. This sequence of events in the inducible 1C11 cell line offers the possibility of exploring cross-talks between the signaling pathways of these three G-protein coupled receptors within a complete serotonergic phenotype.

Many studies have established that 5-HT1B/1D receptors are negatively coupled with adenylate cyclase through Gs (GTP-binding protein) (4). Acting as terminal or somatodendritic autoreceptors, they mediate and modulate serotonergic neurotransmission (5). They can also function as terminal heteroreceptors by inhibiting, after agonist stimulation, the release of other neurotransmitters such as acetylcholine, glutamate, dopamine, norepinephrine, and α-amino butyric acid (6–8). Thus, signals interfering with the Gs-coupled 5-HT1B/1D system may act widely on neuronal responses. In addition, 5-HT1B/1D receptors are involved in cardiovascular functions, vasospasm, and migraine (9). Because of both the pivotal role of 5-HT1B/1D receptors in neurotransmission and the clinical impact of 5-HT1B/1D receptors agonists like sumatriptan (10) in the acute treatment of migraine, studying the regulatory aspects of 5-HT1B/1D receptor function is of great importance.

Two distinct receptors of the 5-HT2 class are also induced during the serotonergic differentiation of 1C11 cells. 5-HT2A and 5-HT2B receptors display a strong amino acid sequence homology and share close but distinct pharmacological profiles (11). Each of these receptors has been described as mediating phosphatidylinositol (PI) hydrolysis through activation of phospholipase C (PLC). The 1,2-diacylglycerol (DAG) thus obtained activates PKC (12), whereas inositol 1,4,5-triphosphate

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‡ The abbreviations used are: 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxamidotryptamine; AA, arachidonic acid; BSA, bovine serum albumin; Bt2cAMP, dibutyryl-cAMP; CCA, cyclohexanecarboxylic acid; DAG, 1,2-diacylglycerol; DMEM, Dulbecco’s modified Eagle’s medium; DO1, (≥)-1,2,5-dimethoxy-4-iodophenyl-2-aminopropane; FSEA, forskolin-stimulated cAMP accumulation; G, GTP-binding protein; HBSS, Hanks balanced salt solution; IP1, inositol phosphates; IP3, inositol 1,4,5-triphosphate; P1, phosphatidylinositol; PKC, protein kinase C; PLA2, phospholipase A2; PLC, phospholipase C.

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(IP3) increases intracellular Ca\(^{2+}\) (13). Nevertheless, the 5-HT2B receptor fails to significantly stimulate PI hydrolysis in rat stomach (14) as well as in rat vasculature (15). This suggests that signaling processes coupled to 5-HT2B receptors, but distinct from PLC, may also occur in vivo. Accordingly, Launay et al. (16) have reported a 5-HT2B-dependent activation of the p21ras/MAP kinase signaling cascade. In addition, 5-HT2 receptor subtypes are found in regions of the hippocampus and cerebral cortex where 5-HT stimulates the neuronal secretion of arachidonic acid (AA) (17, 18). Both 5-HT2A and 5-HT2C, which is a third member of the 5-HT2 receptor family, are likely to be involved in this release because the stable transfection of CHO cell lines with the corresponding human cDNAs activates the phospholipase A2 (PLA2)-mediated AA release (19, 20). The PLA2/AA pathway could also be sensitive to 5-HT2B as indicated by the colocalization of this receptor with the 5-HT2A and 5-HT2C ones in the hippocampus (21–23).

In this report, we establish that the 5-HT2B and 5-HT2A receptors of 1C11* cells are coupled with a PLA2-mediated release of arachidonic acid. Moreover, activation of the 5-HT2B receptor inhibits the 5-HT1B/1D receptor function, via a cyclooxygenase dependent AA metabolite. This 5-HT2B-mediated inhibition of the 5-HT1B/1D function can be blocked by a concomitant 5-HT2A activation.

**EXPERIMENTAL PROCEDURES**

**Materials**—Forskolin was purchased from Calbiochem (San Diego, CA); myo-[\(^{3}H\)]inositol (\(\sim 20.9 \text{ Ci/mmol}\); \(^{3}\text{H}\)AA (\(\sim 57 \text{ mCi/mmol}\)) from NEN Life Science Products; 5-carboxamidotryptamine (5-CT), 5-HT HCl, and DOI from Research Biochemicals (Natick, MA). Indomethacin came from Biomol Research Laboratories (Plymouth Meeting, PA); and mepacrine, melittin, and hygromycin came from Sigma. Rolipram and LY266070 were synthesized in Hoffmann-La Roche AG (Basel, Switzerland) according to the procedure described by Audia et al. (24) for LY266070. All tissue culture reagents and HBSS (Hanks’ balanced salt medium) supplemented with 5-HT-depleted fetal calf serum (10%) (16) were used 2 days (1C11*d2 cells) (5-HT1B/1D and 5-HT2B) or 4 days (1C11*d4 cells) (5-HT1B/1D, 5-HT2B, and 5-HT2A) after addition of the inducers. Cells were maintained in DMEM (Dulbecco’s modified Eagle’s medium) supplemented with 5-HT-depleted fetal calf serum (10%) (16) and 300 \(\mu\)g/ml hygromycin. For all experiments, cells were seeded onto 12- or 24-well tissue culture vessels at a density of \(4 \times 10^{4}\) cells/cm\(^2\). After a 24-h plating period, cells were washed with HBSS and grown for 24-h, before all experiments, in serum-free medium (DMEM/F-12 (1:1) with 5 \(\mu\)g/ml insulin, 5 \(\mu\)g/ml transferrin, 30 nm sodium, 20 nm progesterone, and 100 \(\mu\)g/ml putrescine).

**Measurement of 5-HT1B/1D Receptor Response**—Cells were washed twice with HBBS (with calcium and magnesium) containing 10 mm HEPES (pH 7.4) and then preincubated in 500 \(\mu\)l of 10 mm HEPES (pH 7.4) (wash buffer) per well for 15 min in a CO\(_2\) incubator (5% at 37 °C). Where indicated, inhibitors were added during this preincubation period. 5-HT1B/1D receptor-mediated response was followed by measuring (15 min at 37 °C) the 5-HT-induced inhibition of cAMP accumulation in the presence of 1 \(\mu\)M forskolin and 0.1 mm rolipram, a phosphodiesterase inhibitor. Cellular cAMP content was measured by radioimmunoassay (1) and normalized to protein content as determined by the bicinchoninic acid assay (Pierce, Chichester, UK). For each experiment, data were expressed according to the response obtained with 1 \(\mu\)M forskolin (100%).

**IP Accumulation Measurements**—Cells were labeled with 1 \(\mu\)C/ml myo-[\(^{3}H\)]inositol in serum-free medium for 24 h at 37 °C. Total IP (inositol monophosphate, inositol bisphosphate, and IP3 are collectively referred to as IP) accumulation in response to agonist stimulation in the presence of 20 mm LiCl for 10 min at 37 °C was determined as described previously (25). Radiolabeled IP was separated according to the ion exchange method of Berridge et al. (26).

**Arachidonic Acid Release**—Cells were labeled with 0.1 \(\mu\)C/ml [\(^{14}C\)]AA for 4 h at 37 °C (5% CO\(_2\)). Under these conditions, more than 90% of total radioactivity was taken up by the cells. After labeling, cells were washed three times with HBSS containing calcium and magnesium supplemented with 20 mm HEPES and 0.1% BSA (bovine serum albumin) (experimental medium). For calcium-free experiments, cells were washed with the same experimental medium without calcium. Between washes, cells were incubated for 5 min at 37 °C. After the last wash, cells in 1 ml of experimental medium were exposed to the indicated drugs, and aliquots (100 \(\mu\)l) were removed after 10 min for [\(^{14}C\)]measurement.

**Data Analysis**—Concentration-response data were fitted by non-linear regression to the model: \(R = R_{\text{max}}/[1 + (EC50/\{A\})^n]\), where \(E\) is the measured response at the given agonist concentration \(A\), \(R_{\text{max}}\) is maximal response, \(EC50\) is the concentration of agonist producing half-maximal response, and \(n\) is the slope index. The non-parametric Kolmogorov-Smirnov test (KS test) was used for statistical comparisons.

**RESULTS**

**The Activation of 5-HT2B Receptor Subtypes Increases AA Release via PLA2 Activation in 1C11*d2 Cells**—1C11 cells were preincubated with 0.1 \(\mu\)C/ml [\(^{14}C\)]AA for 4 h, washed, and later exposed either to 5-HT or to the 5-HT2 receptor agonist (\(\sim\))1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Total [\(^{14}C\)] release from the cells into the medium was measured after 10 min. With undifferentiated 1C11 cells, no [\(^{14}C\)] release could be detected. In contrast, at day 2 of the serotonegenic differentiation, when 5-HT2B and 5-HT1B/1D receptors had become induced, a basal level of [\(^{14}C\)] release (48 ± 5 nm) was observed. In addition, 1C11*d2 cells released higher amounts of [\(^{14}C\)] radioactivity in response to 5-HT or DOI (Fig. 1A). As assessed by high performance liquid chromatography, [\(^{14}C\)] AA contributes to 90% of the total [\(^{14}C\)] release. This release increased with the drug concentration, with a maximal response to 5-HT reaching 126 ± 10% (108.5 ± 4.8 nm) above the basal release and an associated \(EC_{50}\) value of 1.67 \(\mu\text{M}\) (\(p\) \(EC_{50} = 5.78 ± 0.14\); mean ± S.E., \(n = 4\)). DOI behaved as a partial agonist by eliciting a maximal response of 87 ± 9% above basal release with an \(EC_{50}\) value of 29 nm (\(p\) \(EC_{50} = 7.54 ± 0.12\); mean ± S.E., \(n = 6\)). These two \(EC_{50}\) values are similar to those previously obtained for 5-HT and DOI-mediated IP3 accumulation in 1C11*d2 cells (27). After incubation of 1C11*d2 cells for 10 min with 2.5 \(\mu\)g/ml melittin, a direct activator of PLA2 (28), the release of [\(^{14}C\)] radioactivity increased up to 252 ± 19% above the basal release (mean ± S.E., \(n = 4\)).

To determine whether the observed [\(^{14}C\)]AA release originated from activation of PLA2 or derived from the PLC/DAG lipase pathway, [\(^{14}C\)]-labeled 1C11*d2 cells were exposed for 15 min to 100 \(\mu\text{M}\) mepacrine, a PLA2 inhibitor, prior to the addition of agonists. As shown in Fig. 1B, this completely inhibited the effect of up to 10 \(\mu\text{M}\) 5-HT on the release of [\(^{14}C\)] radioactivity. The involvement in [\(^{14}C\)] release of 5-HT2B receptors via PLA2 coupling could be further evidenced by using DOI treatment (1 \(\mu\text{M}\)). DOI-mediated AA release was also fully blocked by mepacrine (Fig. 1B). Mepacrine, however, had no significant effect on DOI-mediated PI hydrolysis. Cellular IP accumulation measured after DOI treatment in the presence of 100 \(\mu\text{M}\) mepacrine, amounted to 124 ± 18% above the basal level (mean ± S.E., \(n = 5\), \(p > 0.05\)). Accumulation was very similar in the absence of mepacrine (117 ± 16%). Mepacrine alone had no effect on IP accumulation, as well as on basal AA release.

Unlike phospholipase C or phospholipase D, cytosolic PLA2 requires extracellular calcium for its activity (29). Accordingly, when calcium was removed from the incubation medium of 1C11*d2 cells, 5-HT-mediated AA release was reduced to 11 ± 7% above the basal level (mean ± S.E., \(n = 4\)). We verified that the basal [\(^{14}C\)] release level was not sensitive to the deprivation of calcium. All these data indicate that the 5-HT2B receptor of 1C11*d2 cells is coupled with the cytosolic PLA2 activity.

The PLA2-AA Signal Transduction Pathway Mediated by 5-HT2B Activation Inhibits the 5-HT1B/1D Receptor Function—5-HT1B/1D receptors are linked to G\(_i\) mediated inhibi-
obtained with a 10-min treatment with either 5-HT (10 μM) or melittin (2.5 μM). These data suggest that a cyclooxygenase-activated PLA2, which is triggered by either 5-HT2B receptor stimulation or the direct action of DOI but also enhanced the 5-CT-mediated inhibition of FScA (Fig. 2). *2d cells were treated with either 5-HT (10 μM) or DOI (1 μM) in the presence or absence of 100 μM mepacrine (means ± S.E. of 4 (5-HT) or 6 (DOI) experiments are shown, p < 0.01 versus corresponding control; stars indicate statistically significant differences).

FIG. 1. Activation of 5-HT2B receptor increases AA release via PLA2 activation in 1C11*d2 cells. 1C11*d2 cells were incubated for 4 h with [14C]AA. [14C] radioactivity release was later measured following a 10-min activation of the 5-HT2B receptor with either 5-HT or DOI. A, increase of released [14C] radioactivity above basal level as a function of either 5-HT or DOI concentration. B, released radioactivity obtained with a 10-min treatment with either 5-HT (10 μM) or DOI (1 μM) in the presence or absence of 100 μM mepacrine (means ± S.E. of 4 (5-HT) or 6 (DOI) experiments are shown, p < 0.01 versus corresponding control; stars indicate statistically significant differences).

As shown in Fig. 2, 5-CT (5 nM) inhibits FScA by 50%. The extent of inhibition remains similar with higher 5-CT concentrations. Activation of 5-HT2B receptors by 1 μM DOI completely abolished the 5-CT-mediated inhibition of FScA (Fig. 2). This behavior could be related to PLA2 since the direct addition of melittin (2.5 μg/ml) mimicked the action of DOI on the 5-HT1B/1D receptor signaling pathway (Fig. 2). In the presence of 100 μM mepacrine, this effect of DOI was not observed any more. This indicates that the PLA2/AA signaling cascade governs the 5-HT2B-dependent inhibition of the 5-HT1B/1D receptor pathway.

Since multiple pathways including cyclooxygenase, lipoxygenase, cytochrome P-450, and autooxidation (30) may couple AA metabolism with the 5-HT1B/1D receptor response, we investigated the effect of indomethacin, a cyclooxygenase inhibitor. Addition of a 2 μM amount of this substance not only blocked the action of DOI but also enhanced the 5-CT-mediated inhibition of FScA (Fig. 2). 1C11*d2 cells were treated with either 100 μM mepacrine or 2 μM indomethacin prior to the addition of melittin (2.5 μg/ml). In both cases, the 5-CT-mediated inhibition of FScA by 50% was reasserted because the effect of DOI was not observed any more on the 5-CT-mediated inhibition of FScA (48 ± 5% and 47 ± 4%, in the presence or absence of DOI, respectively; mean ± S.E., n = 4). This behavior suggests that the newly expressed 5-HT2B receptor can antagonize the negative regulation exerted by 5-HT2B on the 5-HT1B/1D function. This was reasserted because the effect of DOI on the 5-CT-mediated inhibition of FScA was restored in the presence of 5 nM ketanserin (not shown).

To know whether the blocking by 5-HT2A of the 5-HT2B-dependent regulation occurred through the PLA2 pathway, 1C11* d4 cells were at first treated with melittin (2.5 μmol/ml)
cells were incubated for 4 h with [14C]AA. [14C] radioactivity both coupled with the PLA2/AA signal transduction pathway. Prior treatment with 5-CT (5 nM) alone or with 5-CT plus either DOI (1 nM), ketanserin (5 nM), or LY266070 (1 nM), are compared. Ordinate values correspond to the increase of released [14C] radioactivity above the basal release value measured in the absence of 5-HT (means ± S.E.; stars indicate statistically significant differences versus corresponding control experiments).

FIG. 3. 5-HT2A and 5-HT2B receptors coexpressed at day 4 are both coupled with the PLA2/AA signal transduction pathway. 1C11* cells were incubated for 4 h with [3H]AA. [3H] radioactivity release was further measured after a 10-min activation with 10 μM 5-HT. Either 1C11*d2 (5-HT2B receptor) or 1C11*d4 (5-HT2B and 5-HT2A receptors) were treated with 5-HT. Values obtained with 5-HT alone, or with 5-HT after prior addition of either mepacrine (100 μM), ketanserin (5 nM), or LY266070 (1 nM), are compared. Ordinate values correspond to the increase of released [3H] radioactivity above the basal release value measured in the absence of 5-HT (means ± S.E.; stars indicate statistically significant differences versus corresponding control experiments).

for 10 min. As shown in Fig. 4, this only induced a small relative decrease (16 ± 4%) of the 5-CT-mediated inhibition of FScA. In contrast, when the cells were pretreated with both melittin and DOI, the inhibition of cAMP accumulation increased by 46 ± 9% above controls (100%) (mean ± S.E., n = 4; stars indicate statistically significant differences versus corresponding control experiments).

FIG. 4. The activation of 5-HT2A receptors in 1C11*d4 cells blocks the AA-dependent inhibition of the 5-HT1B/1D receptor function mediated by 5-HT2B receptors. The cAMP accumulation triggered in 1C11*d4 cells by forskolin stimulation was measured after prior treatment with 5-CT (5 nM) alone or with 5-CT plus either DOI (1 μM), melittin (2.5 μg/ml), or both DOI and melittin. Ordinate values are expressed as percentage of cAMP obtained in the presence of forskolin alone (means ± S.E., n = 4; stars indicate statistically significant differences versus corresponding control experiments).

These results strongly indicate that the 5-HT2A-mediated DOI effect can only be partly accounted for by the PLA2 signaling cascade and that additional transduction pathway(s) must also be involved in the control of 5-HT1B/1D receptor function within the 1C11 serotonergic cell line.

DISCUSSION

So far, the diversity of 5-HT receptors observed in vivo or in heterogeneous primary cultures has rendered difficult the determination of their precise role in the signaling networks that mediate the 5-HT physiological functions. Consequently, most of the possible functional coupling(s) associated with one given receptor subtype could be defined only by using cDNA-transfected heterologous cell lines. With newly established neuronal cell lines expressing a definite set of receptors, studies may be carried out in conditions closer to in vivo physiological circumstances, particularly concerning active stoichiometries and pathways of G protein coupling. The 1C11 neural-like stem cell is at present a unique in vitro clonal model able to acquire three 5-HT receptors during its serotonergic differentiation program (1). Therefore, this model appears suitable to define the transductional pathways related to the activation of all 5-HT1B/1D, 5-HT2B, and 5-HT2A receptors within the same serotonergic context. The sequential onset of these three receptors along the differentiation process makes it possible to follow the functional interaction(s) between their associated signaling cascades at day 2, when 5-HT1B/1D and 5-HT2B receptors are coexpressed, and at day 4, when 5-HT2A has also been induced.

The in vivo signaling pathways that mediate the physiological functions of 5-HT2B receptors remain largely unknown (14, 15). In the present study, we have investigated the PLA2/AA pathway because 5-HT was already known to stimulate the direct release of AA via 5-HT2 receptor subtypes in hippocampal neurons (18, 31). As early as day 2 of the 1C11 differentiation, the activation of 5-HT2B receptors with 5-HT or DOI stimulates the release of AA. The PLA2 inhibitor mepacrine completely inhibits this behavior while having no effect on the 5-HT2B-mediated PI hydrolysis (Fig. 1).

Furthermore, the 5-HT2B receptor-mediated AA release observed here may be responsible for the direct activation of the PLA2 activity and does not derive from the phospholipase C- or D-induced PLA2 activation. Indeed, it was totally dependent on extracellular calcium. Thus, inside the 1C11* cells, in addition to its role in activation of PI hydrolysis (27), p21ras/MAP kinase signaling (16), and NO signaling,² the 5-HT2B receptor can also control AA release through the activation of PLA2.

All members of the 5-HT2 receptor family have been detected in those regions of the cerebral cortex and hippocampus where 5-HT stimulates the release of AA (17, 18, 23). Moreover, after activation of 5-HT2C receptors, PLA2 and cyclooxygenase/lipoxigenase pathways contribute to the formation of cGMP in rat choroid plexus (32). Thus, it is likely that 5-HT2 receptor subtypes mediate in the brain some of the 5-HT physiological functions through the PLA2/AA pathway, but independently of the turnover of phosphoinositides.

On day 2 of 1C11 cell differentiation, the intracellular 5-HT and tryptophan hydroxylase activity becomes measurable. At the same time, 5-HT1B/1D (1500 GTI binding sites/cell) and 5-HT2B receptors (2200 DOI binding sites/cell) are induced (1). The simultaneous presence of these two functional 5-HT receptor renders the 1C11*d2 cells competent to integrate the different signaling inputs mediated by 5-HT. The present study shows that inside 1C11*d2 cells, the 5-HT2B-mediated PLA2 signaling cascade fully represses the negative Gs coupling of 5-HT1B/1D receptors with adenylate cyclase (Figs. 2 and 5A). The direct activation of PLA2 by melittin mimics the inhibitory effect exerted by the 5-HT2B receptor on the 5-HT1B/1D-mediated transduction pathway. Mepacrine antagonizes the action of melittin on the 5-HT1B/1D response and prevents the 5-HT2B-mediated inhibition of 5-HT1B/1D signaling. Furthermore, indomethacin, which inhibits the oxidative metabolism of AA through the cyclooxygenase pathway, completely blocks the inhibition of 5-HT1B/1D receptor function triggered by melittin-directed activation of PLA2. Indomethacin also blocks the 5-HT2B receptor inhibitory effect and furthermore potentiates the 5-CT-mediated inhibition of FScA caused by DOI. All these results imply that the actors of the cyclooxygenase metabolism, such as prostaglandins or thromboxanes, intracellularly mediate the interaction between the PLA2/AA and the adenyl cyclase signaling cascades.

Interestingly, similar potentiating effects of indomethacin have been previously observed in the case of (i) the 5-HT2C receptor-mediated cGMP formation (150% of the control level)² J. M. Launay, unpublished data.
in the choroid plexus (32), and (ii) the control of 5-HT1B/1D receptor pathway in CHO cells stably transfected by human 5-HT2C receptor cDNA (20). The mechanisms underlying such effects of indomethacin have not yet been elucidated. Regarding 1C11*d2 cells, the enhancement of 5-HT1B/1D function in response to both indomethacin and 5-HT2B activation might reflect an increase of the negative coupling between adenylate cyclase and 5-HT1B/1D. Several signaling pathways could be involved. For instance, when cyclooxygenase activity is blocked by indomethacin, the AA produced by 5-HT2B activation might be converted into a new signal. This signal could activate the 5-HT1B/1D signaling response through another metabolic pathway involving either lipooxygenase, cyclochrome P-450 or autooxidation. Accordingly, preliminary experiments with some selective inhibitors of these pathways indicate that the cyclochrome P-450 monoxygenase metabolism could be involved. In fact, the addition of either miconazole or SKF-325A, two inhibitors of the cyclochrome P-450 system, inhibits the antioxidation of their PLA2/AA pathway (Fig. 3). Despite its coupling with a signaling pathway also recruited by 5-HT2B receptors, 5-HT2A receptor activation prevents the negative regulation exerted by 5-HT2B receptors on the 5-HT1B/1D function (Fig. 5B). The rescue of the 5-HT1B/D response at day 4 implies that the DOI-induced 5-HT2A activation either blocks or neutralizes the 5-HT2B cyclooxygenase-dependent negative action also triggered by addition of DOI. The coupling of two 5-HT2 receptor subtypes with PLA2 in the 1C11*d4 serotonergic cells, independent of PI hydrolysis, corroborates the few reports dealing with 5-HT-stimulated release of AA, as observed in vivo (18) or in vitro with CHO cell lines stably transfected with human 5-HT2A or 5-HT2C cDNAs (19, 20). CHO cells are known to endogenously express 5-HT1B-like receptors. In the 5-HT2A/CHO cell line, 5-HT2A receptor activation has no effect on 5-HT1B signaling. Thus, it is likely that, in 1C11*d4 cells, 5-HT2A receptors can abrogate the 5-HT2B receptor-mediated inhibition of 5-HT1B/1D function by blocking the production of some cyclooxygenase product or its effect, instead of directly acting on the 5-HT1B/1D coupling.

Interestingly, in 1C11*d4 cells, the 5-HT2A-mediated PLA2 signaling cascade only slightly accounts for the blocking action of 5-HT2B receptors. Indeed, PLA2 activation by melittin induces a minor relative decrease (~16%) of cAMP accumulation, when compared with the complete (~46%) rescue of the 5-HT2B mediated inhibition of FScA after DOI treatment (Fig. 4). Consequently, a direct activation of the 5-HT2A receptors is needed to antagonize the negative regulation by 5-HT2B of the 5-HT1B/1D coupling. Therefore, the control of the 5-HT1B/1D function must involve some additional 5-HT2A-mediated transduction pathway(s). These are distinct from the PLC and PLA2 signaling pathways already functional in the 1C11 cell line.

In brain tissue, AA and its metabolites can act as either intracellular or intercellular messengers. Eicosanoids released from cells recognize specific cell receptors (33) and mediate many transcellular actions. They play several roles in neuronal signal transduction (33, 34) and seem to be involved in long term potentiation synapticle plasticity, ion channel regulation, neurosecretion, and presynaptic inhibition (30). Moreover, eicosanoids accumulate in the brain during strokes, seizures, or other pathophysiological processes (35, 36). Nevertheless, the biological significance of a 5-HT-mediated release of AA in the nervous system is presently unknown. This study has established that in non-transfected 1C11 cells, 5-HT2 receptor-generated eicosanoids support cross-talks between the three 5-HT receptors. Whether, in vivo, a single neuron has each of the 5-HT2A, 5-HT2B, and 5-HT1B/1D receptors is presently unknown. It has been shown, however, that these receptors colocalize in the same brain regions, especially in the hippocampus (22, 23, 37). Actually, AA release is coupled to 5-HT2 receptors in this area. It may be that in such places some of the 5-HT2 receptor functions are mediated through the diffusion of the oxidative metabolites of their PLA2/AA coupling. Such metabolites would direct the transduction of signals between cells and modulate neuronal or neurovascular activity.

The main contribution of this study is the demonstration of cross-talks between three 5-HT receptors naturally induced in
the course of a serotonergic program. The antagonistic roles of 5-HT2A and 5-HT2B receptors in the regulation of 5-HT1B/1D function are well demonstrated. Links between the regulation of 5-HT1B/1D receptor function and anxiety, depressive or obsessive disorders, and migraine pathogenesis are not yet fully known. It is, however, worth noting that selective 5-HT1B/1D/1F receptor agonists, like sumatriptan or zolmitriptan (10), are effective in the acute treatment of migraine (38). A number of 5-HT2 receptor antagonists are used as prophylactic antimigraine agents, but which 5-HT2 receptor subtype mediates these prophylactic effects is still unclear. A role has been proposed for the 5-HT2C receptor since the 5-HT2A preferential antagonist, ketanserin, is not effective (39). Nevertheless, only trace amounts of 5-HT2C receptor mRNA are detected in meningeal tissues. On the other hand, organ bath experiments have recently suggested that 5-HT2B receptor located on the endothelial cells of meningeal blood vessels would trigger migraine headache, probably via NO formation (41). Thus, 5-HT1B/1D and 5-HT2B receptors are presently the best candidates for involvement in migraine. In this context, a cross-talk analysis between these two 5-HT receptor subtypes as well as 5-HT2A, all present in various meningeal tissues, can be carried out at a cellular level with 1C11* cells.

Finally, the induction of the three 5-HT1B/1D, 5-HT2B, and 5-HT2A receptors is an essential part of the autonomous serotonergic differentiation program of 1C11. These receptors could possibly act as autoreceptors, thus promoting the regulation of the serotonergic phenotype by 5-HT. In fact, the synthesis, metabolism, and storage of 5-HT in 1C11* cells are downregulated by external 5-HT. We now aim at identifying intracellular targets related to the activation of 5-HT receptors and at analyzing the contribution of 5-HT-mediated signals to the control of the serotonergic functions.

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