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Serotonin stimulates phospholipase A_2 and the release of arachidonic acid in hippocampal neurons by a type 2 serotonin receptor that is independent of inositolphospholipid hydrolysis

(phorbol ester/lysophosphatidylcholine/extracellular calcium)

CHRISTIAN C. FELDER*†, ROBERT Y. KANTERMAN‡, ALICE L. MA*, AND JULIUS AXELROD*

*Laboratory of Cell Biology, Section on Pharmacology, National Institute of Mental Health, and ‡Howard Hughes Medical Institute–National Institutes of Health Research Scholars Program, Bethesda, MD 20892

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ABSTRACT **Serotonin (5-HT) stimulated the release of arachidonic acid in hippocampal neurons cocultured with glial cells but not in glial cultures alone. Similar results were observed for the 5-HT-stimulated release of inositol phosphates. These results suggest a neural but not glial origin of both responses. Pharmacological studies suggested that release of arachidonic acid and inositol phosphates was mediated by a type 2 5-HT (5-HT_2) receptor. 5-HT-stimulated release of arachidonic acid was also detected in cortical neurons, which contain high levels of 5-HT_2 receptors, but not striatum, spinal cord, or cerebellar granule cells, which have very low levels or are devoid of 5-HT_2 receptors. The phorbol ester phorbol 12-myristate 13-acetate augmented the 5-HT-stimulated release of arachidonic acid but inhibited the 5-HT-stimulated release of inositol phosphates. 5-HT-stimulated release of arachidonic acid, but not inositol phosphates, was dependent on extracellular calcium. 5-HT stimulated the release of [^{3}H]lysophosphatidylcholine from [^{3}H]choline-labeled cells with no increase in the release of [^{3}H]choline or phospho[^{3}H]choline. These data suggest that 5-HT stimulated the release of arachidonic acid in hippocampal neurons through the activation of phospholipase A_2, independent of the activation of phospholipase C.**

The excitable membranes of the central nervous system are enriched in 20-carbon unsaturated fatty acids, particularly arachidonic acid (1). Arachidonic acid serves as a precursor to a number of biologically active acid lipids, including prostaglandins, leukotrienes, and thromboxanes. Arachidonic acid and its eicosanoid metabolites are released after activation of certain neurotransmitter receptors and may play a role in neurotransmission. Recent studies have demonstrated (2–4) receptor-mediated release of eicosanoids in brain cells in primary culture. *N*-Methyl-D-aspartate stimulated the release of arachidonic acid, 11-HETE, and 12-HETE (where HETE is hydroxyeicosatetraenoic acid) in striatal cells (2) as well as arachidonic acid in cerebellar granule cells (3). Bradykinin stimulated arachidonic acid mobilization from dorsal root ganglion neurons (4). It is not clear from these studies whether the release of arachidonic acid is independent of inositolphospholipid turnover and whether the response is neural or glial in origin. Recent studies demonstrated α-adrenergic receptor stimulation caused arachidonic acid release from spinal cord, hippocampal, and cortical neurons, but not from glia cells, grown in primary culture that was independent of inositolphospholipid turnover (5).

Central serotonin (5-HT) receptors are involved in sleep, depression, hallucinations, anxiety, sexual behavior, memory, appetite control, and thermoregulation (6). 5-HT receptors exist as several subtypes and transduce their signals by way of multiple intracellular messengers (7). Types 1A, 1B, and 1D 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D}) modulate adenylate cyclase activity and types 1C and 2 5-HT (5-HT_{1C} and 5-HT_2) receptors increase inositolphospholipid turnover. The mechanism of signal transduction for 5-HT type 3 (5-HT_3) receptors is presently unknown. 5-HT-stimulated prostaglandin production has been suggested in rat brain homogenates (8), but these studies did not address the cell type involved in the response and a single large dose (500 μM) was used and, therefore, could be nonspecific. 5-HT-stimulated release of prostacyclin was observed in bovine smooth muscle cells, but the response was not assigned to a 5-HT receptor subtype (9). In this study, we demonstrate that 5-HT stimulates the release of arachidonic acid in hippocampal neurons but not glial cells through a 5-HT_2 receptor. The release of arachidonic acid appears to be mediated through activation of phospholipase A_2 and is independent of inositolphospholipid turnover.

MATERIALS AND METHODS

Materials. [5,6,8,9,11,12,14,15-^{3}H(N)]Arachidonic acid was purchased from New England Nuclear and [^{3}H]inositol was from American Radiolabeled Chemicals (Saint Louis). Phorbol 12-myristate 13-acetate (PMA) was purchased from Calbiochem; pertussis toxin was from List Biological Laboratories (Campbell, CA); adrenergic and serotonergic ligands were from Research Biochemicals (Natick, MA). All other reagents were from Sigma. Dulbecco's modified Eagle's medium (DMEM) was from Whittaker M.A. Bioproducts; minimal essential medium (MEM) was purchased from GIBCO; and Eagle's #2 medium was purchased from the National Institutes of Health Media Unit. Eicosanoids were obtained from either Biomol (Plymouth Meeting, PA) or Caymen Chemical (Ann Arbor, MI).

Cell Culture. Hippocampal glial cultures were established from whole hippocampus from 17-day gestational C57BL/6J mouse embryos as described (10) except that cultures were plated on Nunclon multidish (Rosklide, Denmark) 24-well plates. Hippocampal neural/glial cocultures were plated over confluent glial cell beds and were maintained identically as glial cultures except that 5% (vol/vol) horse serum was substituted for 10% (vol/vol) fetal calf serum in the growth medium. The cells were used for biochemical assays between

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Abbreviations: 5-HT, serotonin; 5-HT_{1A} receptor, 5-HT_2 receptor, etc., type 1A 5-HT receptor, type 2 5-HT receptor, etc.; PMA, phorbol 12-myristate 13-acetate; DOI, (*R*)-(−)-2,5-dimethoxy-4-iodo-phenylisopropylamine; TFMPP, *m*-trifluoromethylphenylpiperazine; 8-OH DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; MCPP, *m*-chlorophenylpiperazine; HETE, hydroxyeicosatetraenoic acid.

†To whom reprint requests should be addressed.

4 and 9 days in culture but were viable for up to 4 weeks in culture.

Measurement of [^{3}H]Arachidonic Acid Release. Hippocampal glial or neural/glial cocultures were incubated with [^{3}H]arachidonic acid (0.20 μCi per well; 1 Ci = 37 GBq) to isotopic equilibrium (18–24 hr) and the release of [^{3}H]arachidonic acid was measured as shown (46).

Measurement of [^{3}H]Inositol Phosphate Release. Hippocampal glial or glial/neuronal cocultures were incubated with [^{3}H]inositol (0.5 μCi per well) to isotopic equilibrium (18–24 hr). Prior to the addition of experimental agents the cells were washed once with 500 μl of Eagle's #2 medium supplemented with 20 mM Hepes and 10 mM LiCl. The experimental agents were added in a final volume of 250 μl and the reaction was allowed to proceed for 15 min at 37°C unless otherwise indicated. The reaction was stopped by the addition of 250 μl of ice-cold stop solution (1 M KOH/18 mM $Na_2B_4O_7$/3.8 mM EDTA/7.6 mM NaOH) followed by a neutralization solution containing 7.5% (vol/vol) HCl. The released inositol phosphates were separated by anion-exchange chromatography as described (11). Unless otherwise indicated, data were reported as cpm of total released inositol phosphates.

Measurement of [^{3}H]Lysophosphatidylcholine. Cells were labeled 18–24 hr with [^{3}H]choline in 24-well plates. Wells were washed twice with Eagle's #2 medium supplemented with 20 mM Hepes. After a 2-min incubation with ligands, the [^{3}H]lysophosphatidylcholine was extracted as reported (12) and analyzed by TLC (13). Spots corresponding to authentic lysophosphatidylcholine standards were scraped and measured for radioactivity by using a β-scintillation spectrophotometer.

HPLC Profile of Arachidonic Acid and Eicosanoids Released from Neural/Glial Cocultures. Hippocampal neural/glial cocultures were plated in 10-cm plates and labeled overnight with 10 μCi of [^{3}H]arachidonic acid. The cells were washed twice with Eagles #2 media and incubated with or without 10 μM 5-HT for 10 min at 37°C in the presence of an additional 1 μCi of [^{3}H]arachidonic acid. The supernatant was collected and injected onto a reverse phase C_{18} guard column located in the injector sample loop. The guard column was then washed with 3 ml of distilled water containing 20% (vol/vol) methanol (pH 4.0). The sample contained on the guard column was injected on to the main Porosil reverse-phase C_{18} column and was eluted with a series of isocratic gradients of acidified water, acetonitrile, and methanol, as described (14). Radioactive eicosanoids were detected and quantified with a flow-through tritium detector (Radiomatic Instruments and Chemical, Tampa, FL). Radioactive sample peaks were identified by comparison with authentic nonradioactive standards detected by ultraviolet absorbance.

RESULTS

5-HT Stimulates the Release of Arachidonic Acid Independent of Phospholipase C from Neural but not Glial Cells. In view of the observation that α-adrenergic receptors can mediate the release of arachidonic acid from neural cells in culture (5), we examined the effect of another biogenic amine, 5-HT. 5-HT stimulated the release of [^{3}H]arachidonic acid and [^{3}H]inositol phosphates from cocultured neural/glial cells. In contrast, 5-HT failed to stimulate their release from glial cultures devoid of neurons (Fig. 1). These results indicate that the 5-HT-stimulated responses were neural in origin. 5-HT stimulated the release of arachidonic acid from cocultured glial/neural cells with a similar potency to the release of inositol phosphates (EC_{50} for arachidonic acid = 342 nM; EC_{50} for inositol phosphates = 341 nM).

The simultaneous release of arachidonic acid and inositol phosphates by 5-HT suggests that both responses were generated by a common metabolic pathway. Previous studies have suggested that arachidonic acid could be released by the action of diglyceride lipase on diacylglycerol, a product of inositolphospholipid hydrolysis (15). This possibility seems unlikely since the phorbol ester PMA simultaneously stimulated the release of arachidonic acid and inhibited the release of inositol phosphates in these cells. PMA alone did not stimulate the release of arachidonic acid but potentiated the 5-HT-stimulated arachidonic acid release (Fig. 2). In contrast, PMA completely inhibited the 5-HT-stimulated release of inositol phosphates (Fig. 2) and had no effect on basal inositol phosphate release. The diglyceride lipase inhibitor, RHC 80267 (16), had no effect on 5-HT-stimulated release of arachidonic acid up to 100 μM (data not shown), further eliminating this pathway as a source of 5-HT-stimulated release of arachidonic acid. The time course of 5-HT-stimulated release of [^{3}H]arachidonic acid reached a maximum at 5 min and returned to basal levels by 60 min of stimulation (Fig. 3). 5-HT-stimulated inositol phosphate release increased linearly for 30 min and reached a plateau by 60 min, the last time point tested (Fig. 3).

5-HT-Stimulated Release of Arachidonic Acid Is Mediated by a 5-HT$_2$ Receptor. Functional pharmacological studies suggest that the release of arachidonic acid was mediated by a 5-HT$_2$ receptor (Fig. 4). Both spiperone, a 5-HT$_2$ receptor antagonist, and SCH 23390, a 5-HT$_2$ receptor and dopamine-1 receptor antagonist (17), blocked 5-HT-stimulated arachidonic acid release. Propranolol (a β-adrenergic receptor antagonist), prazosine (an α-adrenergic receptor antagonist), and yohimbine (an α_2-adrenergic receptor antagonist) had no

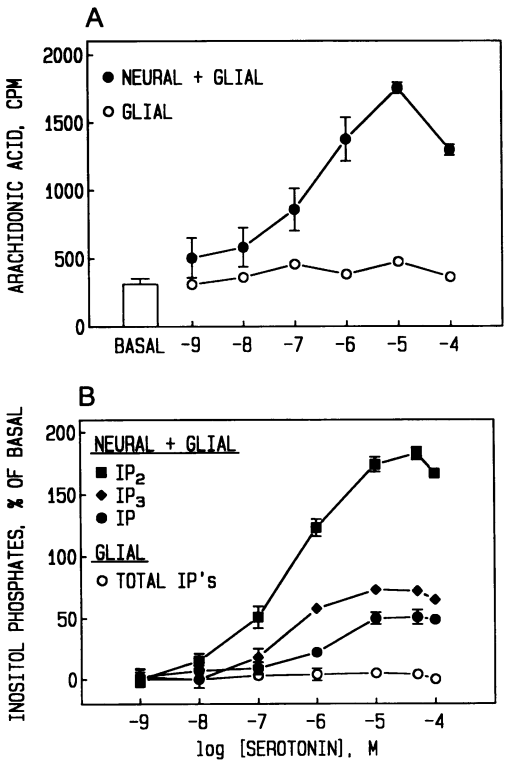

FIG. 1. 5-HT stimulated release of arachidonic acid (*A*) and inositol phosphates (*B*) in neurons but not glia in hippocampal cell cultures. Arachidonic acid release was measured in hippocampal neural/glial cocultures or in cultures devoid of neurons. The data represent the mean ± SEM of at least three experiments performed in triplicate. Arachidonic acid at zero time (mean = 275 cpm) has been subtracted from all values as a blank correction. Inositol phosphate release is represented as the percent stimulation over a basal mean value of 3536 ± 146 cpm for inositol phosphate (IP), 335 ± 20 cpm for inositol bisphosphate (IP_2), and 350 ± 17 cpm for inositol trisphosphate (IP_3).

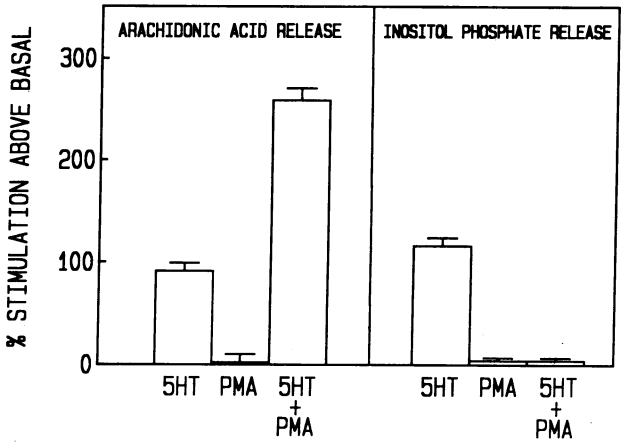

FIG. 2. The phorbol ester PMA augments 5-HT-stimulated release of arachidonic acid but inhibits release of inositol phosphates. 5-HT at 10 μM stimulated the release of arachidonic acid, which was augmented by 10 nM PMA. 5-HT at 10 μM stimulated the release of total inositol phosphates collected as one fraction. PMA at 10 nM inhibited completely the 5-HT-stimulated response when preincubated with the hippocampal cells for 30 min. PMA alone at the same concentration had no effect on release of arachidonic acid or inositol phosphates. Data represent the mean ± SEM of at least three experiments performed in triplicate.

effect on 5-HT-stimulated arachidonic acid release (Fig. 4*A*). Similar results were seen for the release of inositol phosphates (data not shown). (*R*)-(−)-2,5-Dimethoxy-4-iodophenylisopropylamine (DOI), a purported 5-HT_{2A} agonist (18), slightly stimulated the release of arachidonic acid at 100 μM, consistent with its lower affinity for 5-HT_{2B} compared to 5-HT_{2A}. Similar results were seen for DOI-stimulated release of inositol phosphates (EC_{50} for inositol phosphates = 10 μM; EC_{50} for arachidonic acid = 10 μM). *m*-Trifluoromethylphenylpiperazine (TFMPP) [a nonselective 5-HT_1 receptor agonist (19)], 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH DPAT) [a 5-HT_{1A} receptor agonist (20, 21)], and dopamine failed to stimulate the release of arachidonic acid. Isoproterenol, a β-adrenergic receptor agonist, slightly increased the release of arachidonic acid. Similar results were seen for the release of inositol phosphates (data not shown). These data suggest that the release of arachidonic acid and inositol phosphates was due to the stimulation of a 5-HT_2 receptor.

5-HT receptor subtype was further established using the 5-HT_2 receptor antagonists spiperone (22, 23), ketanserin (24, 25), and *m*-chlorophenylpiperazine (MCPP) (19) (Fig. 5). Both spiperone and ketanserin were potent antagonists for

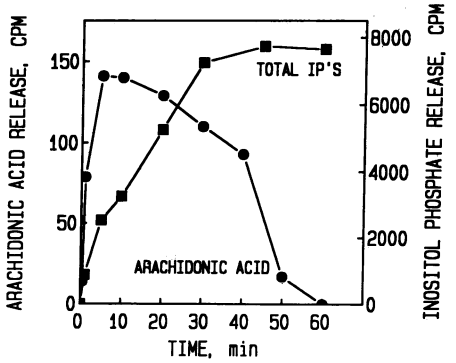

FIG. 3. Time course of 5-HT-stimulated release of arachidonic acid and inositol phosphates. Hippocampal neural/glial cocultures were incubated with 10 μM 5-HT as indicated and either arachidonic acid or total inositol phosphates (IPs) released were measured. Data are representative of at least three experiments performed in triplicate.

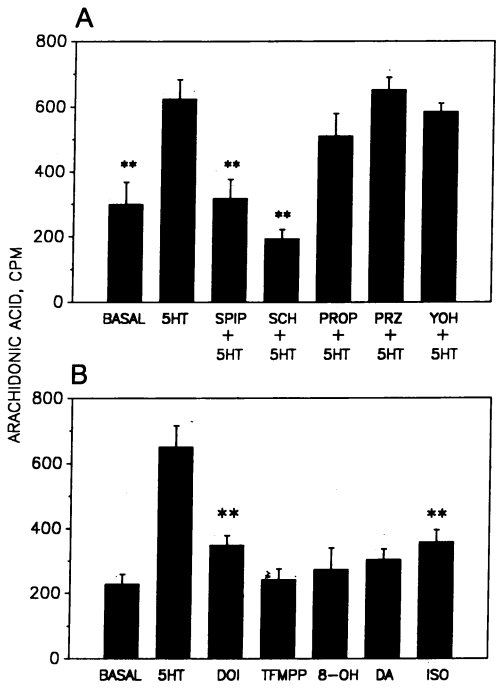

FIG. 4. Functional pharmacological analysis suggest that a 5-HT_2 receptor subtype mediates release of arachidonic acid. (*A*) SPIP, spiperone; SCH, SCH 23390; PROP, propranolol; PRZ, prazosine; YOH, yohimbine. (*B*) DA, dopamine; ISO, isoproterenol. Data represent the mean ± SEM of three experiments performed in triplicate. **, $P > 0.01$ by analysis of variance, Dunnet's test comparison with 5-HT alone in *A* and comparison with basal release in *B*. All ligands were added at a concentration of 10 μM.

5-HT-stimulated arachidonic acid release (IC_{50} for spiperone = 109 pM; IC_{50} for ketanserin = 2 nM) consistent with 5-HT_2 antagonist effects. MCPP also blocked the response that fit to a two-site model with IC_{50} values of 50 pM and 121 nM, respectively. Spiperone, ketanserin, and MCPP had no effect on the release of arachidonic acid when added alone up to 10 μM (data not shown). MCPP alone weakly stimulated the release of inositol phosphates at concentrations above 100 nM (EC_{50} = 10 μM) with a maximal 50% increase over basal stimulation at 100 μM. MCPP blocked 5-HT-stimulated release of inositol phosphates 50% at 100 μM (data not shown).

5-HT-Stimulated Release of Arachidonic Acid Is Mediated by Phospholipase A_2. As shown above, the 5-HT-stimulated

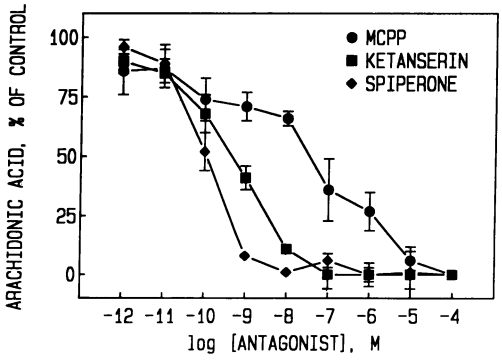

FIG. 5. 5-HT_2 receptor antagonists block 5-HT-stimulated release of arachidonic acid in a concentration-dependent manner. Ketanserin, spiperone, and MCPP block 5-HT-stimulated (10 μM) release of arachidonic acid. Data represent the mean ± SEM of three experiments performed in triplicate. EC_{50} values: spiperone, 109 pM; ketanserin, 2 nM; MCPP, 50 pM and 121 nM fit to a two-site model by nonlinear least squares analysis using GraphPAD software (GraphPAD Software, San Diego).

Table 1. 5-HT stimulates the release of [^{3}H]lysophosphatidylcholine from [^{3}H]choline-labeled hippocampal cells

Stimulating compound(s)	[^{3}H]Lysophosphatidylcholine, cpm
None	2190
5-HT (10 μM)	5283
5-HT (10 μM)/spiperone (0.1 μM)	2202

Cells were grown in 24-well plates and labeled overnight with [^{3}H]choline (1 μCi per well). Cells were stimulated for 2 min and lysophosphatidylcholine was extracted and analyzed. Eight wells of a 24-well plate were combined for each data point. Data are the mean of two experiments performed in duplicate.

release of arachidonic acid was not a consequence of inositolphospholipid hydrolysis. An alternate mechanism might involve 5-HT-receptor-activated phospholipase A_2 and release of lysophosphatidylcholine and arachidonic acid from phosphatidylcholine. Hippocampal neural/glial cocultures were prelabeled overnight with [^{3}H]choline to label phosphatidylcholine and the release of [^{3}H]lysophosphatidylcholine was measured by TLC (Table 1). 5-HT stimulated the release of [^{3}H]lysophosphatidylcholine, which was blocked by spiperone.

5-HT-Stimulated Release of Arachidonic Acid Is Dependent on Extracellular Calcium. Phospholipase A_2, unlike phospholipase C or phospholipase D, requires extracellular calcium for activity (26). 5-HT failed to stimulate the release of arachidonic acid in the absence of extracellular calcium (data not shown). These data further suggest that the release of arachidonic acid is mediated by phospholipase A_2.

5-HT-Stimulated Arachidonic Acid Release Correlates with Presence of 5-HT$_2$ Receptors. Neural/glial cocultures and glial cultures from several brain regions were tested for 5-HT-stimulated release of arachidonic acid. 5-HT stimulated the release of arachidonic acid in cerebral cortical neural/glial cocultures but failed to stimulate release in cerebral glial cultures. 5-HT failed to stimulate the release of arachidonic acid in striatal and spinal cord neural/glial cocultures as well as in cerebellar granule cells (data not shown). 5-HT$_2$ receptors have been localized in regions of the hippocampus and cerebral cortex, but only low levels have been identified in striatum, cerebellum, or spinal cord (27).

5-HT Stimulates an Increase in All Detectable Eicosanoid Metabolites from Hippocampal Neural/Glial Cocultures. HPLC analysis of supernatants from hippocampal neural/glial cocultures detected 6-keto-prostaglandin $F_{1\alpha}$ (metabolite of prostacyclin), thromboxane B_2, prostaglandin E_2, prostaglandin D_2, leukotrienes B_4, C_4, D_4 and E_4, and small amounts of 15-, 12-, and 5-HETEs (Fig. 6). 5-HT stimulated a small increase (10–20%) in all detected eicosanoid metabolites and arachidonic acid over basal levels. In contrast to the neural/glial cocultures, 5-HT failed to stimulate an increase in eicosanoid metabolites in glial cultures devoid of neurons.

DISCUSSION

5-HT stimulated the release of arachidonic acid in hippocampal neurons cocultured with glial cells but not in glial cultures alone. Similar results were observed for the 5-HT-stimulated release of inositol phosphates. These results suggest a neural origin for 5-HT-stimulated release of arachidonic acid and inositol phosphates. Our findings demonstrate a signaling pathway for 5-HT involving release of arachidonic acid in neural cells by way of a 5-HT$_2$ receptor.

Pharmacological analysis of 5-HT-stimulated release of arachidonic acid and inositol phosphates suggest that the responses were mediated by a 5-HT$_2$ receptor. α- and β-adrenergic and dopamine receptor agonists had little or no

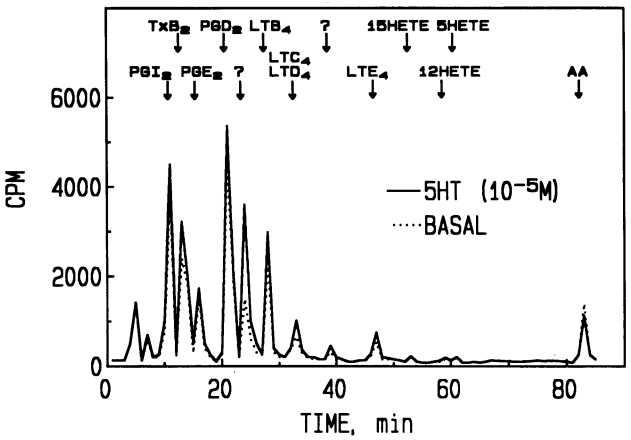

FIG. 6. HPLC profile of eicosanoids released from hippocampal cells with and without 5-HT. 5-HT at 10 μM stimulated an increase (10–20%) in the release of all eicosanoids from hippocampal neural/glial cocultures labeled with [^{3}H]arachidonic acid (AA). Samples were collected directly onto a C_{18} guard column located in the sample loop. Arrows indicate the elution time of authentic nonradioactive standards. Data are representative of at least five similar HPLC profiles. TxB_2, thromboxane B_2; PGD_2, PGI_2, and PGE_2, prostaglandins D_2, I_2, and E_2, respectively; LTC_4, LTD_4, LTE_4, leukotrienes C_4, D_4, and E_4, respectively.

effect on the responses. TFMPP, an indiscriminate 5-HT$_1$ receptor agonist, and 8-OH DPAT, a 5-HT$_{1A}$ receptor agonist, failed to stimulate both responses. Both MCPP and TFMPP, 5-HT$_2$ antagonists and 5-HT$_{1C}$ and 5-HT$_{1B}$ agonists, blocked both 5-HT-stimulated responses yet did not increase either response when added alone. SCH 23390 blocked both responses, consistent with its 5-HT$_2$ > 5-HT$_1$ receptor antagonist properties (17). Ketanserin and spiperone, potent 5-HT$_2$ antagonists, blocked both responses at low nanomolar concentrations. These data suggest 5-HT$_2$ receptors mediate the release of both arachidonic acid and inositol phosphates.

The presence of two subtypes of the 5-HT$_2$ receptor, 5-HT$_{2A}$ and 5-HT$_{2B}$, has been suggested based on binding studies with the agonist DOI (18). In contrast, shifts in binding curves upon addition of nucleotides suggested that multiple 5-HT$_2$ receptor sites are actually high- and low-affinity forms of the same receptor (28). In these studies, both release of arachidonic acid and inositol phosphates were weakly stimulated by DOI (EC_{50} = 10 μM for both). It is not apparent from these limited studies the nature of the 5-HT$_2$ receptor involved in the release of arachidonic acid. More insight will be possible through the study of the expressed 5-HT$_2$ receptor in a host cell devoid of endogenous 5-HT receptors.

A correlation exists between 5-HT$_2$ receptor localization and 5-HT-stimulated release of arachidonic acid. 5-HT$_2$ receptors have been localized in regions of the hippocampus and cerebral cortex (27) where 5-HT stimulated the release of arachidonic acid from neurons. We were unable to detect 5-HT-stimulated release of arachidonic acid in cerebellum, spinal cord, and striatum, where very low levels of 5-HT$_2$ receptors have been measured (27).

Several metabolic pathways exist for the receptor-mediated generation of arachidonic acid from membrane phospholipids. Previous studies have shown that phosphatidylcholine, phosphatidylserine, and phosphatidylinositol are rich in arachidonic acid in the second position of their glycerol backbone, making them good candidates as substrates for hormone regulated phospholipases. Predominantly phosphatidylcholine and phosphatidylinositol are labeled with added arachidonic acid in brain tissue (29). Phospholipase A_2 can hydrolyze phosphatidylcholine directly to release free arachidonic acid and yield lysophosphatidylcholine. Indeed, 5-HT stimulated the release of arachidonic acid

and lysophosphatidylcholine that was blocked by the addition of the 5-HT_2 antagonist spiperone in hippocampal neurons. The 5-HT-stimulated release of arachidonic acid was dependent on extracellular calcium, a characteristic of phospholipase A_2 (26).

The addition of the phorbol ester PMA inhibited 5-HT-stimulated release of inositol phosphates coincident with enhancement of 5-HT-stimulated arachidonic acid release; therefore, it is unlikely that inositolphospholipid-specific phospholipase C is involved in the generation of arachidonic acid. Arachidonic acid could also be generated by the hydrolysis of diacylglycerol by diglyceride lipase, with diacylglycerol originating from the action of phospholipase C on phosphatidylcholine (15). It is also conceivable that arachidonic acid could arise from receptor-mediated activation of phosphatidylcholine-specific phospholipase D to yield phosphatidic acid. Arachidonic acid would then be liberated by the action of diglyceride lipase on phosphatidic acid. These two pathways do not appear to play a major role in the generation of arachidonic acid in the hippocampal neuron since there was no detectable increase in the release of phospho[^{3}H]choline or [^{3}H]choline from [^{3}H]choline-labeled cells. Furthermore, 5-HT-receptor-mediated release of arachidonic acid was totally dependent on extracellular calcium, a characteristic not associated with either phospholipase C or D (30–32). In addition, the diglyceride lipase inhibitor, RHC 80267, did not block 5-HT-stimulated release of arachidonic acid.

Recent evidence has suggested that receptors are coupled to phospholipase A_2 through a guanine nucleotide binding protein (33). Receptor-mediated activation of phospholipase A_2 can be both pertussis toxin-sensitive (34) and -insensitive (35). Pertussis toxin binds to and inactivates certain guanine nucleotide binding proteins by catalyzing ADP-ribosylation of their α subunits. In our cells, neither 5-HT-stimulated release of arachidonic acid or inositol phosphates was sensitive to pertussis toxin when the neurons were exposed to 10 ng/ml for 18 hr (data not shown). Therefore, it is not clear if either of these two effector enzymes are linked to the 5-HT receptor through guanine nucleotide binding proteins in this system.

Analysis of the eicosanoids produced in hippocampal cultures demonstrated that several prostaglandins, leukotrienes, and small amounts of HETEs were produced under basal conditions. 5-HT stimulated a modest increase in all detected metabolites in cultures containing neurons but not in glial cultures. The physiologic role of 5-HT-receptor-stimulated release of arachidonic acid and subsequent eicosanoid formation in the hippocampus is not clear. Several other neurotransmitters have been shown to stimulate arachidonic acid mobilization in neurons, including norepinephrine, histamine, bradykinin, and glutamate (2–5, 36). Arachidonic acid and one of its lipoxygenase metabolites 12-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12-HPETE) can inhibit ion conductances and cause presynaptic inhibition of neurotransmitter release (37) presumably through inhibition of Ca^{2+}/calmodulin-dependent protein kinase II, a protein thought to be involved in neurotransmitter release (38). Arachidonic acid may also play a role in long-term potentiation (39), may activate potassium channels in cardiac and smooth muscle cells (40, 41), stimulates the release of intracellular calcium (42), and stimulates protein kinase C (43). Arachidonic acid and its eicosanoid metabolites have also been implicated in a number of pathophysiological processes, including trauma, hypoxia, ischemia, hypoglycemia, electroconvulsive shock, and fever (44), and are released in brain during stroke and seizures (45). 5-HT may potentially mediate some of these processes through receptor-generated arachidonic acid or eicosanoids.

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