5-HT induces an arachidonic acid-sensitive calcium influx in rat small intrapulmonary artery

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5-HT induces an arachidonic acid-sensitive calcium influx in rat small intrapulmonary artery. Am J Physiol Lung Cell Mol Physiol 286: L1228–L1236, 2004. First published January 29, 2004; 10.1152/ajplung.00265.2003.—5-Hydroxytryptamine (5-HT) is a potent pulmonary vasoconstrictor and contributes to hypoxic pulmonary vasoconstriction and pulmonary arterial hypertension. Small intrapulmonary vessels are very sensitive to hypoxia and play a major role for blood flow regulation in the lung. Thus we have investigated the mechanisms involved in the calcium signal to 5-HT in small intrapulmonary artery (IPA). Effects of 5-HT were examined in isolated IPA (external diameter <250 μm) from rat. Digital imaging with fura-PE3 was used to record intracellular calcium concentration ([Ca2+]i) and to follow external diameter of the vessels. 5-HT induced a sustained ([Ca2+]i) variation that was sensitive to the inhibitor of the 5-HT2A receptors, ketanserin, and insensitive to voltage-dependent L-type calcium channel blockers (nifedipine and nicardipine) or voltage-independent calcium channel antagonists (LOE-908, SKF-96365, and gadolinium). The calcium response to 5-HT was also not modified by a sarcoplasmic reticulum Ca2+/ATPase inhibitor (cyclopiazonic acid; CPA), which depletes intracellular calcium stores. CPA alone activated a capacitative calcium channel that was sensitive to LOE-908 and insensitive to SKF-96365 and gadolinium. The sustained calcium signal to 5-HT was partly blocked by inhibitors of arachidonic acid production (RHC-80267 and isotetrandrine) and mimicked by application of exogenous arachidonic acid. These results suggest that activation of a noncapacitative, arachidonic acid-sensitive, receptor-operated calcium channel contributes to 5-HT-induced sustained calcium increase in small IPA.

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many agonists [endothelin-1 (ET-1), ATP, and angiotensin II (ANG II)] induce calcium oscillations in rat pulmonary artery cells (13, 14). On the other hand, via the activation of the 5-HT2A receptors, mainly involved in rat pulmonary vasoconstriction (36), 5-HT can produce arachidonic acid through the activation of PLA2 in cell lines and neuronal cells (34). Therefore, we hypothesized that 5-HT would produce arachidonic acid that in turn could be involved in calcium signal.

We believe that increasing attention should be given to small IPA. Indeed, small IPA are very important for blood flow regulation in the pulmonary vascular bed under both physiological and pathological conditions. They constrict in response to hypoxia, whereas large conduit pulmonary arteries constrict and then dilate or do not respond to the same stimulus (3, 18).

Thus we investigated the effect of 5-HT on contraction and [Ca^{2+}] in rat small IPA. We designed an experimental protocol involving an integrated arterial model. Because 5-HT is a potent pulmonary vasoconstrictor and strongly involved in PAH, we explored the calcium sources possibly involved in the 5-HT-induced calcium signal and the role of arachidonic acid.

**METHODS**

**Tissue preparation.** Wistar male rats (8–10 wk) were stunned and then killed by cervical dislocation according to the animal care and use local committee (Comité d’éthique régional d’Aquitaine). Heart and lungs were removed and placed into Krebs-HEPES physiological solution. IPA with an external diameter of 228.6 ± 8.5 μm (n = 22) were isolated from the left lung. These vessels were cleaned of adventitial tissue under binocular control. Arteries were then cannulated at both ends with glass microprobes, secured with 10–0 nylon monofilament suture, and placed in a microvascular chamber (Living Systems, Burlington, VT). The vessels were maintained in a no-flow state and held at a constant transmural pressure of 10 mmHg. The length of the vessel was kept small (997.3 ± 111.5 μm; n = 10) to...
restrict the number of pulmonary branches to one branch at the maximum. A peristaltic pump and a pressure sensor were used to compensate any leak and maintain constant the transmural pressure. When intraluminal pressure was increased from 0 to 15 mmHg, the external diameter was increased by ~8% of the initial diameter when the pressure applied was 0 mmHg (Fig. 1C, n = 10). These results confirmed that the vessels were pressurized. The chamber was superfused at a rate of 2 ml/min with normal Krebs-HEPES solution gassed with air and maintained at 37°C.

Intracellular calcium and external arterial diameter recordings. An analog of fura 2, the fura-PE3-AM (40), was mixed with an equal volume of a 20% solution of pluronic acid in DMSO. Arteries were incubated in the microvascular chamber with 1 μM fura-PE3-AM for 90 min at 37°C in Krebs-HEPES solution. As previously mentioned (9, 16), 3 μM fluo 4-AM were used instead of fura-PE3 for experiments with LOE-908 because LOE-908 was found to disturb measurement of intracellular calcium by absorbing the light at excitation wavelengths for fura-PE3. The loading protocol was similar. The respective control calcium responses to 5-HT and KCl were also recorded from fluo 4-AM-loaded vessels. The chamber was then placed on the stage of an inverted epifluorescence microscope (Olympus IX70) equipped with a ×40, UApo/340–1.15 W water-immersion objective (Olympus). The source of excitation light was a xenon arc lamp (175 W), and excitation wavelengths were selected by a monochromator (Life Science Resources). Digital images were sampled at 12-bit resolution by a fast-scan, cooled charge-coupled device camera (CoolSNAP fx Monochrome, Photometrics). Fura-PE3 was alternately excited at 345 and 380 nm, and ratios of the resulting images (345/380) were produced every 20 s. Fluor 4 was excited at 494 nm, and images were produced every 20 s. Regions of interest were drawn on the vascular wall to determine calcium signal from smooth muscle cells (Fig. 1D). To check that fura-PE3 loading of the endothelium was absent, we verified that vasorelaxing agents, such as ACh, decreased intracellular calcium in the smooth muscle (Fig. 2, A and B). The vessels were preconstricted with either high-K⁺ solution (40 mM KCl) or 10 mM phenylephrine. ACh-induced decrease in calcium confirmed that the calcium signal recorded was specific to the smooth muscle. Moreover, as previously demonstrated in other vascular preparations (20), we verified the absence of an endothelial calcium signal by stimulating the microvessels with 0.1, 1, and 10 μM ACh (n = 5). All the images were background subtracted. All imaging was controlled by Universal Imaging software, including metafluor and metamorph, and operated on a pentium III PC.

Fig. 2. Selective fura-PE3 loading in the smooth muscle cells and viability of the preparation. A: typical trace showing simultaneous recording of intracellular calcium (top) and contraction (bottom) induced by high-K⁺ solution (40 mM) in the presence or not of 1 and 10 μM ACh (horizontal bars). B: mean data ± SE of similar experiments when the vessels were preconstricted with 40 mM high-K⁺ solution (a, open bar, n = 5) or 10 μM phenylephrine (Phe; b, open bar, n = 6). ACh (0.1, 1, and 10 μM) was applied in the presence of the preconstricting agent (a and b, solid, gray, and light gray bars, respectively). Data are expressed as a percentage of the calcium response induced by the preconstricting agent (KCl in Ba and Phe in Bb). *, ** Significant difference vs. the control calcium response to 40 mM KCl or 10 μM Phe when P < 0.05 and 0.01, respectively. Viability of the preparation was tested by recording the contraction to various contracting agents, namely high-K⁺ solution (D, 80 mM KCl, open bar, n = 10), 10 μM 5-HT (D, solid bar, n = 10), 10 μM angiotensin II (ANG II; C and D, gray bar, n = 5), and 0.1 μM endothelin-1 (ET-1; D, light gray bar, n = 7). Data are means ± SE in D and are expressed as a percentage of the initial diameter. C: typical trace of a simultaneous recording of the intracellular calcium (top) and contraction (bottom) when 10 μM ANG II was bath applied (horizontal bar). For typical traces, calcium data were expressed by the ratio of 345/380 signals, and contraction data were expressed by the percentage of the initial diameter (A and C).
Because of the diffuse fluorescence on pictures at a single wavelength, three lines were drawn on the ratio picture of the resulting digital images (345/380) to determine external diameter of the vessels. The mean number of fluorescent pixels crossing those lines was produced by metaphorm software and used for external diameter recording (Fig. 1E).

**Experimental protocol and controls.** High-K\(^+\) solution (80 mM) was first bath applied and then washed out before perfusion of 10 \(\mu\)M 5-HT. 5-HT (10 \(\mu\)M) was then washed out and drugs were superfused during 30 min before a second dose of 10 \(\mu\)M 5-HT was added to the bath still in the presence of the drugs. Only one experimental condition was tested on each vessel. The first response to 5-HT was considered as the control response. Time-matched controls were performed: drug perfusion was replaced with normal Krebs-HEPES superfusion, and the second calcium and contractile responses to 5-HT were not significantly different from the first responses \((n = 8)\). To determine the viability of the vessels, we bath applied several potent vasoconstrictors well known for pulmonary artery \((10 \mu\)M ANG II and 0.1 \(\mu\)M ET-1, Fig. 2, C and D). ANG II and ET-1 induced a contractile response with an amplitude that was, respectively, 76.7 \(\pm\) 3.7 and 75.2 \(\pm\) 3.2% of the initial external diameter \((n = 5–7)\).

**Solutions.** Krebs-HEPES solution contained in mM: 118.4 NaCl, 4.7 KCl, 2 CaCl\(_2\), 1.2 MgSO\(_4\), 4 NaHCO\(_3\), 1.2 KH\(_2\)PO\(_4\), 10 HEPES, and 6 d-glucose, pH 7.4, with NaOH. For calcium-free solution, calcium was removed from Krebs-HEPES solution, and 0.4 mM EGTA was added. For high-K\(^+\) solution (80 mM), 80 mM NaCl was substituted for the same amount of KCl in Krebs solution.

**Data analysis and statistics.** The concentration-response curves were fitted with a sigmoidal equation to determine the concentration of 5-HT inducing half of the maximal effect \((EC_{50})\). Data points were fitted by using Origin 6 software (Microcal).

All results are expressed as means \(\pm\) SE. Because on each microvessel the first response to 5-HT was considered as the control response \(\text{(see Experimental protocol and controls)}\), a paired \(t\)-test was used to determine significance. It was concluded significant when \(P < 0.05\). \(N\) represents the number of arteries.

**Chemicals and drugs.** General salts were from Merck Eurolab (Fontenay-sous-Bois, France). Nitrendipine was from Calbiochem (Meudon, France), isetradtrindine and RHC-80267 were from Biomol (Le Perray-en-Yvelines, France), and ketanserin was from Tocris (Illkirch, France). Fluo 4-AM and pluronic acid were purchased from Molecular Probes (Leiden, Netherlands). LOE-908 was a kind gift from Boehringer Ingelheim Pharma (Biberach, Germany). All other compounds were purchased from Sigma (St Quentin Fallavier, France). All drugs were dissolved in water except cyclopiazonic acid (CPA), nicardipine, nitrendipine, isetradtrindine, RHC-80267, fluripe3-AM, and fluo 4-AM, which were dissolved in 100% DMSO such that the final concentration was \(\leq 0.1\%\) and had no effect on the calcium and the contractile responses to 5-HT.

**RESULTS**

**Effect of 5-HT on \([Ca^{2+}]_i\), and contraction of rat IPA.** Bath application of 5-HT induced a concentration-dependent intracellular calcium increase and vasoconstriction \((\text{Fig. 3, A and B})\). The maximum effect of 5-HT occurred at 30 \(\mu\)M for the calcium response and the vasoconstriction, and the EC\(_{50}\) were similar \((1.4 \pm 0.4 \mu\)M vs. 2.4 \(\pm\) 1 \(\mu\)M, respectively). 5-HT \((10 \mu\)M) induced a sustained calcium signal that slightly preceded a sustained contraction \((\text{Fig. 3C})\), indicating that the calcium signal recorded is not an artifact linked to the movement of the vessel. An antagonist of the 5-HT\(_{2A}\) receptors, 0.1 \(\mu\)M ketanserin, completely inhibited the calcium response to 10 \(\mu\)M 5-HT \((n = 4)\), demonstrating that the effect of 5-HT is a receptor-mediated effect.
Role of L-type calcium channels in 5-HT-induced calcium response of rat IPA. We then focused on the sources of calcium involved in the calcium response induced by 10 μM 5-HT. Because voltage-gated calcium channels are very important in the presence of L-type calcium channel antagonists. After 30 min of superfusion of 1 μM nitrendipine, the calcium response to high-K+ solution (80 mM) was reduced by 86%, whereas the calcium response to 5-HT was not significantly modified (Fig. 4, A and B). The same experiment was performed in the presence of another L-type calcium channel blocker, 1 μM nicardipine. The response to 80 mM KCl was reduced by 88%, but the calcium response to 10 μM 5-HT was not significantly affected (Fig. 4C).

According to these results, although voltage-gated L-type calcium channel blockers were present, they could not explain the calcium response to 5-HT, so we addressed the hypothesis of the involvement of intracellular calcium sources.

Role of intracellular calcium stores in 5-HT-induced calcium response of rat IPA. Intracellular calcium stores were depleted by incubating the vessels with a specific SR Ca2+-ATPase (SERCA) blocker (10 μM CPA) for 30 min in calcium-free solution (Fig. 5A). A transient increase in intracellular calcium was observed in calcium-free solution when 10 μM CPA was added to the bath, thus confirming depletion of the SR (data not shown). Calcium (2 mM) was then introduced into the bath, inducing a sustained calcium increase (Fig. 5Aa). This sustained calcium increase was observed in 68% of the vessels tested (21 out of 31 vessels). These results indicated the presence of a store-operated calcium channel (SOCC), also named a capacitative calcium entry (CCE), in isolated small IPA. 5-HT (10 μM) was then applied and induced an additive sustained calcium increase (Fig. 5Ab) that was not significantly different from the one observed in control conditions in the same vessel (Fig. 5B). All these results demonstrate the activation of a nonvoltage-gated calcium influx by 5-HT.

Effect of nonvoltage-gated calcium channel inhibitors. Because 68% of the vessels demonstrated the presence of SOCC or CCE in isolated small IPA, we tested the effect of different antagonists of nonvoltage-gated calcium channels, such as 10 μM LOE-908, 10 μM SKF-96365, and 10 μM gadolinium, on CCE and 5-HT-induced calcium increase in the presence of 10 μM CPA and 1 μM nitrendipine. CCE was significantly inhibited by LOE-908 (n = 5; Fig. 6Aa) but was not blocked by SKF-96365 (n = 6) or gadolinium (n = 5; Fig. 6, Ba and Ca). 5-HT-induced sustained calcium increase was not significantly modified by LOE-908 (n = 8) or SKF-96365 (n = 7) or gadolinium (n = 7; Fig. 6, Ab, Bb, and Cb).

Role of arachidonic acid in the activation of 5-HT-activated noncapacitative calcium influx. To determine the role of arachidonic acid, we inhibited the main pathways of arachidonic acid production (see Introduction). In normal Krebs-HEPES solution, 50 μM RHC-80267, a selective antagonist of DAG lipase (21), significantly inhibited the calcium response to 10 μM 5-HT by 65% (n = 8; Fig. 7Aa). Isotretinoin (10 μM), a blocker of the G protein-activated cPLA2 (37), significantly reduced the calcium response to 10 μM 5-HT by 48% (n = 5; Fig. 7Ab). When those blockers were applied together at once, their effects were not additive since they had significantly blocked the calcium signal to 5-HT by 49% (n = 9; Fig. 7Ac). To further demonstrate the role of arachidonic acid, we directly bath applied arachidonic acid at 1, 3, 10, 30, and 100 μM. In three of six vessels, arachidonic acid induced a dose-dependent calcium increase with a maximal effect at 10 μM (Fig. 7Ba). The maximal calcium response to arachidonic acid has a similar amplitude (39% of the control calcium response induced by 5-HT) to the arachidonic acid-sensitive part of the calcium signal to 5-HT (Fig. 7A). Finally, 10 μM 5-HT, bath applied when 100 μM arachidonic acid achieved its maximal effect, induced an additional calcium response, which had an
Fig. 5. Role of the sarcoplasmic reticulum in 5-HT-induced calcium increase. A: in the presence of 1 μM nitrendipine and 10 μM cyclosporine acid (CPA), changing the bath solution from calcium free to 2 mM calcium induced an intracellular calcium increase (a), and addition of 10 μM 5-HT activated an additive calcium increase (b). B: mean ± SE of the 5-HT-induced sustained calcium response in the absence or in the presence of nitrendipine and CPA (open and solid bars, respectively). The number of vessels used are noted in parentheses. Data are expressed by the ratio 345/380 signals for a typical trace and by the amplitude of the calcium signal (Δ ratio 345/380 nm) for the mean data.

Fig. 6. Effect of voltage-independent calcium channel antagonists on 5-HT-induced calcium signal. In the presence of 10 μM CPA and 1 μM nitrendipine, when changing the solution from calcium free to 2 mM calcium, calcium influx was reduced by 10 μM LOE-908 (Aa) but not by 10 μM SKF-96365 (Ba) and 10 μM gadolinium (Gd³⁺; Ca). In the same experimental conditions, the additive 5-HT-induced calcium increase was not modified by LOE-908 (Ab), SKF-96365 (Bb), and Gd³⁺ (Cb). Data are shown in the absence of any voltage-independent calcium channel antagonists (open bars, A–C) and in the presence of LOE-908 (solid bar, A), SKF-96365 (gray bar, B), or Gd³⁺ (light gray bar, C). The number of vessels used are noted in parentheses. *Significant inhibition when P < 0.05. Data are expressed by the amplitude of the calcium signal (Δ ratio 345/380 nm). For LOE-908, data are expressed by the Δ fluorescence recorded at the excitation wavelength for fluo 4 (494 nm).
amplitude of 48% of the control calcium signal to 5-HT. The total calcium increase induced by 100 \mu M arachidonic acid and 10 \mu M 5-HT was not significantly different from the control calcium response to 5-HT (Fig. 7Bb).

DISCUSSION

The experiments demonstrated that 5-HT increased [Ca^{2+}]_{i} and constricted the rat intrapulmonary microvessels (external diameter of \sim 250 \mu m) with an EC_{50} of \sim 2 \mu M. Even though a capacitative calcium influx was identified, the calcium signal induced by 5-HT was characterized as a noncapacitative receptor-operated calcium influx. This sustained calcium increase was also independent of L-type voltage-gated calcium channels and SR but dependent on arachidonic acid production.

Two distinct calcium sources have been described in smooth muscle: intracellular calcium sources (mainly SR and mitochondria) and extracellular calcium sources that will induce [Ca^{2+}]_{i} increase by activating calcium channels in the plasma membrane (mainly voltage-gated, capacitative, and noncapacitative voltage-independent calcium channels) (25). Because CPA, a blocker of the SERCA in pulmonary artery (12), did not affect the 5-HT-induced sustained calcium signal in calcium-containing solution, we excluded the intracellular calcium sources. However, Yuan et al. (41) described an intracellular calcium signal to 5-HT in rat pulmonary artery (see Introduction). Our experimental conditions may have failed to detect the transient calcium signal due to SR because ratio images were captured every 20 s. Relative contributions of extracellular calcium and calcium stores according to vessel sizes have already been previously demonstrated, predicting a major importance of calcium fluxes for small vessels (10, 22). Indeed, contraction in arterioles has often been shown to be sensitive to L-type voltage-gated calcium channel inhibitors (7, 30). Although the inhibition of the calcium response to 80 mM KCl by nicardipine or nitrendipine indicated the presence of L-type voltage-gated calcium channels in isolated small IPA, our experiments demonstrated no involvement of those channels in 5-HT-induced sustained calcium increase.

CPA experiments revealed the presence of a CCE, activated by store depletion. Such calcium entry had already been shown in main conduit and small IPA (12, 33, 35). The molecular identity of those channels is still not proved, although hypothesis raises toward the involvement of transient receptor potential (TRP) proteins (25). So far, there are no specific pharmacological tools to inhibit CCE, but many substances, such as lanthanides, gadolinium, LOE-908, SKF-96365, and trifluoromethylphenylimidazole (8, 25, 33, 42) have been used to

Fig. 7. Role of arachidonic acid in 5-HT-induced calcium increase. In the presence of a diacylglycerol lipase inhibitor, 50 \mu M RHC-80267 (Aa, gray bar), or a G protein-activated phospholipase A_{2} antagonist, 10 \mu M isotetrandrine (Ab, light gray bar), or both (Ac, solid bar), the calcium signal induced by 5-HT is similarly reduced by about one-half. Arachidonic acid produced a dose-dependent calcium increase (Ba). In addition to the calcium response to 100 \mu M arachidonic acid, 10 \mu M 5-HT induced a further calcium increase that was additive to the response to arachidonic acid (Bb, solid bar). Open bars indicate the control responses to 10 \mu M 5-HT. The number of vessels used are noted in parentheses. *Significant inhibition when \( P < 0.05 \). Data are expressed by the amplitude of the calcium signal (\( \Delta \) ratio 345/380 nm) for the mean data.
block such nonselective cation channels, and the effects of these compounds were as varied as the number of studies. For instance, CCE has been commonly shown to be insensitive to LOE-908 and inhibited by SKF-96365 in various cells, including vascular smooth muscle cells (16, 33, 42). However, one study described an inhibition of CCE by LOE-908 in human endothelial cells (8), confirming variations according to tissues and/or species. Unfortunately, these authors did not investigate the effect of SKF-96365 on the CCE they observed.

It has been suggested that this channel would be a heteromultimer composed of subunits encoded in TRP genes, which could explain the various effects of the pharmacological tools used. In our experimental conditions, in contrast to what has been previously shown in vascular smooth muscle cells, CCE was sensitive to and blocked by 10 μM LOE-908 but insensitive to 10 μM SKF-96365 or 10 μM gadolinium. Thus this is the first time that such sensitivity to those blockers was shown in small IPA, suggesting a specific capacitative channel. Although CCE appeared to be present in small IPA, our study excluded its implication in the 5-HT-induced sustained calcium increase. Several lines of evidence strengthen this statement: 1) 5-HT-induced sustained calcium increase was additive to CCE, 2) the amplitude of that 5-HT-induced sustained calcium increase was similar in the presence and in the absence of CPA, and 3) the sensitivity to LOE-908 was different for CCE and 5-HT-induced sustained calcium increase, suggesting differences in the molecular identity of both channels.

These results demonstrated that 5-HT-induced sustained calcium increase was linked to the activation of a noncapacitative receptor-operated calcium channel. Such a channel has recently been identified in various cell lines, including the vascular smooth muscle cell line A7r5 (5, 29). This channel (ARC) is activated by arachidonic acid and shows many similarities with the channel activated by 10 μM 5-HT in rat small IPA. First, ARC is activated by a receptor, and the current passing through this channel (I_{ARC}) is a noncapacitative current additive to CCE, activated when CCE is fully activated (27). ARC is permeable to calcium (28), blocked by 10 μM isotetrandrine in HEK-293 cells (29, 37), sensitive to 8–50 μM exogenous arachidonic acid (5, 28), inhibited by 50 μM RHC-80267, and activated by 50 μM 5-HT in A7r5 (5, 31). However, in contrast to what we observed, 30 μM LOE-908 blocks I_{ARC}, but not CCE, in A7r5 (31). Only three out of six vessels responded to arachidonic acid, but it is well known that exogenous arachidonic acid has different activity than endogenous arachidonic acid because of the presence of fatty acid-binding proteins (4). Thus this is the first time that a noncapacitative arachidonic acid-activated, calcium-permeable channel activated by 5-HT is shown in vascular tissue such as rat small IPA.

From our experiments, we can speculate on the mechanisms involved in arachidonic acid production and in the calcium signal to 10 μM 5-HT. RHC-80267 (50 μM) is a very specific blocker of the DAG lipase and does not modify phospholipase C (PLC) and PLAs activities (21). Thus the nonadditive effect of RHC and isotetrandrine applied simultaneously cannot be explained by a nonspecific effect of RHC on PLAs. Isotetrandrine (10 μM), a blocker of a G protein-activated PLAs, has also been shown to be specific, and although it stimulates PLAs through the Gs-subunit of Gs protein in pancreatic acini or the α-subunit of G protein in peritoneal mast cells, 10 μM isotetrandrine does not affect PLC activity and DAG production (1, 37, 39). However, since the effect of isotetrandrine on G protein appeared different according to cell type, we can hypothesize that isotetrandrine decreases DAG production through its effect on G protein in rat pulmonary arteries. This would imply DAG production as the major source of arachidonic acid production, a mechanism very close to what has been observed in A7r5 cells (5, 31). 5-HT would activate PLC-β, inducing IP3 production, which would be responsible for a possible transient calcium increase. DAG production would be degraded into arachidonic acid by DAG lipase, and arachidonic acid would stimulate directly or indirectly a calcium-permeable channel responsible for the sustained calcium signal.

In summary, our results demonstrate that CCE was present and a noncapacitative receptor-operated calcium channel sensitive to arachidonic acid contributed to the calcium response to 5-HT. Thus this is the first time the mechanisms involved in the calcium signal to 5-HT in small IPA are elucidated.

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