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Signalling pathways involved in the contractile response to 5-HT in the human pulmonary artery

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ABSTRACT: Serotonin (5-hydroxytryptamine; 5-HT) is a potent pulmonary vasoconstrictor and mitogenic agent whose plasma level is increased in pulmonary hypertensive patients. Thus, we explored the signalling pathways involved in the contractile response to 5-HT in human pulmonary arteries (HPAs).

Intact and β-escin permeabilised rings from HPAs mounted in an organ bath system were used to assess both tension and myofilament Ca^{2+}-sensitisation. Microspectrofluorimetry was used for intracellular Ca^{2+} recordings in cultured HPA smooth muscle cells.

Voltage-operated Ca^{2+} channel blockers (nitrendipine and nifedipine) partially reduced the contraction to 5-HT. Thapsigargin or cyclopiazonic acid (CPA), known to deplete sarcoplasmic reticulum Ca^{2+} stores, also partially inhibited the contraction, whereas removal of extracellular Ca^{2+} under these conditions further inhibited the contraction. Changing from Ca^{2+}-free to Ca^{2+} containing solution, in the presence of nitrendipine and CPA, a protocol known to stimulate store-operated Ca^{2+} channels, induced HPA contractions that were blocked by nickel. Nickel or gadolinium also reduced the contraction to 5-HT. Finally, 5-HT increased intracellular Ca^{2+} responses in cultured HPA smooth muscle cells and myofilament Ca^{2+}-sensitisation in HPA rings.

Collectively, these results indicate that voltage-operated and voltage-independent Ca^{2+} channels, as well as Ca^{2+} release and myofilament Ca^{2+}-sensitisation, participate in 5-HT-induced contraction in HPAs.

KEYWORDS: Calcium, contraction, human pulmonary artery, myofilament calcium sensitivity, 5-HT

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Serotonin (5-hydroxytryptamine; 5-HT) is mainly stored in the platelets but is also locally released in the lung by pulmonary neuroendocrine cells, neuroepithelial bodies and pulmonary arterial endothelial cells [1–3]. 5-HT is a potent pulmonary vasoconstrictor whose high circulating concentration is clinically associated with pulmonary arterial hypertension (PAH), an often fatal disease. In animal models of PAH, 5-HT-induced hyperreactivity and mitogenic effects have been reported in pulmonary arteries [4, 5]. In human pulmonary arteries (HPAs), while numerous studies have explored the role of 5-HT in vascular remodelling associated with smooth muscle hyperplasia [6, 7], few studies have been performed on the contractile effect of 5-HT [8, 9]. Nevertheless, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2B} receptors have been detected in HPA, and the contractile effect of 5-HT appeared to be mainly mediated by the 5-HT_{1B} receptors and also by the 5-HT_{2A} receptors [6, 8–12]. Despite the fact that pulmonary arterial vasoconstriction is an important early component of PAH, the current knowledge about transduction pathways involved in the 5-HT-induced vasoreactivity remains incomplete for HPAs.

Owing to the low availability of human tissue, we previously studied the contractile response to 5-HT in rat intrapulmonary arteries and we demonstrated that there were regional differences in 5-HT-induced contraction [13]. Since calcium is essential for smooth muscle contraction, we addressed the relative contribution of calcium pools involved in the vasoreactivity to 5-HT. In small vessels, 5-HT activates voltage-independent calcium channels to a larger extent than it does voltage-dependent calcium channels (L-type calcium channels) [5, 13–15]. Calcium release from intracellular calcium stores (sarcoplasmic reticulum) also contributes to 5-HT-induced contraction in rat pulmonary arteries [13–15]. Studies from other groups have been performed on calcium signalling and ion channels in cultured human

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pulmonary arterial smooth muscle cells (PASMCs) but none of these studies have linked these channels and/or signalling pathways to the effect of 5-HT on PASMCs. In human PASMCs, various channels have been detected, such as potassium, chloride and calcium channels, including L-type voltage-gated Ca^{2+} channels, and receptor-operated and store-operated Ca^{2+} channels [16]. Taking into account the transduction pathways activated by 5-HT in rat intrapulmonary arteries, most of these channels could be involved in the signalling associated to 5-HT in HPAs.

Aside from isolated PASMCs, there is considerable interest for more integrated models that allow the study of cells within their microenvironment. In addition, differences have been observed in the *in vitro* pulmonary arterial vasoreactivity to 5-HT between humans and other mammals [17]. Consequently, owing to the critical role of 5-HT in pulmonary vascular disease, study of vasoreactivity to 5-HT in HPAs is clinically relevant. In the present study, we thus investigated the contractile response to 5-HT in distal human pulmonary arterial rings and the associated signal transduction pathways, including calcium signalling and calcium sensitivity of the contractile apparatus.

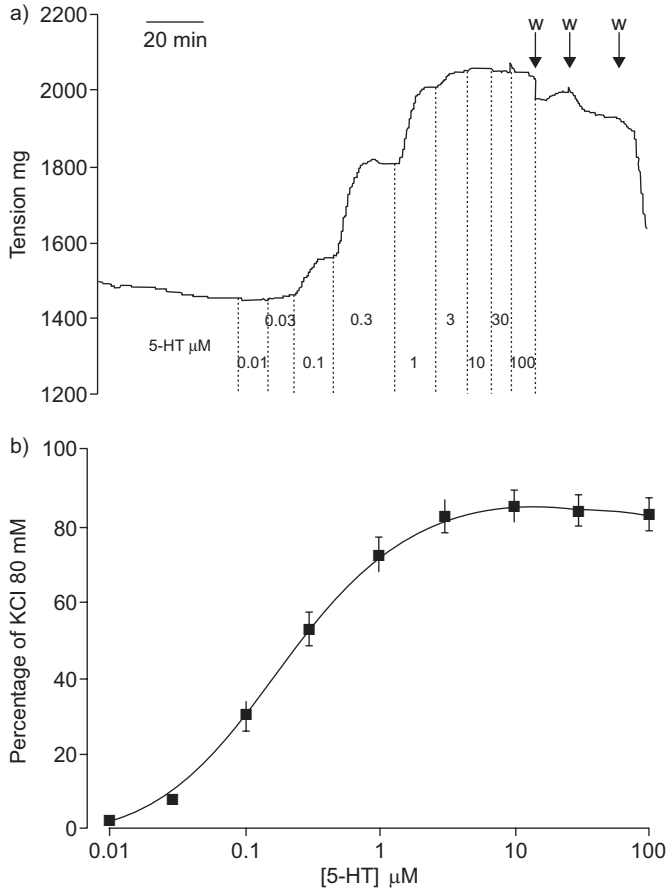

FIGURE 1. Cumulative concentration–response curve to serotonin (5-hydroxytryptamine; 5-HT) (0.01–100 μM). a) Typical trace of a plot of tension against time and as a function of cumulative 5-HT concentrations. W: washout. b) Mean concentration–response curve to 5-HT. Data are presented as mean±SEM for 99 rings and 40 patients, and are expressed as a percentage of the high potassium solution (80 mM)-induced response. The EC_{50} value (concentration of agonist which produces half maximal tension) for 5-HT on human pulmonary arteries was 0.44±0.05 μM.

METHODS

HPA preparation

The study was approved by the ethics committee of our institution, and informed consent was obtained from each subject. The investigation conformed to the principles outlined in the Declaration of Helsinki. The population under study comprised 67 patients, including 42 males and 25 females with mean±SD age 62±9 yrs (range 45–78 yrs). Oxygen tension from pre-operative blood samples was 84±1.3 mmHg (range 71.8–98.3 mmHg). Human lung arteries were obtained from patients undergoing surgery for lung carcinoma. After lobectomy and transport in sterile physiological saline solution, lung samples, distant from the malignant lesion, were dissected by the pathologist. The absence of tumoural infiltration was retrospectively established in all tissue sections by the pathological analysis. Tissue samples were immediately placed in Krebs–HEPES solution containing: 118.4 mM NaCl, 4.7 mM KCl, 2 mM $CaCl_2$, 4 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 10 mM HEPES and 6 mM glucose, previously bubbled with 21% O_2 (pH 7.4) at 22°C. After removal of the connective tissues, arterial rings (inner diameter 0.5–4 mm) were used as fresh tissue or cultured in individual wells of 24-well culture plates containing DMEM-F12 culture medium (1 mL per well) supplemented with 0.3% penicillin (100 IU·mL^{-1}) and streptomycin (0.1 mg per well). Culture plates were placed in a humidified incubator at 37°C, under 21% O_2 and 5% CO_2. Some rings were maintained in culture for 1–2 days. The contractile responses to 5-HT were not modified under those conditions.

Isometric tension measurements

Arterial rings were mounted in isolated organ baths, containing Krebs–HEPES solution at 37°C and bubbled continuously with 21% O_2. As previously determined, an initial load of 0.8–1.5 g was applied to arterial rings, according to arterial diameter. Tissues were allowed to equilibrate for 1 h in Krebs–HEPES solution and washed out every 15 min. At the outset of each experiment, K^+-rich (80 mM) solution was applied in order to obtain a reference contraction, which was used to normalise subsequent contractile responses. Contractile properties to 5-HT were tested by constructing a cumulative concentration–response curve (CCRC) to 5-HT (10 nM to 100 μM). When indicated, drugs were preincubated for 30 min, and then CCRC to 5-HT was determined in the presence of the drug. Endothelial function was tested on each ring by relaxation with 10 μM carbamylcholine or 5 μM A23187 on 0.3 μM phenylephrine-induced preconstricted pulmonary arterial rings. In our hands, in both laboratories (in France and Canada), we did not observe any relaxation to carbamylcholine or A23187, indicating that the properties of the contraction to 5-HT in the present study were related to the smooth muscle. Calcium-free bath solution was prepared by substituting 2 mM $CaCl_2$ by 0.4 mM EGTA in Krebs–HEPES solution. As previously described, passive and active tensions were assessed using transducer systems coupled to IOX software (EMKA Technologies, Paris, France) or Polyview software (Grass Astro Med., West Warwick, RI, USA) to facilitate data acquisition and analysis [18, 19].

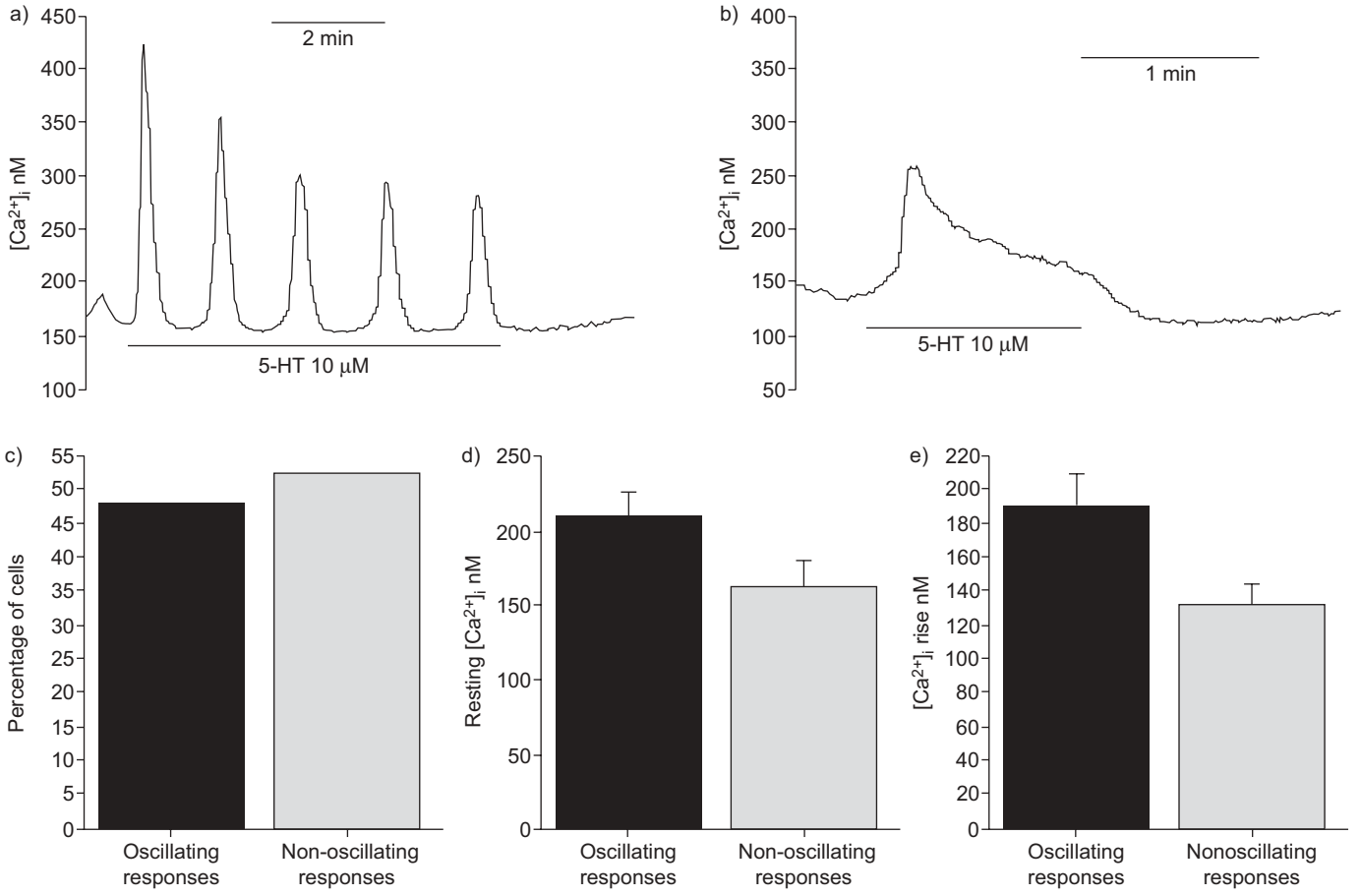

FIGURE 2. Effect of 10 μM serotonin (5-hydroxytryptamine; 5-HT) on the intracellular calcium concentration ($[Ca^{2+}]_i$) in cultured smooth muscle cells (SMCs) from human pulmonary arteries. a and b) Typical traces showing various profiles of the calcium responses to 5-HT. c) The percentage of cells observed for each profile. d) The resting $[Ca^{2+}]_i$ values and e) the amplitude of the calcium rises in response to 5-HT. n=24 SMCs tested for oscillating calcium responses and n=22 SMCs tested for non-oscillating calcium responses. Data are presented as mean±SEM.

Cell culture

As previously described [13], HPAs were initially cut into several pieces (1–2 mm^2) and placed at the bottom of individual wells of six-well culture plates containing culture medium (DMEM–HEPES supplemented with 1% penicillin–streptomycin, 1% sodium pyruvate and 1% nonessential amino acids) enriched with 10% fetal calf serum. Isolated cells with trypsin-EDTA (one or two passages) were plated on glass cover slips. PASMCs were growth-arrested for 48 h by using serum-free culture medium supplemented with 1% insulin–transferrin–selenium before they were used for immunofluorescent labelling or intracellular calcium measurements. Immunostaining with the monoclonal antibody anti-α-smooth muscle actin and the polyclonal antibody anti-calponin 1/2/3 was positive for all cells demonstrating the presence of a population of smooth muscle cells (data not shown).

Intracellular calcium measurements

As previously described [20], isolated cells were loaded with 2 μM indo-1 penta-acetoxymethylester (indo-1/AM) in Krebs–HEPES solution at room temperature for 40 min and then washed. Briefly, the cells were placed on the stage of an inverted epifluorescence microscope (Nikon Diaphot; Nikon, Champigny sur Marne, France) equipped with a ×40 oil immersion fluorescence objective. Loaded cells were excited at 355 nm and the emitted fluorescence signal was collected at 405 and 480 nm by two separate photometers (P100; Nikon). The fluorescence ratio (F405/F480) was calculated and recorded online as a voltage signal. The intracellular free calcium concentration ($[Ca^{2+}]_i$) was estimated from the F405/F480 after Ca^{2+} calibration for indo-1/AM determined within cells as previously described [20].

Permeabilisation with β-escin

Before permeabilisation, we assessed the viability and reactivity of the tissue by recording the contraction induced by high potassium (80 mM) and 5-HT (10 μM) in normal Krebs–HEPES solution. The ring was then incubated for 20 min in low Ca^{2+} relaxing solution containing: 87 mM KCl, 5.1 mM $MgCl_2$, 5.2 mM NaATP, 10 mM creatine phosphate, 2 mM EGTA and 30 mM PIPES, brought to a pH of 7.2 with KOH at 23°C, followed by treatment with 50 μM β-escin in relaxing solution for 35 min at 23°C. Ca^{2+} stores were depleted by the addition of 10 μM A23187. The arterial ring was then washed several times with fresh relaxing solution containing 5 mM EGTA. Tension developed by the permeabilised tissue was measured in activating solutions containing 5 mM EGTA, 1 μM calmodulin and specified amount of $CaCl_2$ to yield the desired free Ca^{2+}

concentration, (pCa= -log[Ca^{2+}]). Step increases in free Ca^{2+} from pCa 9 to pCa 6 were used to induce reproducible tension responses, indicating a successful permeabilisation of the tissue under these conditions, as previously described [19]. The arterial ring was challenged with pCa 6 before the addition of 10 μM 5-HT and 10 μM guanosine 5′-O-(γ-thio)triphosphate to the bath.

Drugs and chemical reagents

All salts were diluted in distilled water except A23187, cyclopiazonic acid (CPA), indo-1/AM, nitrendipine, nifedipine, SB204741 and thapsigargin (TG), which were dissolved in dimethylsulfoxide (DMSO). The maximal concentration of DMSO was <0.1%, and had no effect on the calcium and mechanical responses of HPAs.

Data analysis and statistics

Results are expressed as mean±SEM; n indicates the number of rings or cells used and N indicates the number of patients for each set of experiment. CCRC to agonists without drugs (control) were performed on each patient. Statistical analyses were performed using unpaired t-tests, as well as ANOVA for global comparisons of the curves. Values of $p<0.05$ were considered significant. Data curve fittings were performed using Origin 6 software (Microcal, Paris, France). CCRC to agonists were fitted to the logistic equation:

$$T=((T_0-T_{max})/(1+(X/EC_{50})^p))+T_{max}$$

where T, T_{max} and T_0 are, respectively, the amplitude of tension developed and the relative maximum and minimal tensions for a given agonist concentration normalised to the 80 mM KCl responses, X is the concentration of agonist used, EC_{50} is the concentration of agonist which produces half maximal tension, and p is the slope of the curve.

RESULTS

Contractile and calcium responses to 5-HT in HPAs

As shown in figure 1, 5-HT induced a concentration-dependent contraction on HPA rings with a maximal contraction for 5-HT 10 μM and an EC_{50} value of 0.44±0.05 μM (n=99, N=40).

We then studied the effect of 5-HT on $[Ca^{2+}]_i$ in isolated PASMCs from HPA. In cultured PASMCs from the same segment of artery, 5-HT (10 μM) increased $[Ca^{2+}]_i$ with various profiles characterised by oscillations (47.8% of the cells) or a transient phase followed by a sustained phase (52.2% of the cells), the relative amplitude of each component of the calcium response being variable (fig. 2a–c; n=46, N=3). Whatever the

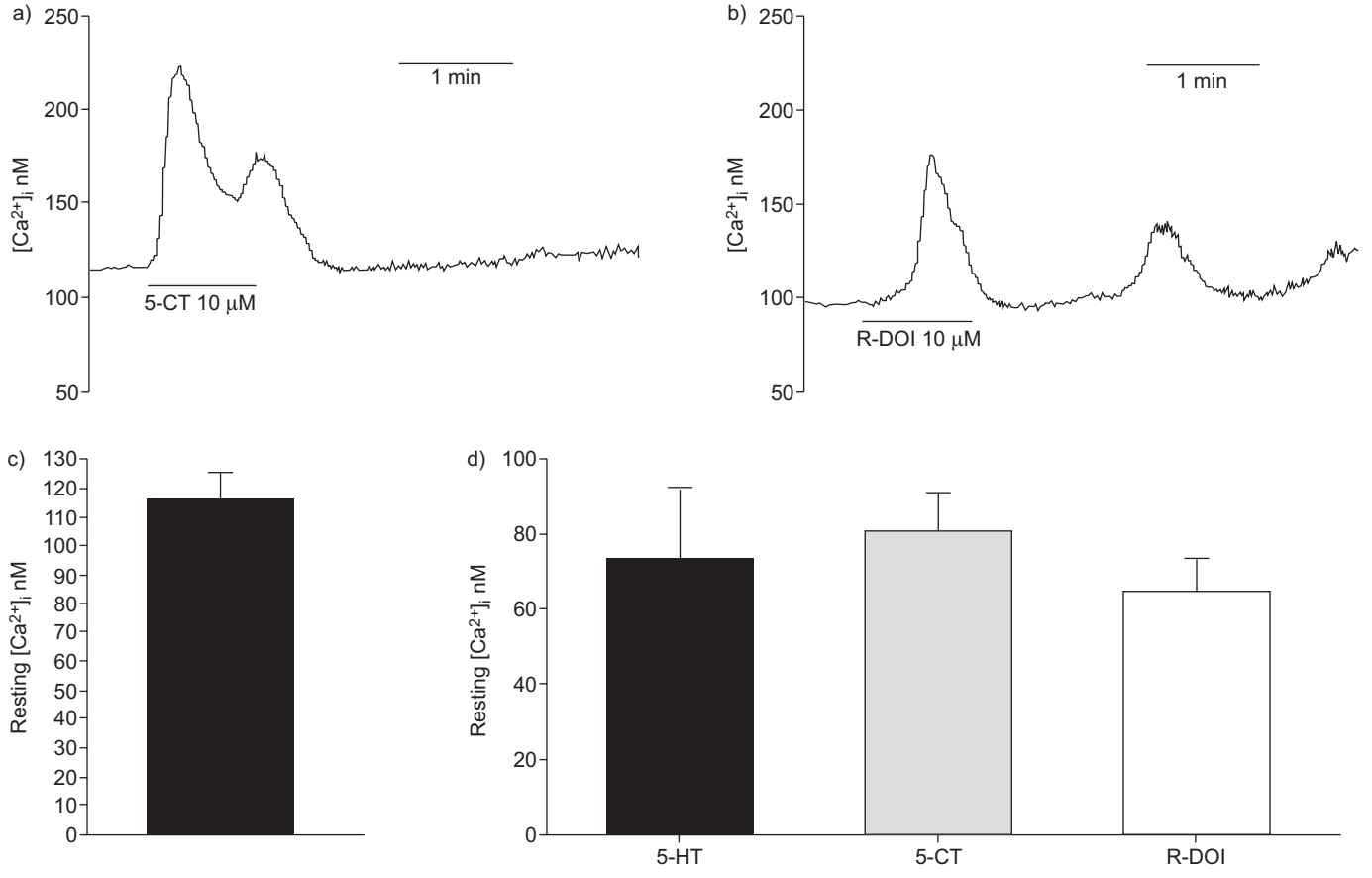

FIGURE 3. Effect of 5-HT_1 and 5-HT_2 receptors agonists on the intracellular calcium concentration in cultured smooth muscle cells (SMCs) from human pulmonary arteries. Typical traces showing the calcium responses to a) 5-carboxamidotryptamine (5-CT), a 5-HT_1 receptor agonist, and to b) (R)(-)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (R-DOI), a 5-HT_2 receptor agonist. c) Resting intracellular free calcium concentration ($[Ca^{2+}]_i$) values (n=52 SMC tested). d) The amplitude of the calcium rises in response to serotonin (5-hydroxytryptamine; 5-HT) (n=7), 5-CT (n=22) and R-DOI (n=23) (all 10 μM). Data are presented as mean±SEM.

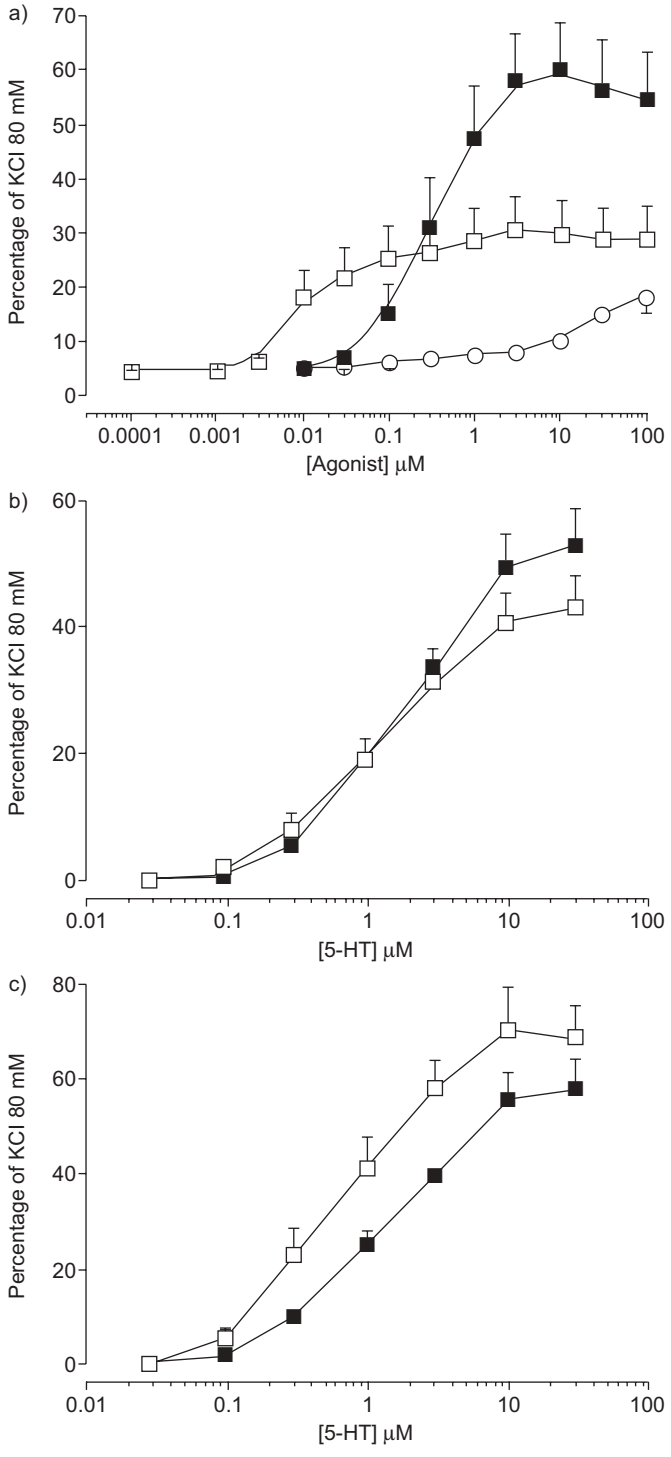

FIGURE 4. Asssessment of 5-HT_1 and 5-HT_2 receptors, as well as serotonin (5-hydroxytryptamine; 5-HT) transporter, on the cumulative concentration–response curve (CCRC) to 5-HT in human pulmonary arteries. a) The CCRC to 5-HT (■), 5-carboxamidotryptamine (□), a 5-HT_1 receptor agonist, and (R)(-)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (○), a 5-HT_2 receptor agonist. b) The effect of 1 μM SB204741, a 5-HT_{2B} receptor antagonist, on the CCRC to 5-HT (n=18, N=3). ■: control; □: SB204741. c) The effect of 1 μM citalopram, an antagonist of the 5-HT transporter, on the CCRC to 5-HT (n=8, N=3). ■: control; □: citalopram. Data are presented as mean±SEM and contraction is expressed as a percentage of the K^+-rich (80 mM) solution-induced response.

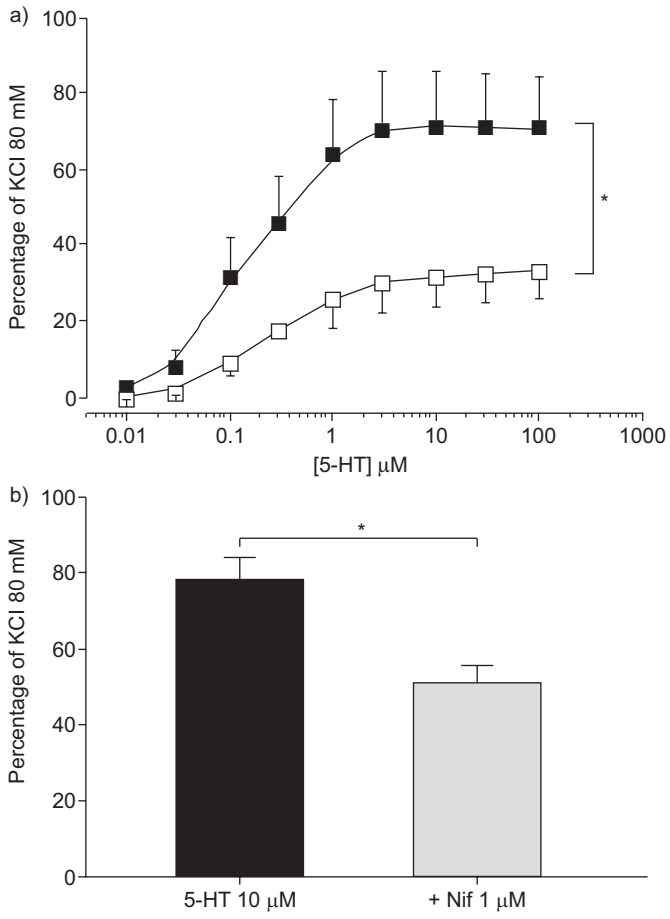

FIGURE 5. Role of L-type voltage-gated calcium channels in serotonin (5-hydroxytryptamine; 5-HT)-induced contraction in human pulmonary arteries. a) Concentration–response curves to 5-HT were performed in the presence of nitrendipine 1 μM, a specific L-type voltage-gated calcium channel blocker. ■: control; □: nitrendipine 1 μM. b) The effect of nifedipine 1 μM on the contractile response to 10 μM 5-HT (34.5% inhibition). Contraction is expressed as a percentage of the K^+-rich (80 mM) solution-induced response. Each value represents the mean±SEM for 12–24 rings and five patients. *: $p<0.05$.

time course of the calcium responses to 5-HT, the mean basal $[Ca^{2+}]_i$ was 209±17 and 161.7±19 nM and the mean amplitude of the $[Ca^{2+}]_i$ rise was 189.8±19.9 and 131.3±12.5 nM for oscillating and non-oscillating calcium responses, respectively (fig. 2d and e; n=46, N=3).

Role of 5-HT_1 and 5-HT_2 receptors as well as 5-HT transporter in the calcium and contractile responses to 5-HT

5-Carboxamidotryptamine (5-CT) 10 μM, a 5-HT_1 receptor agonist and (R)(-)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (R-DOI) 10 μM, a 5-HT_2 receptor agonist, both induced an oscillating calcium signal in cultured human PASMCs (fig. 3a and b). The resting calcium level was 116±8.9 nM (fig. 3c; n=52, N=3). The amplitude of the calcium rises was not significantly different for 5-HT, 5-CT and R-DOI (fig. 3d, n=7–23, N=3). In HPA rings, dose–response curves to R-DOI or 5-CT induced pulmonary arterial contractions whose amplitudes were half the amplitude of the contractions to 5-HT. However, the sensitivity to 5-CT (EC_{50}

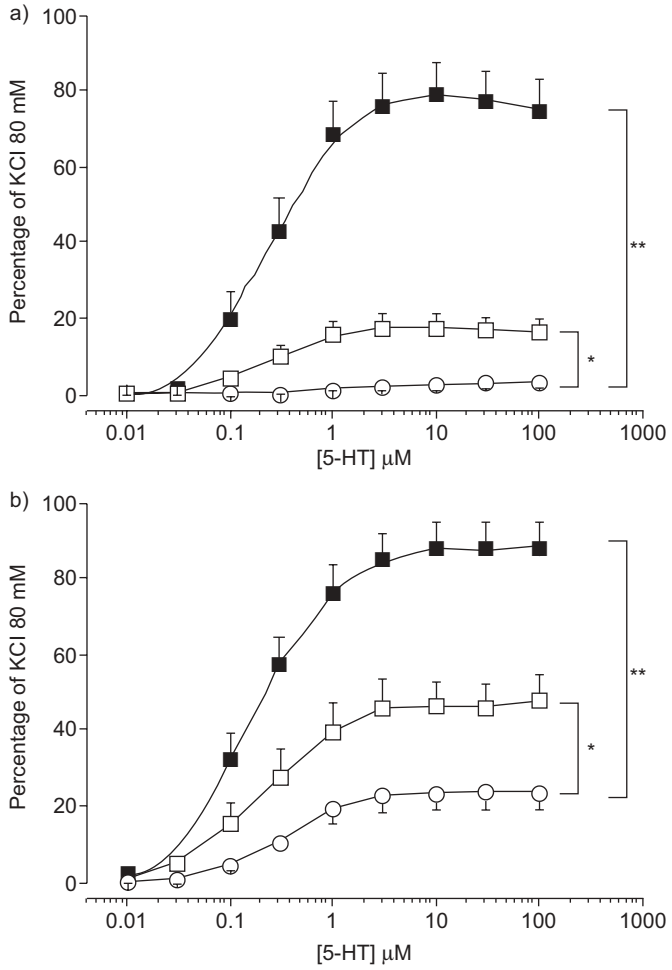

FIGURE 6. Role of intracellular calcium from the sarcoplasmic reticulum on the contractile response to serotonin (5-hydroxytryptamine; 5-HT). a) Cyclopiazonic acid (CPA) 10 μM or b) thapsigargin (TG) 1 μM, two specific blockers of the sarcoplasmic reticulum Ca-ATPases, partially decreased the contractile response to 5-HT (□). In the absence of extracellular calcium, CPA and TG further decreased the contractile response to 5-HT (○). Contraction is expressed as a percentage of the K^+-rich (80 mM) solution-induced response. ■: control. Each value represents the mean±SEM for 10–26 rings and 6–10 patients. *: $p<0.05$; **: $p<0.01$.

0.12±0.06 μM; n=13, N=5) was significantly higher than the sensitivity to 5-HT (EC_{50} 0.52±0.17 μM; n=7, N=5), whereas the sensitivity to R-DOI (EC_{50} 19.76±4.51 μM; n=12, N=5) was significantly lower than the sensitivity to 5-HT (fig. 4a). In the presence of 1 μM SB204741, an antagonist of the $5\text{-}HT_{2B}$ receptors, the contraction to 5-HT was not modified (fig. 4b). Finally, 1 μM citalopram, an inhibitor of the 5-HT transporter, had no significant effect on the CCRC to 5-HT (fig. 4c), suggesting that the contraction in HPAs may depend mainly on the activation of 5-HT receptors.

Role of the main calcium sources in the contractile response to 5-HT

In order to determine the transduction pathways involved in the contractile response to 5-HT, we then focused on the role of: 1) the extracellular calcium sources, namely L-type voltage-gated and/or voltage-independent calcium channels; and 2) the intracellular calcium sources, namely the sarcoplasmic reticulum.

In the presence of 1 μM nitrendipine or 1 μM nifedipine, two L-type voltage-gated calcium channel inhibitors, the maximal contraction to 5-HT was inhibited by 53.43% and 34.5%, respectively (fig. 5a and b; n=12–16, N=5) attesting the contribution of the L-type voltage-gated calcium channels to the 5-HT-induced contraction. Calcium-free solution also showed a partial inhibiting effect on the contraction to 5-HT (inhibition of 31.51%; n=25, N=8; data not shown). In the presence of 1 μM TG or 10 μM CPA, two specific sarcoplasmic reticulum Ca^{2+}–Mg ATPase inhibitors which thus deplete sarcoplasmic reticulum calcium stores, the maximal contraction to 5-HT was also partially decreased (fig. 6; n=25–26, N=8–10; $p<0.01$). In both conditions, the residual contraction to 5-HT was further decreased in the absence of extracellular calcium (fig. 6; n=11–14, N=6).

Since voltage-independent calcium channels were shown to be important in the contractile response to 5-HT in rat intrapulmonary arteries [5, 14], we then examined the role of a store-operated calcium channel (SOCC) and its role in the contractile response to 5-HT in HPA. In the presence of CPA (10 μM) and nitrendipine (1 μM), switching from a Ca^{2+}-free to a 2 mM $CaCl_2$-containing solution induced a contraction that was strongly blocked by 0.5 mM Ni^{2+}, a nonspecific inhibitor of calcium entry (fig. 7a and b; n=9, N=3). The same concentration of Ni^{2+} (0.5 mM) inhibited the CCRC to 5-HT by 50% (fig. 7c; n=24, N=5). In contrast, Ni^{2+} (0.5 mM) did not modify the contractile response to high potassium (80 mM) solution (n=10, N=3), whereas nifedipine blocked the same contraction by 78% (fig. 7d; n=10, N=4), which indicates that L-type voltage-gated calcium channels are not sensitive to Ni^{2+} in HPAs. Gadolinium (Gd^{3+}) 100 μM, another inhibitor of non-voltage-gated calcium channels also blocked by 51.8% the contractile response to 5-HT (10 μM) and the addition of nifedipine (1 μM) had an additive effect and abolished the contraction (fig. 7e; n=12, N=4). Altogether, these results demonstrated the presence of SOCCs and L-type voltage-gated calcium channels, which are both involved in the contraction to 5-HT in HPAs.

Effect of 5-HT on Ca^{2+} sensitivity

In β-escin-permeabilised HPA rings [19], a single pCa of 6 (1 μM free Ca^{2+}) followed by addition of 10 μM 5-HT resulted in stepwise tension increases, probably related to a significant increase in Ca^{2+} sensitivity of the myofilaments (fig. 8a). This 5-HT sensitisation to a Ca^{2+} clamp at pCa 6 produced a 36% increase in tone (fig. 8b; n=17, N=3).

DISCUSSION

5-HT (0.01–100 μM) induces a concentration-dependent contraction in HPAs. $5\text{-}HT_1$ and $5\text{-}HT_2$ receptors are present and functional and 5-HT transporter may not participate in the contraction in HPAs. Moreover, this contraction is sensitive to: 1) voltage-operated (nifedipine and nitrendipine) and 2) non-voltage-operated (Ni^{2+} and Gd^{3+}) calcium channel blockers; as well as 3) drugs that deplete intracellular Ca^{2+} stores (TG and CPA). Stimulation of SOCCs also induces a contraction. Finally, Ca^{2+} sensitivity of the contractile apparatus is enhanced by 5-HT. Thus, the present study demonstrates, for the first time,

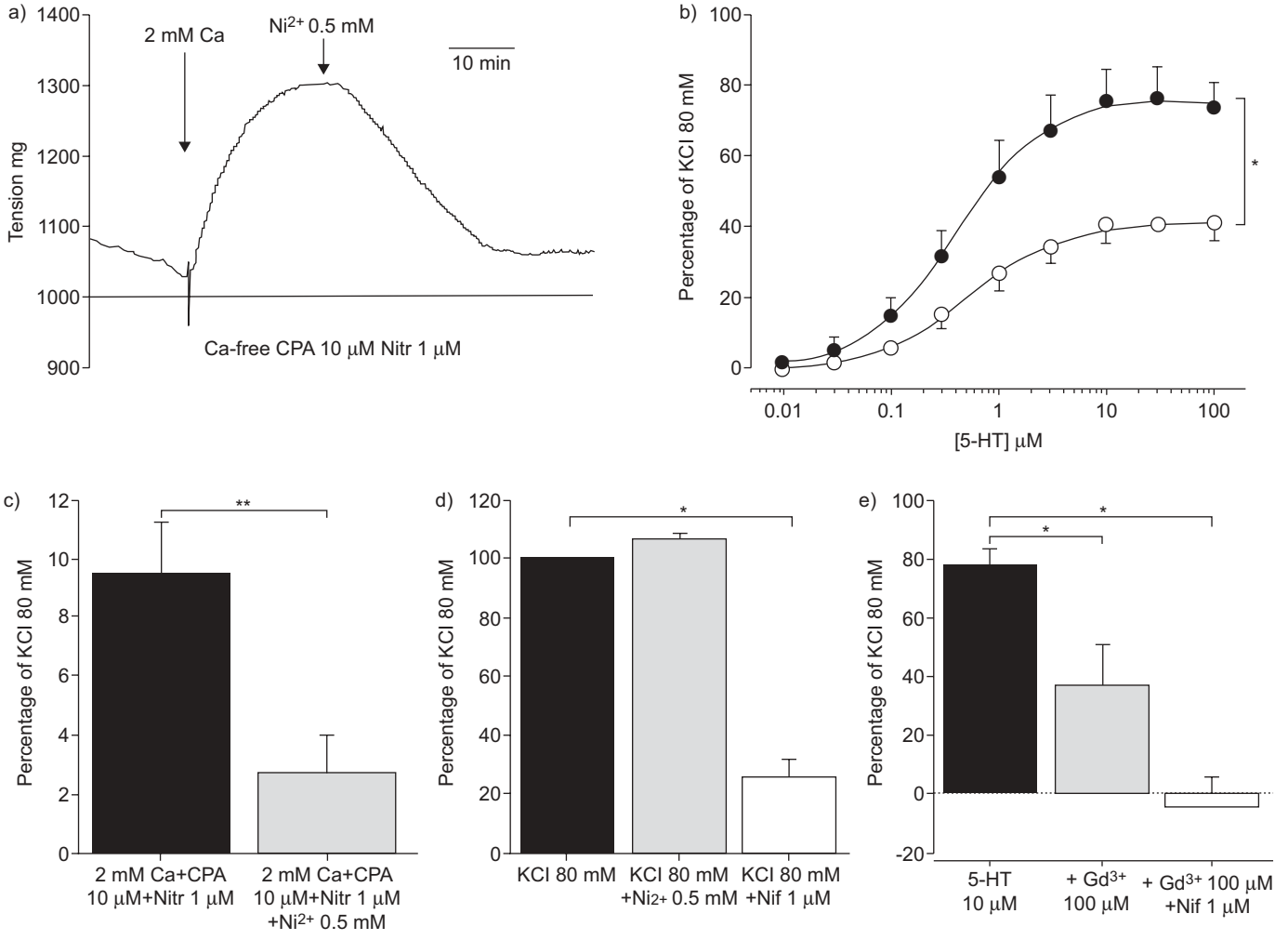

FIGURE 7. Presence of voltage-independent calcium influxes and their role in the contractile response to serotonin (5-hydroxytryptamine; 5-HT). a) In the presence of 1 μM nitrendipine (Nitr) and 10 μM cyclopiazonic acid (CPA), changing the bath solution from calcium-free to 2 mM calcium induced a contraction. This contraction was abolished by 0.5 mM Ni^{2+}. c) The mean±SEM of the inhibitory effect of Ni^{2+} on the contraction (n=9 for both experiments). b) The effect of 0.5 mM Ni^{2+} (○) on the contraction to 5-HT (0.01–100 μM) (●: control); d) the effect of 0.5 mM Ni^{2+} on the contraction to a depolarising high potassium (80 mM) solution is shown. d) Nifedipine (Nif) 1 μM strongly blocked the contraction to high potassium (80 mM) solution (n=10 for both experiments). e) The effect of voltage-independent and voltage-dependent Ca^{2+} channel blockers on 5-HT-induced contractions in human pulmonary arteries (HPAs). HPA rings were pre-contracted with 10 μM 5-HT prior to the addition of the voltage-independent Ca^{2+} channel blocker, 100 μM gadolinium (Gd^{3+}) (resulting in 51.8% inhibition), and the voltage-dependent Ca^{2+} channel blocker, 1 μM nifedipine, alone or combined (104.3% inhibition). Data are presented as mean±SEM for 9–24 rings and 3–5 patients. In panels b, c and d, contraction is expressed as a percentage of the K^+-rich (80 mM) solution-induced response. *: $p<0.05$; **: $p<0.01$.

that: 1) Ca^{2+} influxes through both L-type voltage-gated calcium channels and SOCCs; and that 2) Ca^{2+} release from the sarcoplasmic reticulum and 3) Ca^{2+} sensitisation of the myofilaments are the main components of the contractile response to 5-HT in HPAs.

In previous studies on the reactivity of HPA to 5-HT, EC_{50} values varied from 0.1 to 0.39 μM, which is consistent with the current results (EC_{50} 0.44 μM) [8, 9, 12]. It should be noted that, regarding CCRC to 5-HT in intrapulmonary arteries from male Wistar rats, EC_{50} values varied from 0.8 to 7.4 μM [13], which is higher than the EC_{50} values in HPAs. Although human pulmonary arterial contraction could be induced by 5-HT_1 and 5-HT_2 agonists, SB204741, a 5-HT_{2B} antagonist, did not modify the contraction to 5-HT (fig. 4). Such a result regarding 5-HT_{2B} receptors may not be very surprising since it has been previously shown that 5-HT_{2B} receptors induce a relaxation mediated by endothelial 5-HT_{2B} receptors in pig pulmonary arteries [21].

We have observed different patterns of calcium response to 5-HT 10 μM in cultured smooth muscle cells from HPAs (fig. 2). It is noteworthy that these calcium signal time courses are similar to those we reported in rat pulmonary arteries [5]. In freshly dissociated or cultured smooth muscle cells from the same segment of rat pulmonary arteries, different smooth muscle cell phenotypes have been shown to correlate with the activity of different potassium channels [22] or different profiles of Ca^{2+} responses to hypoxia or ATP, which mobilises intracellular calcium *via* G protein receptor activation [23].

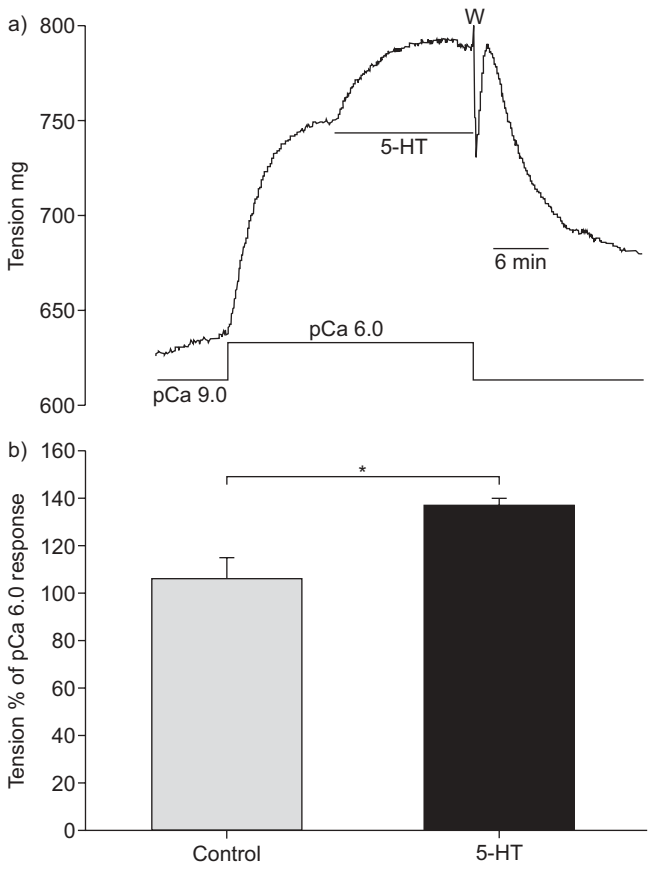

FIGURE 8. Effect of serotonin (5-hydroxytryptamine; 5-HT) treatment on the Ca^{2+} sensitivity of permeabilised human pulmonary arteries (HPAs). a) Typical recording displaying the tension induced by a step to pCa 6 followed by the addition of 10 μM 5-HT after permeabilisation procedure of HPA at pCa 9. b) Quantitative analysis of the mean tonic responses to pCa 6 and 10 μM 5-HT expressed as a percentage of pCa 6 response (n=17, N=3). These results suggest that 5-HT is able to increase the Ca^{2+} sensitivity of the myofilaments in HPAs. W: washout. *: $p<0.05$.

The various patterns of calcium responses to 5-HT observed in the present study may, therefore, be related to different PASMC phenotypes which might relate to their physiological status. Since 5-HT_1 and 5-HT_2 agonists both induce intracellular calcium variations (fig. 3), the various profiles of the calcium responses to 5-HT may well be due to different expression levels of the 5-HT_1 and 5-HT_2 receptors. 5-HT (10 μM) induced a calcium signal characterised by oscillations in about half of the smooth muscle cells from HPAs. In a more integrated model, such as thin lung slices cut from mouse lungs, 5-HT also induced an oscillating Ca^{2+} signal in response to 5-HT [24]. Cultured smooth muscle cells from HPAs may, therefore, be a good model for the study of calcium signalling, which is essential for many of the pathological processes in pulmonary arteries.

The contractile response to 5-HT was partially inhibited by intracellular calcium store depletion, and extracellular calcium removal further inhibited the contraction. Since both effects were additive, both components are involved. Nifedipine and nitrendipine, two L-type voltage-gated calcium channel inhibitors partially blocked 5-HT-induced contraction, suggesting that L-type calcium channels are involved. This result is in accordance with a previous study that demonstrated a partial effect of nifedipine (3 μM) on the contractile response to 5-HT in HPAs [25]. In rat PASMCs, it has been shown that 5-HT decreases the activity of voltage-gated K^+ channels, thus inducing membrane depolarisation [26]. Such an effect involves 5-HT receptors and Kv1.5. Since Kv1.5 is also expressed in human PASMCs [27], the activation of L-type voltage-gated Ca^{2+} channels by 5-HT observed in HPAs could well be linked to a depolarisation induced by the inhibition of Kv1.5.

The contraction induced by re-addition of extracellular Ca^{2+} after CPA treatment is characteristic of the activation of a SOCC. Such channels are known to be permeable to Ca^{2+} and/or Na^+, leading to depolarisation and consequently to L-type voltage-gated calcium channel activation. These channels are also sufficiently permeable to calcium to induce a rise in $[Ca^{2+}]_i$ and contraction [16]. In the current study, the effect of nifedipine and gadolinium on the contractile response to 5-HT were additive, suggesting that calcium influx from both L-type voltage-gated calcium channels and SOCC are both involved. As previously described in rat PASMCs, we demonstrated in HPAs that the SOCC component was insensitive to nitrendipine, a dihydropyridine L-type voltage-gated calcium channel antagonist, but sensitive to gadolinium and nickel [28, 29]. The SOCC has also been described in cultured human PASMCs and this conductance is insensitive to nifedipine, another dihydropyridine calcium channel antagonist, but fully inhibited by 0.5 mM Ni^{2+} [30]. By means of siRNA or antisense oligonucleotides, the contribution of the canonical transient receptor potential (TRPC)1 and 4 proteins to endogenous SOCC was demonstrated in human cultured PASMCs [31, 32]. Hence, in rat primary cultured PASMCs, we have previously shown that 5-HT activates a transient receptor potential vanilloid (TRPV)4-like calcium influx, potentially involved in PASMC proliferation [20]. Such calcium influx is strongly blocked by extracellular calcium removal or the presence of Ni^{2+} 0.3 mM [20]. Consequently, stimulation of TRPC1–4 and/or TRPV4 by 5-HT may contribute to calcium entry and contraction in HPA.

Since we have previously shown that Rho-kinase plays a role in the contraction to 5-HT in rat pulmonary arteries [13], we addressed effect of 5-HT on the Ca^{2+} sensitivity of the contractile apparatus in permeabilised HPA rings. In the presence of a fixed Ca^{2+} concentration (pCa 6), 10 μM 5-HT increased the calcium sensitisation of the contractile apparatus (fig. 8). We previously used similar protocol to successfully permeabilise small HPAs and thus demonstrated that 20-HETE decreased the Ca^{2+} sensitivity of the myofilaments [19]. 5-HT also increased calcium sensitisation of the contractile apparatus in rabbit renal arteries [33]. These results suggest that 5-HT may modulate key effectors involved in the Ca^{2+} sensitisation process in HPAs.

Altogether, the present data show that the contractile response to 5-HT in HPA is characterised by the contribution of various signalling pathways, including: 1) calcium influx through both L-type voltage-gated and non-voltage-gated (potentially SOCC) calcium channels; 2) intracellular Ca^{2+} release from the sarcoplasmic reticulum; and 3) calcium sensitisation of the contractile apparatus. Since 5-HT reactivity plays a crucial role

in PAH, the present findings in human tissue may be clinically relevant for the identification of novel therapeutic targets.

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STATEMENT OF INTEREST

None declared.

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